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Lung Cancer Epidemiology in Korea

Clinically Significant Unclassified Variants in BRCA1 and BRCA2 Genes among Korean Breast Cancer Patients
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Development of a Community-Based Palliative Care Model for Advance Cancer Patients in Public Health Centers in Busan, Korea

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Purpose
A feasible palliative care model for advance cancer patients is needed in Korea with its rapidly aging population and corresponding increase in cancer prevalence. This study describes the process involved in the development of a community-based palliative care (CBPC) model implemented originally in a Busan pilot project.

Materials and Methods
The model development included steps I and II of the pilot project, identification of the service types, a survey exploring the community demand for palliative care, construction of an operational infrastructure, and the establishment of a service delivery system. Public health centers (including Busan regional cancer centers, palliative care centers, and social welfare centers) served as the regional hubs in the development of a palliative care model.

Results
The palliative care project included the provision of palliative care, establishment of a support system for the operations, improvement of personnel capacity, development of an educational and promotional program, and the establishment of an assessment system to improve quality. The operational infrastructure included a service management team, provision teams, and a support team. The Busan Metropolitan City CBPC model was based on the principles of palliative care as well as the characteristics of public health centers that implemented the community health projects.

Conclusion
The potential use of the Busan CBPC model in Korea should be explored further through service evaluations.

Key words
Chronic disease, Neoplasm, Patients, Palliative care, Pilot projects

Introduction

In Korea, 28.6% of all deaths are cancer-related, but only 13.8% of the population receives palliative care before death [1,2]. To improve palliative care, Korea has implemented policies aimed at increasing its use to 20%, increasing the number of palliative care beds from 1,000 to 1,400, and providing a variety of palliative care services, including a palliative care consultation system and a home-based palliative care system by 2020 [1]. In addition, to build social consensus and systems for palliative care, a payment system was initiated in July 2015 to guarantee that the health insurance system covers the cost of inpatient palliative care for terminally cancer patients. The "Palliative Care and the Decision Regarding Life-Sustaining Treatment of End-of-life Patients..."
“Bill” was passed in 2016 and was set to be enacted in 2018 [3]. On the other hand, the Korean palliative care system is limited in usage and short in duration.

The scope of palliative care was recently expanded to include patients with end-stage heart disease, respiratory problems, kidney failure, liver diseases, and neurological disorders. With the increase in the elderly population, the inclusion of an increasing number of patients with non-cancer end-stage illnesses is expected to widen the scope of palliative care and the number of beneficiaries. Palliative care is not an end-stage care provided for patients for whom active treatment has been discontinued; rather, it involves physical symptom control and psychosocial and spiritual support. Therefore, it is an inclusive care approach that should begin at a life-threatening disease diagnosis [4]. Since the 2000s, the interest in palliative care has emerged from human rights and social justice perspectives, and the need for healthcare policy to ensure that every member of society benefits from the service has been emphasized [5]. The service should be easily accessible and universal to ensure that anyone can use it. In addition, a good strategy based on community involvement is required, along with an increase in community knowledge and public awareness of palliative care. The World Health Organization (WHO) suggests that palliative care strategies should combine palliative and primary care in communities that use public healthcare services [6].

This paper proposes that a community-based palliative care (CBPC) strategy be considered in end-of-life Korean care strategies because the use and accessibility of palliative care is particularly low there. In 2009, Busan, Republic of Korea, conducted a pilot CBPC project centered on public health centers (i.e., community-level primary healthcare facilities). Public health centers were selected as the focal points for allowing the use of various community resources investing in early approaches to end-of-life care and social support because they are public primary healthcare institutions at which community members first make contact and receive medical care. Palliative care provision must satisfy the fundamental concepts of healthcare delivery systems (i.e., accessibility, continuity, and standardized quality services). The Busan Metropolitan City CBPC pilot project, which was conducted between 2009 and 2014, established a basic framework for the structure, personnel, and service content required to provide CBPC based on other healthcare delivery systems [7]. In January 2015, Busan applied the system developed in the pilot project and began a CBPC project in the city’s public health centers. To develop and standardize the CBPC service model, Busan created a CBPC center (CBPCC) that acted as a control tower and provided service standardization, directives, education, execution strategy development, and quality control.

This paper describes the processes involved in establishing the Korean CBPC model. The study aimed to develop a CBPC model based on the Busan CBPC pilot project, the demand for palliative care, and previous studies. The specific objectives were as follows: to determine the community demand and identify suitable types of CBPC services, to establish an operational infrastructure for CBPC task performance, and to establish a CBPC service delivery system.

Development of CBPC Model

The model was developed during steps I and II of the pilot project. The developmental process of the CBPC model included identification of the types of services required to meet the CBPC demand, and the establishment of an operational infrastructure and service delivery system. The study received financial support from Busan and the Korean Ministry of Health, Welfare, and Family Affairs Research and Development Fund. The study was approved by the Catholic University of Pusan Research Ethics Committee (CUPIRB-2014-059).

1. Geographical region and target population

The project was conducted in Busan, the second largest city in Korea, which has a population of 3.41 million (2014) and an area of 764.43 km². The city includes 16 districts and counties. In 2014, the Korean cancer mortality rate was 150.9 per 100,000 people per year, and that of Busan Metropolitan City was 175.2 per 100,000 people per year, the highest rate in Korea [8]. The four palliative care centers in Busan provide a total of 87 beds, which represents 49% of the total number of beds required based on the criterion that stipulates 25 beds per 500,000 people [9]. Korean palliative care centers currently provide only inpatient services, and palliative care staff members include physicians who have completed 60 hours of standardized training in palliative care, as well as palliative nurses, social workers, chaplains, and volunteers who have received 30 hours of training.

As the study aimed to develop a CBPC model centered on public health centers, a system for service provision was established by selecting home-based patients receiving end-of-life care who were registered at the public health centers as the target population. Public health centers were selected as the regional hubs for CBPC model development because they have sufficient medical personnel, community resources, and networks, and the local residents can access them readily. The scope of the project and characteristics of the target population were as follows:

- The study included home-based patients receiving end-
of life care, who were registered at Busan public health centers between January and December 2015, their families, and their service providers.
- Sixteen Busan public health centers served as regional hubs for CBPC model development.
- Regional cancer centers, palliative care centers, and social welfare centers in Busan were involved in model development.
- The independence of each organization was respected. On the other hand, the CBPCC that housed the model development team was responsible for research/development decision making and regulation.

2. Model development team

The initial model development team consisted of two nursing professors with expertise in palliative care. These experts proposed a palliative care project centered on public health centers and conducted a pilot project from 2009 to 2014. In 2015, when the project was expanded to include all of Busan Metropolitan City, a full model development team, consisting of six professors in nursing, social work, business administration, and medicine, was formed.

Busan consigned the CBPC project to the model development team, which managed the planning and execution unit within the service management team. In particular, the model development team set the vision, goals, and strategies of the project and made decisions regarding the crucial aspects of model development involving infrastructure for execution, service standards, and outcome measures.

3. Developmental steps

1) Pilot project step I (2009-2014)

In 2008, a research team specializing in palliative care submitted a CBPC project proposal to the Health Improvement Department of Busan and persuaded the city’s health administrators to support it. In 2009, the model development team was entrusted with the project and formed a research support committee. A pilot project center began in 2009 and included one public health center; however, the number of participating public health centers had increased to six by the 2014 project completion date. During the pilot project, Busan supplied administrative and financial support. The regional cancer centers provided technical support, and public health centers played the role of service providers. The model development team supervised, planned, and regulated the project.

During the study period, the model development team assembled an early framework for an operating model based on palliative care recipients, service level, labor standards for palliative care teams, personnel education, and consortium establishment [10]. Busan accepted the model development team’s request for continuous adaptation, development, financial support, and evaluation based on the access of home-based cancer patients to palliative care and the effective use of healthcare resources. Furthermore, Busan recognized that the CBPC project increased the quality of life for home-based cancer patients and their families and was valuable as an end-of-life care system that was easily accessible to a highly vulnerable low-income population. Accordingly, in 2015, the city decided to expand the project to include public health centers across Busan.

2) Pilot project step II (2015)

Since January 2015, the CBPC project included 16 public health centers in Busan Metropolitan City as regional hubs and applied the preliminary operating model developed in Step I to establish a CBPC model. The model development team requested a grant from the Ministry of Health, Welfare, and Family Affairs’ Health Technology Research and Development Project for the development of a CBPC service system, for which it received 2 years of research funding for 2015 and 2016. Busan established the CBPCC to effectively conduct the CBPC project and entrusted the model development team with the responsibility of supervising, planning, and evaluating the project. The research support committee that operated during the pilot project was reorganized to include Busan regional cancer centers, the Social Service Network, legal experts, religious leaders, and journalists.

Results of CBPC Model Development

1. Community demand for palliative care

During step I, palliative care services were designed based on the demands of experts and home-based patients receiving end-of-life care in the community in which the pilot project had been conducted. In step II, 802 home-based cancer patients registered at public health centers in Busan and 651 center staff members to assess the palliative care needs were surveyed (Table 1).

Most home-based cancer patients (68.8%) and public health center personnel (96.6%) responded that they desired to receive palliative care services. The most preferred type of visiting care personnel was nurses (85.5% of the home-based patients, and 83.4% of the community health center personnel), and the preferred types of home-based palliative care services were psychological/emotional support (72.1% of
Table 1. Demand for palliative care from public health center personnel and home-based cancer patients registered at public health centers

<table>
<thead>
<tr>
<th>Area</th>
<th>Item</th>
<th>Home-based cancer patients (%) (n=802)</th>
<th>Public health center personnel (%) (n=651)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention to use palliative care</td>
<td>Use</td>
<td>68.8</td>
<td>96.6</td>
</tr>
<tr>
<td></td>
<td>Not use</td>
<td>31.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Preferred home-based palliative care service provider</td>
<td>Nurse</td>
<td>85.5</td>
<td>83.4</td>
</tr>
<tr>
<td></td>
<td>Volunteer</td>
<td>17.9</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Social worker</td>
<td>15.0</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>Doctor</td>
<td>14.5</td>
<td>24.7</td>
</tr>
<tr>
<td>Preferred home-based palliative care service</td>
<td>Psycho-emotional support</td>
<td>72.1</td>
<td>82.6</td>
</tr>
<tr>
<td></td>
<td>Medical support</td>
<td>69</td>
<td>64.4</td>
</tr>
<tr>
<td></td>
<td>Educational information</td>
<td>60.6</td>
<td>57.4</td>
</tr>
<tr>
<td></td>
<td>Socioeconomic support</td>
<td>48.2</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>Spiritual support</td>
<td>6.9</td>
<td>41.9</td>
</tr>
<tr>
<td></td>
<td>Family support</td>
<td>4.7</td>
<td>50.2</td>
</tr>
<tr>
<td>Preferred place of death</td>
<td>Home</td>
<td>49.4</td>
<td>63.7</td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>28.6</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>Palliative care facility</td>
<td>12.3</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Nursing care facility</td>
<td>7.1</td>
<td>4.8</td>
</tr>
</tbody>
</table>

*a*Multiple response.

Fig. 1. Community-based palliative care service infrastructure.

patients and 82.6% of staff) and medical support (69% of patients and 64.4% of staff). Home-based cancer patients preferred their homes (49.4%) and hospitals (28.6%), while public health center personnel preferred their homes (63.7%) and palliative care facilities (18.6%) as the places of death.

2. Derived objectives and content

The following goals and business plans for the CBPC project were established based on the pilot project and survey results regarding the service demands from the main project stakeholders.

The project’s goals were to provide end-of-life, home-based hospices, and palliative care for patients and their families with public health centers as the community hubs, and to improve the quality of life for both patients and their families. The business plan was as follows.

(1) Provide CBPC
(2) Establish a support system for CBPC operation
(3) Train personnel involved in CBPC
(4) Develop educational and promotional programs to raise the CBPC awareness
(5) Create an assessment system to improve the CBPC quality
3. Infrastructure organization

The following groups were established to manage the CBPC project (Fig. 1).

1) Service management team

The service management team was a part of the CBPCC and included planning and management, education, and human resource support units.

The planning and management unit established the project’s vision, goals, and strategies and made decisions regarding the infrastructure for execution, service content, and outcomes. The unit included palliative care coordinators, social workers, and six professors specializing in palliative care nursing, social work, and business administration. They held weekly planning and management meetings and made decisions regarding service direction.

The educational unit was responsible for providing the education and information required to allow the CBPC teams at 16 public health centers to perform their tasks. The unit included four professors specializing in palliative care, nursing practitioners in palliative care, social workers, and other expert consultants.

The human resource support unit was responsible for supporting the personnel required to provide CBPC. One of the responsibilities of the service management team was to support palliative nurses, social workers, and volunteers to ensure that public health centers could provide palliative care. The educational unit trained palliative nurses before dispatching them to public health centers, along with social workers and volunteers, when public health centers required staff or support. Because Korean public health centers implement several community healthcare projects and employ insufficient numbers of palliative care professionals, a system was established through which trained nurses and social workers were dispatched to maintain the palliative care quality. Palliative nurses all had more than 2 years’ hospital work experience and had either completed 60 hours of standardized training in palliative care or had obtained palliative care nursing certification. Social workers who had completed standard training in palliative care were also hired and were dispatched to public health centers where needed.

CBPCC staff that provides first-hand services consisted of a total of 13 members: nine palliative nurses, two palliative social workers, and two administrative workers.

2) Service provision teams

Palliative care teams were formed to provide comprehensive palliative care in public health centers. These teams consisted of a public health center manager, a physician, a palliative nurse, a social worker, a chaplain, and volunteers (Fig. 2) [10]. Public health center managers had the overall administrative responsibility. The physicians provided medical services, and the officer responsible for family health performed the administrative tasks. The staff working on the project for home-based cancer patients planned and executed the budget, handled the business aspects of palliative care provision in public health centers, and managed the volunteers. The manager of the healthcare home-visit team and home-visiting nurses identified and referred home-based cancer patients requiring palliative care to the palliative care team, and a chaplain provided spiritual support. Palliative nurses and social workers were responsible for case management. They also held weekly case management meetings with the service management team to prioritize the tasks and make decisions regarding resource distribution from a problem-solving perspective. The service provision team members were also responsible for other healthcare tasks in public health centers. CBPC project team meetings were held quarterly to allow the nurses to report the status of palliative care recipients and discuss the issues requiring resolution.

The CBPC project was based on the operating principles
Fig. 3. Case management pathway.

for the effective utilization of community personnel and material resources with minimal investment from public health centers. Accordingly, the palliative care teams were established by adjusting the task assignments in public health centers, rather than hiring additional staff. On the other hand, palliative nurses and social workers who provided the care recipients with direct services were a part of the CBPCC and performed the tasks as service provision team members while receiving education and support in recipient management from the center. One palliative nurse was assigned to two public health centers and provided services by visiting the registered palliative care recipients at home. Two social workers were involved in managing cases to which patients at one of the 16 public health centers had been referred because of the socioeconomic need and the need for support from a palliative nurse (Fig. 2) [10].

Out of the 490 patients treated at the 16 health centers from January to December 2015, 485 (99%) were terminally ill cancer patients and five (1%) were terminally ill non-cancer patients.

3) Service support team

The service support team was responsible for the administrative, financial, and technical consultations. This team consisted of the research support, working, and medical support committees.

- The research support committee included Busan regional cancer center personnel, the Social Service Network, legal experts, religious leaders, and journalists. The committee discussed the project-related issues and mainly provided financial and administrative support. Committee meetings were held at least biannually.
- The working committee regulated and discussed everyday project execution and consisted of officers managing everyday CBPC project implementation at public health centers, palliative nurses, and service management teams in palliative care centers. The committee members held meetings at least biannually where needed.
- The medical support committee served as a support system providing medical service consultations at public
health centers to control the patients’ symptoms and pain. The committee included three physicians who specialized in palliative care and were responsible for providing education and consultations regarding the management of end-of-life symptoms for public health center physicians.

4. Case management and referral system

1) Case management pathway

The specific case management pathways for palliative care recipients referred to service provision teams were as follows (Fig. 3).

The referred patients were registered as case management recipients by a palliative nurse after they gave informed consent. After registration, a palliative nurse and a social worker initially assessed patients’ service demands and provided services according to prioritized care plans. The visiting intervals were determined according to the service demand, and a hospice volunteer was dispatched once or twice per week to provide physical, psychological, and spiritual help.

If community medical support was required, medical support from a physician and home visits were arranged by the service provision teams.

Palliative care recipients showing symptoms necessitating hospitalization while receiving palliative care were referred to a consortium palliative care center or hospital and treated as inpatients. Case management pathways were created to ensure that the patients would be referred back to the CBPC service provision teams if their symptoms improved and they were discharged. A bereavement service was provided when the care recipients died, and family members who were not at high risk of bereavement were removed from the system after 13 months.

Patients and families were provided with a phone number providing access to a 24-hour, on-call service for use when emergencies occurred or they required counseling outside of normal business hours. Palliative nurses were responsible for this service.

2) Referral system

The primary referral path for CBPC service recipients was
accessed via home-visit teams or nurses working on a project for home-based cancer patients who referred patients registered in their systems to palliative nurses. In addition, patients and their families could contact the CBPC service office directly, and the social welfare center could refer patients who had been discharged from palliative care centers.

Patients referred to palliative nurses received care after providing informed consent and completing registration. A consortium system was established to ensure that the service provision teams referred patients requiring inpatient treatment to palliative care centers or the palliative medical ward of a regional cancer center in the consortium system. If professional services from a social welfare center were required, help was provided via a social economic support referral system. The referral system of a CBPC project was designed to ensure that the regional cancer and palliative care centers maintained close coordinating relationships with the service provision teams, and when the palliative care recipients required medical support, they were admitted to a consortium hospital and referred back to service provision teams upon discharge to continue receiving care (Fig. 4).

5. CBPC service delivery system

During the implementation of a pilot project between 2009 and 2015, the model development team established a service delivery system to provide services for patients requiring palliative care from public health centers (i.e., primary care organizations as regional hubs) (Fig. 4).

The service management team responsible for the project provided the personnel and technological and administrative support required by the service provision teams to manage the project. Public health centers established service provision teams and implemented the project with operational support from the service management team. The primary beneficiaries of the CBPC project included patients who required palliative care and were referred by a project team for home-based cancer patients or a healthcare home-visit team public health center. In addition, patients who were referred from regional palliative care centers, hospitals, or social welfare centers were registered and received comprehensive services from the service provision teams and volunteers following the initial assessment. Palliative nurses who worked with the public health centers to visit and provide direct care to patients discussed with the service management team in weekly case management meetings the type and level of care each patient required.

The service management team developed practical guidelines for the service provision teams at public health centers to provide standardized services and develop strategic plans for education, research, and promotion of the overall project.

The service provision teams identified patients, provided care, and offered direct services by coordinating the required community resources. Finally, the service support team aided the service provision teams regarding general issues concerning palliative care provision (Fig. 4).

Discussion

This paper described the processes involved in establishing the Korean CBPC model developed in a pilot project implemented in Busan Metropolitan City. This model was developed by surveying the regional characteristics and community demand for palliative care to identify the business goals and service content, establishing an operational infrastructure, and establishing a service delivery system.

During the six years of the pilot project before the CBPC project was implemented, the service provision teams’ capacity was developed, the scope and level of service was determined, the patient referral and consortium systems were established in various institutions, and volunteer training and community education were provided to induce community participation. Consequently, the city health administrators recognized that the pilot project, which was based on public health centers in Busan that had an insufficient number of palliative care beds, increased the accessibility of end-of-life care for home-based cancer patients, including those belonging to the highly vulnerable low-income class, and ensured effective utilization of healthcare resources. In 2015, a CBPCC was established as the project was expanded to include public health centers across Busan. The model development team performed service management functions in the CBPCC while developing the model.

Public health centers served as regional hubs in the CBPC project, and each public health center established a palliative care team that functioned as a service provision team. Palliative nurses and social workers from the service provision teams were dispatched by the CBPCC, and other support was provided by public health center personnel by adjusting their job responsibilities. The advantage of this structure was that additional investment was not required to allow public health centers to implement palliative care projects. Moreover, service quality management was possible via the organic relationships between the service management and provision teams, which reduced the burden community member health placed on public health centers that implemented a range of other regional healthcare projects.

The project’s service support team played an advisory role in providing administrative, financial, and technological consultation. The research support committee, based at the
Health Improvement Department of Busan, regional cancer centers, and the Social Service Network, provided support and discussed the issues concerning project implementation. The medical support committee was responsible for educating physicians in public health centers to ensure that the palliative care recipients received drug treatment and medical support when required and provide consultation regarding drug treatment. The working committee consisted of the officers managing the project at public health centers, and it contributed to service standardization and improvements in task effectiveness across all public health centers via regulation and discussion regarding the issues concerning everyday palliative care tasks. The most difficult aspect of implementing the CBPC project was persuading public health centers to agree to implement a palliative care project and provide education regarding the provision of general information and an awareness of end-of-life care to physicians in public health centers and the officers managing the projects. In executing the project, the working and medical support committees played important roles in addressing the challenges, implementing the project effectively, and increasing service satisfaction.

The main pathway for palliative care recipient referral was accessed via project teams for home-based cancer patients and healthcare home-visit teams in public health centers. The educational unit of the service management team educated periodically healthcare home-visit teams and nurses working on projects for home-based cancer patients at public health centers to ensure they would refer potential palliative care recipients to a palliative nurse. Service provision teams provided home-visit services for the referred patients and co-managed these services with healthcare home-visit teams when necessary to guarantee quality services for recipients and effectively utilize the resources available at public health centers. Before the project was implemented, patients receiving end-of-life care and were registered at public health centers received intermittent healthcare rather than palliative care from nurses in healthcare home-visit teams [11]. Specifically, home-based cancer patients registered at public health centers and elderly patients with chronic illnesses registered with healthcare home-visit teams belonged mainly to a highly vulnerable, low-income class and did not receive quality medical services [12]. Therefore, the CBPC project could be evaluated positively as a new health management project implemented by public health centers that provides quality palliative care for low-income community members.

A consortium system was established to ensure that palliative care recipients requiring inpatient medical service were admitted to a consortium palliative medical center and were referred back to service provision teams after discharge to receive continuous palliative care. In 2015, there were only 87 palliative beds in Busan Metropolitan City; therefore, the capacity for inpatient medical care was insufficient [9]. Since July 2015, when the healthcare payment system began to cover inpatient palliative care, the waiting period for admission increased, and prompt admission for the inpatient treatment of case management recipients is difficult when their symptoms deteriorate. Accordingly, the provision of a medical support system, in which physicians in public health centers prescribe drug therapy to manage palliative care of the recipients’ symptoms, is considered essential to help patients live at home for longer. On the other hand, this is currently impossible in Korea, where public institutions, such as public health centers, issue prescriptions for palliative care recipients. Therefore, the model development team will develop an assessment system and guidelines regarding symptoms and drug use in palliative care for physicians in public health centers.

To increase the quality of palliative care recipient case management, palliative nurses dispatched to service provision teams in public health centers held weekly case management meetings with the service management team. The objective of the CBPC project was to develop a practical model in which services are provided based on the public health centers’ circumstances and palliative care principles. Accordingly, the CBPCC dispatched palliative nurses and social workers trained in the principles of palliative care to ensure that the palliative care project would not be burdensome to public health centers that had already implemented a range of other regional healthcare projects. Palliative nurses and social workers dispatched from the CBPCC were the project’s primary service providers, and the service management team’s education unit was responsible for the development and provision of educational programs designed to increase capacity.

The CBPC model is significant in that it suggests a new palliative care system for the consideration of policies regarding end-of-life treatment in Korea, where the health insurance program currently covers inpatient palliative care for end-stage cancer patients. In particular, the CBPC project helped expand the pool of professionals specializing in palliative care by supporting the standardization of palliative care education for service provision teams in public health centers. The system is based on the WHO’s concept of primary palliative care, in which palliative care provided by public medical centers should be cost-effective and accessible to the entire community [13].
Conclusion

The CBPC model is expected to ultimately contribute to improvements in the National Quality of Death Index as a new means of providing universal end-of-life care in an aging society [14]. Therefore, the potential of the CBPC model presented herein and implemented in Korea should be evaluated by research and the development of outcome measures.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References

An Open-Label, Randomized, Parallel, Phase III Trial Evaluating the Efficacy and Safety of Polymeric Micelle-Formulated Paclitaxel Compared to Conventional Cremophor EL-Based Paclitaxel for Recurrent or Metastatic HER2-Negative Breast Cancer

In Hae Park, MD, PhD1
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Tae You Kim, MD, PhD5
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Eun Kyung Cho, MD, PhD6
Yang Soo Kim, MD, PhD7
Hong Suk Song, MD, PhD8
Jae Hong Seo, MD, PhD9
Hun Mo Ryoo, MD, PhD10
Sun Ah Lee, MD11
So Young Yoon, MD, PhD12
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Yong Tai Kim, MD, PhD14
Si Young Kim, MD, PhD15
Mi Ryung Jin, MS16
Jungsil Ro, MD, PhD1

Purpose
Genexol-PM is a Cremophor EL-free formulation of low-molecular-weight, non-toxic, and biodegradable polymeric micelle-bound paclitaxel. We conducted a phase III study comparing the clinical efficacy and toxicity of Genexol-PM with conventional paclitaxel (Genexol).

Materials and Methods
Patients were randomly assigned (1:1) to receive Genexol-PM 260 mg/m² or Genexol 175 mg/m² intravenously every 3 weeks. The primary outcome was the objective response rate (ORR).

Results
The study enrolled 212 patients, of whom 105 were allocated to receive Genexol-PM. The mean received dose intensity of Genexol-PM was 246.8±21.3 mg/m² (95.0%), and that of Genexol was 168.3±10.6 mg/m² (96.2%). After a median follow-up of 24.5 months (range, 0.0 to 48.7 months), the ORR of Genexol-PM was 39.1% (95% confidence interval [CI], 31.2 to 46.9) and the ORR of Genexol was 24.3% (95% CI, 17.5 to 31.1) (ORR: 1.59; 95% CI: 1.14–2.23). The two groups did not differ significantly in overall survival (28.8 months for Genexol-PM vs. 23.8 months for Genexol; p=0.52) or progression-free survival (6.0 months for Genexol-PM vs. 6.7 months for Genexol; p=0.26). In both groups, the most common toxicities were neutropenia, with 68.6% occurrence in the Genexol-PM group versus 40.2% in the Genexol group (p < 0.01). The incidences of peripheral neuropathy of greater than grade 2 did not differ significantly between study treatments.

Conclusion
Compared with standard paclitaxel, Genexol-PM demonstrated non-inferior and even superior clinical efficacy with a manageable safety profile in patients with metastatic breast cancer.

Key words
Polymeric micelle paclitaxel, Cremophor EL-free, Genexol-PM, Metastatic breast cancer
Introduction

Paclitaxel, a chemotherapeutic agent that interferes with microtubule function, is among the most effective treatments for metastatic breast cancer (MBC) [1,2]. Because of its poor solubility in conventional solvent [3], paclitaxel is prepared using polyoxy-35-castor oil (Cremophor EL; CrEL) as a solubilizer [4,5]. Unfortunately, CrEL contributes to hypersensitivity reactions in a substantial number of patients, necessitating premedication prior to paclitaxel administration [6-12].

Genexol-PM is a lyophilized polymeric micellar formulation of paclitaxel that delivers a higher paclitaxel dose to the tumor tissue with lower vehicle-related toxicities than conventional paclitaxel formulations. Unlike CrEL, Genexol-PM is prepared using the low-molecular-weight, biodegradable amphiphilic diblock copolymer mPEG-PDLLA [methoxy-(polyethylene glycol)-block-poly (D,L-lactide)] as a solubilizer [13].

Dose-limiting toxicities of Genexol-PM include neuropathy, myalgia, and neutropenia. Two phase I trials investigated the maximum tolerated dose (MTD) of Genexol-PM: one in the United States that reported an MTD of 435 mg/m², and one trial in Korea that showed an MTD of 390 mg/m². For safety, a dose of 300 mg/m² was recommended [14]. A phase II trial used this dose of Genexol-PM in patients with MBC and reported a high response rate of 58.5%, including complete responses (CRs) in five patients and partial responses in 19 patients [1]. This trial also showed a relatively low rate of myelosuppression even though the dose applied was higher than the conventionally used dose of CrEL-based paclitaxel [1].

In the present multicenter, open-label, randomized, phase III study, we evaluated the non-inferiority of Genexol-PM in terms of clinical efficacy compared to conventional CrEL-based paclitaxel (Genexol, Samyang Biopharmaceuticals Corp.). This trial was registered with ClinicalTrials.gov, number NCT00876486.

Materials and Methods

1. Patient population

We performed a multicenter joint clinical trial in patients with HER2-negative advanced or metastatic invasive breast cancer at 20 institutions in Korea. Patients were eligible if they had not been treated with taxane for recurrent or metastatic disease, and had not relapsed within 1 year of receiving adjuvant paclitaxel or docetaxel treatment. Additional inclusion criteria were an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 with adequate organ function, and disease measurable using the Response Criteria in Solid Tumors (RECIST) ver. 1.0 [15]. Exclusion criteria were central nervous system metastases, current uncontrolled medical conditions that could limit the patient’s ability to undergo study treatment, preexisting peripheral neuropathy above grade 1 according to the National Cancer Institute Common Toxicity Criteria, and history of allergic or hypersensitivity reactions to the study drug or any of its excipients.

All participants gave their written informed consent. The study protocol was approved by the appropriate institutional review boards and independent ethics committees, and was in compliance with Good Clinical Practice, Guidelines of the International Conference on Harmonization, and the Declaration of Helsinki.

2. Treatment

Patients enrolled in the study were randomly assigned (1:1) to a treatment group, with stratification by prior chemotherapy in the recurrent or metastatic setting. On the first day of each cycle, Genexol-PM (Samyang Biopharmaceuticals Corp., Seoul, Korea) was intravenously administered over 3 hours without premedication, which was repeated every 3 weeks. For safety, Genexol-PM administration began at a dose of 260 mg/m², while Genexol was administered at a dose of 175 mg/m². At the discretion of the physician, Genexol-PM dosage could be increased to 300 mg/m² after the first cycle. To minimize hypersensitivity, Genexol administration was preceded by premedications, including dexamethasone and H2-blockers. In the Genexol-PM group, premedications were allowed in cases showing hypersensitivity or when deemed necessary by the investigator.

3. Assessments

Every 6 weeks, routine tumor assessments were performed based on RECIST ver. 1.0 criteria. All partial response (PR) and CR were confirmed with repeat imaging at least 4 weeks later. Such assessments were repeated until the time of disease progression or death. Every cycle included laboratory testing and assessment of ECOG performance status. Adverse events were graded according to the National Cancer Institute’s Common Terminology Criteria for Adverse Events (CTCAE), ver. 3.0.
4. Statistical analysis

The primary objective of this study was to determine the objective response rate (ORR), defined as the fraction of patients whose maximum response was CR or PR based on RECIST ver. 1.0. Secondary objectives included progression-free survival (PFS) and overall survival (OS). This study had 80% power, with a one-sided type I error of 0.025. The pre-defined non-inferiority margin was an absolute difference of 7% in the primary endpoint. After confirming non-inferiority of treatment with Genexol-PM, we tested the superiority hypothesis with the null hypothesis that ORR did not differ between the two groups.

Categorical variables were analyzed using the chi-square test, while non-parametric variables were evaluated with the Wilcoxon rank-sum test. The survival curve was analyzed using the Kaplan-Meier method, and between-group differences were evaluated using the log-rank method. Cox proportional hazard analysis was applied to identify variables that significantly influenced the objective response to the study drug. We also analyzed safety and efficacy endpoints within the per protocol population who received at least one dose of study treatments. All statistical analyses were conducted using SPSS ver. 21 (IBM Corp., Armonk, NY) while applying a two-sided significance level of 5%.

Results

1. Patient population

This study enrolled 230 MBC patients from December 2008 to February 2013 (CONSORT diagram) (Fig. 1), 212 of whom were included in the analyses of clinical efficacies and safety. Median patient age was 49 years (range, 28 to 72 years) in the Genexol-PM group, and 52 years (range, 25 to 78 years) in the Genexol group (p=0.02) (Table 1). The two groups did not significantly differ with regards to hormone receptor status or visceral metastasis. Over 80% of patients received Genexol-PM or Genexol as first-line cytotoxic chemotherapy for advanced MBC (Table 1). The median number of previous chemotherapy treatments was one (range, 0 to 4). Patients in the Genexol-PM group underwent a median of six treatment cycles (range, 1 to 50 cycles), with a relative dose intensity of 246.8±21.3 mg/m² (95.0%). Five patients in the Genexol-PM group received an escalated dose of 300 mg/m² starting in cycle 2. Patients in the Genexol group received a median of six treatment cycles (range, 1 to 34 cycles), with a relative dose intensity of 168.3±10.6 mg/m² (96.2%).

2. Efficacy

The Genexol-PM group showed an ORR of 39.1%, with a 95% confidence interval (CI) of 31.2 to 46.9. The Genexol treatment group showed a lower ORR of 24.3% (95% CI, 17.5 to 31.1), demonstrating that Genexol-PM was non-inferior
<table>
<thead>
<tr>
<th>Variable</th>
<th>Genexol-PM (n=105)</th>
<th>Genexol (n=107)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>49.0 (28.0-72.0)</td>
<td>52.0 (25.0-78.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Menstruation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>61 (58.1)</td>
<td>49 (45.8)</td>
<td>0.10</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>44 (41.9)</td>
<td>58 (54.2)</td>
<td></td>
</tr>
<tr>
<td>ECOG status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>47 (44.8)</td>
<td>31 (29.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>1</td>
<td>53 (50.5)</td>
<td>68 (63.6)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 (4.8)</td>
<td>8 (7.5)</td>
<td></td>
</tr>
<tr>
<td>DFI (yr)</td>
<td>2.2 (0.2-8.7)</td>
<td>3.0 (0.2-9.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>No. target lesions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3</td>
<td>90 (85.7)</td>
<td>94 (87.9)</td>
<td>0.65</td>
</tr>
<tr>
<td>≥ 3</td>
<td>15 (14.3)</td>
<td>13 (12.2)</td>
<td></td>
</tr>
<tr>
<td>Visceral metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71 (67.6)</td>
<td>65 (60.8)</td>
<td>0.30</td>
</tr>
<tr>
<td>No</td>
<td>34 (32.4)</td>
<td>42 (39.3)</td>
<td></td>
</tr>
<tr>
<td>ER or PgR status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>75 (71.4)</td>
<td>82 (76.6)</td>
<td>0.63</td>
</tr>
<tr>
<td>Negative</td>
<td>28 (26.7)</td>
<td>24 (22.4)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (1.9)</td>
<td>1 (0.9)</td>
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<tr>
<td>De novo stage IV breast cancer</td>
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<td>Yes</td>
<td>71 (67.6)</td>
<td>74 (69.2)</td>
<td>0.81</td>
</tr>
<tr>
<td>No</td>
<td>34 (32.4)</td>
<td>33 (30.8)</td>
<td></td>
</tr>
<tr>
<td>Previous chemotherapy*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (15.2)</td>
<td>14 (13.1)</td>
<td>0.70</td>
</tr>
<tr>
<td>No</td>
<td>89 (84.8)</td>
<td>93 (86.9)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as median (range) or number (%). ECOG, Eastern Cooperative Oncology Group; DFI, disease-free interval; ER, estrogen receptor; PgR, progesterone receptor. *Systemic cytotoxic chemotherapy for metastatic disease.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genexol-PM (n=105)</th>
<th>Genexol (n=107)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective response rate*</td>
<td>41 (39.1)</td>
<td>26 (24.3)</td>
<td>0.021</td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial response</td>
<td>41 (39.1)</td>
<td>26 (24.3)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>46 (43.8)</td>
<td>56 (52.3)</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td>7 (6.7)</td>
<td>16 (15.0)</td>
<td></td>
</tr>
<tr>
<td>Not evaluated</td>
<td>11 (10.5)</td>
<td>9 (9.4)</td>
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</table>

Values are presented as number (%). *Objective response rate=complete response+partial response.

(ρ<0.021) and was in fact superior to Genexol with regard to the ORR (ρ<0.016) (Table 2). Clinical variables that influenced ORR included previous chemotherapy for metastatic disease (hazard ratio [HR], 2.41; 95% CI, 0.92 to 6.35; p=0.07) and treatment with Genexol-PM (HR, 2.20; 95% CI, 1.20 to 4.06; p=0.01) (Table 3).

Subgroup analysis revealed a significantly higher response rate to Genexol-PM compared to Genexol among patients with visceral metastasis (HR, 0.35; 95% CI, 0.16 to 0.77; p=0.01) and among patients who were chemotherapy naïve.
Table 3. Clinical variables associated with better response to treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
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<tr>
<td></td>
<td>HR (95% CI)</td>
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</tr>
<tr>
<td>Age (≥ 45 yr vs. &lt; 45 yr)</td>
<td>0.99 (0.52-1.89)</td>
<td>0.97</td>
</tr>
<tr>
<td>Genexol-PM vs. Genexol</td>
<td>2.14 (1.17-3.93)</td>
<td>0.01</td>
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<tr>
<td>Premenopausal vs. postmenopausal</td>
<td>0.98 (0.54-1.77)</td>
<td>0.94</td>
</tr>
<tr>
<td>Visceral involvement (yes vs. no)</td>
<td>1.11 (0.60-2.07)</td>
<td>0.73</td>
</tr>
<tr>
<td>Previous treatment (no vs. yes)</td>
<td>2.29 (0.88-5.95)</td>
<td>0.09</td>
</tr>
<tr>
<td>Hormone receptor (positive vs. negative)</td>
<td>0.97 (0.51-1.85)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>HR (95% CI)</th>
<th>p-value</th>
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<tbody>
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<td>Age (yr)</td>
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<tr>
<td>&lt; 45</td>
<td>62</td>
<td>0.71 (0.23-2.18)</td>
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<tr>
<td>≥ 45</td>
<td>150</td>
<td>0.39 (0.19-0.80)</td>
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<td>Menopausal status</td>
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<tr>
<td>Premenopausal</td>
<td>110</td>
<td>0.46 (0.19-1.09)</td>
<td>0.08</td>
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<td>Postmenopausal</td>
<td>102</td>
<td>0.46 (0.18-1.09)</td>
<td>0.08</td>
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<td>Visceral</td>
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<tr>
<td>Yes</td>
<td>136</td>
<td>0.35 (0.16-0.77)</td>
<td>0.01</td>
</tr>
<tr>
<td>No</td>
<td>76</td>
<td>0.77 (0.28-2.10)</td>
<td>0.61</td>
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<td>Previous treatment</td>
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<tr>
<td>No</td>
<td>182</td>
<td>0.45 (0.24-0.87)</td>
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</tr>
<tr>
<td>Yes</td>
<td>30</td>
<td>0.46 (0.07-3.02)</td>
<td>0.57</td>
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<td>HR status</td>
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<tr>
<td>Positive</td>
<td>157</td>
<td>0.41 (0.20-0.83)</td>
<td>0.01</td>
</tr>
<tr>
<td>Negative</td>
<td>52</td>
<td>0.80 (0.18-2.05)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Fig. 2. Subgroup analysis of overall response rate.

for metastatic disease (HR, 0.45; 95% CI, 0.24 to 0.87; p=0.02) (Fig. 2). We also observed a greater response rate to Genexol-PM among patients over 45 years of age (HR, 0.39; 95% CI, 0.19 to 0.80; p=0.01) and those with hormone receptor-positive disease (HR, 0.41; 95% CI, 0.20 to 0.83; p=0.01) (Fig. 2).

The median follow-up period was 24.5 months (range, 0.0 to 48.7 months). The median OS during this time was 28.8 months (95% CI, 22.8 to 34.8) for the Genexol-PM group and 23.8 months (95% CI, 18.7 to 28.9) for the Genexol group. The between-group difference in OS did not reach statistical significance (p=0.52) (Fig. 3A). We found a longer median PFS in the Genexol-PM group (8.0 months; 95% CI, 6.1 to 9.9) than the Genexol group (6.7 months; 95% CI, 5.6 to 7.8), but this difference did not reach statistical significance (p=0.26) (Fig. 3B).

3. Safety

According to CTCAE ver. 3.0, grade 1 or 2 toxicities comprised over 85% of the adverse events reported in both groups (Table 4). Neutropenia of grade 3 or higher was more common in the Genexol-PM group (68.6%) than in the
Fig. 3. Survival analysis according to treatment. (A) Overall survival. (B) Progression-free survival.

Table 4. Adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Genexol-PM (n=105)</th>
<th>Genexol (n=107)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>0</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>26 (24.8)</td>
<td>28 (26.7)</td>
</tr>
<tr>
<td>Nausea</td>
<td>25 (23.8)</td>
<td>14 (13.3)</td>
</tr>
<tr>
<td>Neuropathy peripheral</td>
<td>13 (12.4)</td>
<td>16 (15.2)</td>
</tr>
<tr>
<td>Constipation</td>
<td>17 (16.2)</td>
<td>22 (21.0)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>11 (10.5)</td>
<td>12 (11.4)</td>
</tr>
<tr>
<td>Asthenia</td>
<td>8 (7.6)</td>
<td>4 (3.8)</td>
</tr>
<tr>
<td>Rash</td>
<td>16 (15.2)</td>
<td>11 (10.5)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>13 (12.4)</td>
<td>9 (8.6)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>13 (12.4)</td>
<td>7 (6.7)</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>5 (4.8)</td>
<td>8 (7.6)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

Genexol group (40.2%) (\(p < 0.01\)), which was expected due to the higher paclitaxel dose administered with Genexol-PM. However, the frequency of febrile neutropenia was similar between the two groups (Table 4). The incidences of ≥ grade 3 peripheral neuropathy and myalgia did not differ significantly according to study treatment (Table 4). Hypersensitivity to the study drugs (of any grade) was more frequent in the Genexol-PM group; therefore, the study protocol was amended to allow premedication in the Genexol-PM arm at the physician’s discretion. Finally, 87 patients (82.9%) received premedications before Genexol-PM administration. One patient dropped out of the study because of severe hypersensitivity to Genexol. All reported toxicities were manageable with conservative care. Similar numbers of patients discontinued study treatment because of adverse events in each group: 16 subjects (15.2%) in the Genexol-PM group and 18 subjects (16.8%) in the Genexol group. No treatment-related deaths occurred in either group.
Discussion

Paclitaxel currently plays a central role in breast cancer treatment. However, the paclitaxel solubilizer CrEL contributes to severe toxicities, including hypersensitivity reactions and peripheral neuropathies [6-12]. The CALGB 9342 trial previously demonstrated that higher doses of conventional paclitaxel did not improve response rates or survival among patients with MBC, primarily due to greater toxicities [16]. Conversely, nanoparticle albumin-bound (nab) paclitaxel at 260 mg/m² resulted in much higher response rates with similar safety profiles compared to conventional paclitaxel at 175 mg/m² in cases of MBC (33% vs. 19%, respectively) [17].

Several randomized trials have investigated the roles of higher paclitaxel doses for breast cancer treatment, with controversial results. The large randomized trial CALGB 40502 reported superior PFS with conventional weekly paclitaxel administration compared to weekly nab-paclitaxel in PFS as the first-line therapy for MBC (11 months vs. 9.3 months; p=0.054), even though conventional paclitaxel was administered at a lower dose [18]. In contrast, in the neoadjuvant setting for early breast cancer, nab-paclitaxel, achieved significantly higher pathological CR compared with conventional paclitaxel (38% vs. 29%, p=0.00065) [19].

Based on the promising results of a phase II clinical trial [1], we compared the clinical efficacy of CrEL-free Genexol-PM with that of conventional solvent-based paclitaxel Genexol-PM. In this context, using a different solubilizer enhanced the efficacy of drug delivery while limiting toxicities, making it possible to safely administer higher doses of paclitaxel. Consistent with previous results, we found a higher response rate for Genexol-PM than Genexol. We further found that the response rate with Genexol-PM was significantly higher among patients of more than 45 years of age, with visceral metastasis, with hormone receptor-positive disease, and in the first-line treatment setting. Although the higher response rate with Genexol-PM was not associated with improved PFS, there was a trend of longer PFS in the Genexol-PM group. It is possible that our study design targeting ORR within a relatively small population may have produced results with underpowered subgroup analysis.

Another important issue was dosing schedule. As a matter of fact, toward the end of patients accrual in the trial, weekly paclitaxel or nab-paclitaxel has become recognized as a preferred schedule in view of its superiority to every three weeks schedule in CALGB 9840, NCT00274456 [20,21]. Current study demonstrated comparable clinical efficacies of Genexol-PM over standard paclitaxel in the same dosing every 3 weeks schedule, although this schedule is not widely preferred one in common practice.

Genexol-PM and conventional paclitaxel showed similar safety profiles. As expected, the Genexol-PM arm of the study showed a higher incidence of neutropenia, but similar rates of peripheral neuropathy were observed in the two groups. Hypersensitivity observed during the study period led to a requirement for premedications in the Genexol-PM group, although the majority of hypersensitivity events were easily manageable in both arms.

Considering potentially higher clinical efficacies and similar toxicity profiles, nab-paclitaxel could replace conventional paclitaxel as the first line therapy of MBC; however, its cost-effectiveness has been issued consistently. The National Institute for Health and Care Excellence (NICE) stated that using nab-paclitaxel (Abraxane) is not cost effective in view of its much higher cost and limited benefits compared to current treatment in the management of pancreatic cancer, although no such data are available in breast cancer [22]. On the other hand, Genexol-PM costs same as a generic version of the older medicine, Taxol, that it would not result in any added burden for the breast cancer patients. In this respect, Genexol-PM would be an easily accessible alternative for MBC treatment.

Conclusion

In conclusion, our present phase III study documented an improved overall response rate to Genexol-PM compared to standard Genexol treatment, with manageable toxicities with both drugs. Genexol-PM allows administration of an increased dose of paclitaxel, offering significantly improved efficacy without compromising patient safety. Further studies of Genexol-PM are warranted, particularly with the use of different schedules, including weekly doses, and in different settings in breast cancer treatment.

Conflicts of Interest

Co-author Mi Ryung Jin is an employee of Samyang Biopharmaceuticals Corporation. All remaining authors have declared that they have no conflicts of interest.
Acknowledgments

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References


Real-World Treatment Patterns among Patients with Advanced Gastric Cancer in South Korea

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   Seoul, Korea

Purpose
The purpose of this study was to understand patient treatment patterns, outcomes, and healthcare resource use in cases of metastatic and/or locally recurrent, unresectable gastric cancer (MGC) in South Korea.

Materials and Methods
Thirty physicians reviewed charts of eligible patients to collect de-identified data. Patients must have received platinum/fluoropyrimidine first-line therapy followed by second-line therapy or best supportive care, had no other primary cancer, and not participated in a clinical trial following MGC diagnosis. Data were summarized using descriptive statistics. Kaplan-Meier analysis was used to describe survival.

Results
Of 198 patients, 73.7% were male, 78.3% were diagnosed with MGC after age 55 (mean, 61.3 years), and 47.0% were current or former smokers. The majority of tumors were located in the antrum/pylorus (51.5%). Metastatic sites most often occurred in the peritoneum (53.5%), lymph nodes (47.5%), and liver (38.9%). At diagnosis, the mean Charlson comorbidity index was 0.4 (standard deviation, 0.6). The most common comorbidities were chronic gastritis (22.7%) and cardiovascular disease (18.7%). Most patients (80.3%) received second-line treatment. Single-agent fluoropyrimidine was reported for 22.0% of patients, while 19.5% were treated with irinotecan and a fluoropyrimidine or platinum agent. The most common physician-reported symptoms during second-line treatment were nausea/vomiting (44.7%) and pain (11.3%), with antiemetics (44.7%), analgesics (36.5%), and nutritional support (11.3%) most often used as supportive care. Two-thirds of inpatient hospitalizations were for chemotherapy infusion. Outpatient hospitalization (31.6%) and visits to the oncologist (58.8%) were common among second-line patients.

Conclusion
Most patients received second-line treatment, although regimens varied. Understanding MGC patient characteristics and treatment patterns in South Korea will help address unmet needs.

Key words
Treatment patterns, Stomach neoplasms, Republic of Korea, Resource use, Observational study

Introduction
Gastric cancer is the fourth most common cancer worldwide and the second most common cause of cancer-related deaths [1,2]. The incidence of gastric cancer is highest in East Asian countries and in some parts of South America, while its occurrence is lower in North America and Africa [1,3]. South Korea has the highest incidence rate of gastric cancer (age standardized, male vs. female: 64.2 and 26.7 per 100,000). Globally, a high proportion of patients are diagnosed with late-stage disease, and 5-year survival rates are < 25% for these patients. Gastric cancer is the second most commonly diagnosed cancer in South Korea, with male and...
female mortality rates of 37.1 and 15.0 per 100,000, respectively. Risk increases with advancing age, history of *Helicobacter pylori* infection and cigarette smoking [2,3].

There is high unmet need in gastric cancer as there are few approved agents, and treatment practices vary widely among countries [1,2], particularly in the second-line treatment setting. There is also limited information available on gastric cancer patient characteristics, healthcare resource use and treatment patterns [1,2,4].

Therefore, this study was conducted to understand treatment patterns, patient outcomes, and healthcare resource use in South Korean patients with metastatic and/or locally recurrent, unresectable gastric cancer (MGC), including cancer of the stomach and gastroesophageal junction with adenocarcinoma histology.

**Materials and Methods**

This study is a retrospective analysis of de-identified patient-level data from medical charts collected via a physician-administered online chart review or face-to-face interviews with physicians who treated gastric cancer patients. Physicians selected at random from a panel of oncologists and referrals to the study by local contacts and elected to participate in the study provided de-identified patient-level data from a random sample of patient charts.

This study received Investigational Review Board (IRB) exemption from the Seoul National University Hospital and from the Western Institutional Review Board.

1. **Data collection**

Per protocol, each physician could provide information from up to 10 patient charts. Inclusion in the study was limited to adult patients (≥18 years) diagnosed with MGC, including cancer of the stomach or gastroesophageal junction with adenocarcinoma histology, on or after January 1, 2009 (until data collection began in 2013). Patients could have been diagnosed with an earlier stage gastric cancer before January 1, 2009. Eligible patients had no other primary malignant tumors and completed platinum/fluoropyrimidine (P/F) first-line therapy (with or without other drugs; e.g., therapy with trastuzumab) after MGC diagnosis. Upon completion of first-line therapy, eligible patients either went on to (1) second-line therapy or (2) best supportive care (BSC) only. Patients were not eligible if they participated in any clinical trials after MGC diagnosis.

The chart abstraction instrument was designed to collect information on physician and patient characteristics, treatment patterns by line of therapy, patient outcomes, and health care resource use. Comorbidities were also collected to allow reporting of patients’ Charlson comorbidity index (CCI). The CCI is a validated tool based on 17 comorbidities that is used to predict the risk of 1-year mortality [5].

2. **Statistical analysis**

Patient characteristics were compared between patients who received second-line therapy and those who received only BSC after first-line therapy. Chi-squared tests were used for comparisons of proportions, and Wilcoxon rank sum tests were used for comparisons of continuous variables. p-values of < 0.05 were considered statistically significant. All data were evaluated descriptively with univariate analysis using SAS ver. 9.3 (SAS Institute Inc., Cary, NC).

Kaplan-Meier analysis was used to describe survival and disease progression. Survival time was calculated from (1) the date of MGC diagnosis to the date of death for all patients, and (2) from the date of initiation of second-line therapy to the date of death for the cohort that received second-line therapy. Patients surviving at the time of data collection were censored at the date of last contact. Patients who were reported to have died, but for whom no dates of death were available, were recorded as having died on the date of last contact.

Rates of disease progression defined as discontinuing any agent in that line of therapy due to disease progression were calculated for each line of therapy. Duration of each line of therapy was defined as the number of days from the first to the last administration of any agent in that line of therapy. Patients with ongoing second-line and third-line therapy were excluded from the calculations of mean duration of second-line and third-line therapy, respectively, but not from Kaplan-Meier analyses of survival and disease progression.

Healthcare resource utilization, including inpatient and outpatient office visits, was calculated for each line of therapy. Observations were excluded from the calculation of each resource use category if it was unknown in what line of therapy they occurred.

**Results**

1. **Physician characteristics**

Thirty physicians that were selected at random from a panel of oncologists and referrals to the study by local contacts and elected to participate in the study provided de-identified patient-level data from 198 patient charts. Most
Table 1. Baseline patient and disease characteristics at diagnosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGC patient</td>
<td>198 (100)</td>
</tr>
<tr>
<td>Age at MGC diagnosis, mean±SD</td>
<td>61.3±9.8</td>
</tr>
<tr>
<td>Age group at MGC diagnosis (yr)</td>
<td></td>
</tr>
<tr>
<td>25-44</td>
<td>12 (6.1)</td>
</tr>
<tr>
<td>45-54</td>
<td>31 (15.7)</td>
</tr>
<tr>
<td>55-64</td>
<td>73 (36.9)</td>
</tr>
<tr>
<td>≥ 65</td>
<td>82 (41.4)</td>
</tr>
<tr>
<td>Male</td>
<td>146 (73.7)</td>
</tr>
<tr>
<td>Body mass index*, mean±SD (kg/m²)</td>
<td>21.4±2.6</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>83 (41.9)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>38 (19.2)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>55 (27.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>22 (11.1)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
</tr>
<tr>
<td>No alcohol use</td>
<td>68 (34.3)</td>
</tr>
<tr>
<td>Light to moderate</td>
<td>94 (47.5)</td>
</tr>
<tr>
<td>Heavy</td>
<td>18 (9.1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>18 (9.1)</td>
</tr>
<tr>
<td>History of <em>Helicobacter pylori</em> infection</td>
<td>8 (4.0)</td>
</tr>
<tr>
<td>Family history of gastric cancer</td>
<td>13 (6.6)</td>
</tr>
<tr>
<td>Charlson comorbidity index (CCI)*, mean±SD</td>
<td>0.4±0.6</td>
</tr>
<tr>
<td>Common comorbidity</td>
<td></td>
</tr>
<tr>
<td>Chronic atrophic gastritis</td>
<td>45 (22.7)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>37 (18.7)</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>28 (14.1)</td>
</tr>
<tr>
<td>Diabetes without chronic complications</td>
<td>24 (12.1)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>16 (8.1)</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>15 (7.6)</td>
</tr>
<tr>
<td>Stage IV at MGC diagnosis</td>
<td>193 (97.5)</td>
</tr>
<tr>
<td>Disease classification at MGC diagnosis</td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>48 (24.2)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>72 (36.4)</td>
</tr>
<tr>
<td>Mixed</td>
<td>13 (6.6)</td>
</tr>
<tr>
<td>Unknown</td>
<td>65 (32.8)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Antrum and pylorus</td>
<td>102 (51.5)</td>
</tr>
<tr>
<td>Fundus and corpus</td>
<td>48 (24.2)</td>
</tr>
<tr>
<td>Gastric cardia</td>
<td>25 (12.6)</td>
</tr>
<tr>
<td>Esophagogastric junction</td>
<td>11 (5.6)</td>
</tr>
<tr>
<td>Whole stomach</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Metastatic site</td>
<td></td>
</tr>
<tr>
<td>Peritoneum</td>
<td>106 (53.5)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>94 (47.5)</td>
</tr>
<tr>
<td>Liver</td>
<td>77 (38.9)</td>
</tr>
<tr>
<td>Bone</td>
<td>22 (11.1)</td>
</tr>
<tr>
<td>Lung</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (4.5)</td>
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</table>

Table 1. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested for HER2/neu gene expression</td>
<td>84 (42.4)</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>8 (9.5)</td>
</tr>
<tr>
<td>HER2 negative</td>
<td>75 (89.3)</td>
</tr>
<tr>
<td>HER2 status unknown</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

MGC, metastatic and/or locally recurrent, unresectable gastric cancer; SD, standard deviation; HER2, human epidermal growth factor receptor 2. a Patients with weight less than 20 kg (n=1) were assumed to have the population average weight of 58 kg. b The CCI was calculated excluding any malignancy (including leukemia and lymphoma) and metastatic solid tumor. c Comorbidities contributing to the CCI are marked. d The proportions of patients with positive, negative, and unknown values for HER2/neu gene expression are only among the tested patients.

physicians (27/30, 90%) specialized in gastric oncology, and the average time in practice was 11.5 years (standard deviation [SD], 5.5 years). Nine of 30 physicians reported affiliation with one of the major cancer centers in South Korea based on a limited set of centers noted in the survey instruments. Data were collected from February 20, 2013, to April 29, 2013. No data identifying the physicians were collected, and only physicians had access to the medical charts during the abstraction process.

2. Patient and disease characteristics

Charts were abstracted for 198 MGC patients. At MGC diagnosis, patients were 61.3 years old (SD, 9.8) on average, and 73.7% were male. Most patients (41.9%) had no history of smoking, while 19.2% and 27.8% were current or former smokers, respectively. Alcohol use was most frequently light to moderate (47.5%), although 9.1% of patients had a history of heavy alcohol consumption. A history of *H. pylori* infection or a family history of gastric cancer was observed in 4.0% and 6.6% of patients, respectively (Table 1).

The most commonly reported comorbidities were chronic atrophic gastritis (22.7%), cardiovascular disease (18.7%), intestinal metaplasia (14.1%), and diabetes without chronic complications (12.1%). Excluding malignancy and metastatic solid tumor diagnoses, the mean CCI was 0.4 (SD, 0.6) (Table 1).
Table 2. Patient status and treatment regimens by line of therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGC patient</td>
<td>198 (100)</td>
</tr>
<tr>
<td>Patient who received first-line chemotherapy treatment</td>
<td>198 (100)</td>
</tr>
<tr>
<td>Patient who received second-line chemotherapy treatment</td>
<td>159 (80.3)</td>
</tr>
<tr>
<td>Patient who received third-line chemotherapy treatment</td>
<td>46 (23.2)</td>
</tr>
<tr>
<td>Patient who received BSC only after first-line treatment</td>
<td>39 (19.7)</td>
</tr>
<tr>
<td><strong>ECOG PS score of first-line patients</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0: Asymptomatic</td>
<td>25 (12.6)</td>
</tr>
<tr>
<td>1: Symptomatic but completely ambulatory</td>
<td>151 (76.3)</td>
</tr>
<tr>
<td>2: Symptomatic, &lt; 50% in bed during the day</td>
<td>19 (9.6)</td>
</tr>
<tr>
<td>4: Bedbound</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td><strong>First-line regimen</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Fluoropyrimidine+platinum (+/- leucovorin)</td>
<td>120 (60.6)</td>
</tr>
<tr>
<td>Capecitabine+platinum</td>
<td>29 (14.6)</td>
</tr>
<tr>
<td>Single-agent fluoropyrimidine (+/- leucovorin)</td>
<td>38 (19.2)</td>
</tr>
<tr>
<td><strong>Reason for initiating second-line therapy</strong></td>
<td></td>
</tr>
<tr>
<td>Tumor progression</td>
<td>152 (95.6)</td>
</tr>
<tr>
<td>Toxicity of first-line therapy</td>
<td>7 (4.4)</td>
</tr>
<tr>
<td><strong>ECOG PS score of second-line patients</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0: Asymptomatic</td>
<td>16 (10.1)</td>
</tr>
<tr>
<td>1: Symptomatic but completely ambulatory</td>
<td>105 (66.0)</td>
</tr>
<tr>
<td>2: Symptomatic, &lt; 50% in bed during the day</td>
<td>35 (22.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (1.9)</td>
</tr>
<tr>
<td><strong>Second-line regimen</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Single agent fluoropyrimidine (+/- leucovorin)</td>
<td>35 (22.0)</td>
</tr>
<tr>
<td>S-1</td>
<td>11 (6.9)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>9 (5.7)</td>
</tr>
<tr>
<td>5-FU</td>
<td>7 (4.4)</td>
</tr>
<tr>
<td>Irinotecan+platinum and/or fluoropyrimide (+/- leucovorin)</td>
<td>31 (19.5)</td>
</tr>
<tr>
<td>Irinotecan, 5-FU, leucovorin</td>
<td>15 (9.4)</td>
</tr>
<tr>
<td>Irinotecan, 5-FU</td>
<td>10 (6.3)</td>
</tr>
<tr>
<td>Fluoropyrimidine+platinum agent (+/- leucovorin)</td>
<td>21 (13.2)</td>
</tr>
<tr>
<td>Capecitabine+platinum agent</td>
<td>9 (5.7)</td>
</tr>
<tr>
<td>5-FU+platinum agent</td>
<td>7 (4.4)</td>
</tr>
<tr>
<td>S-1+platinum agent</td>
<td>5 (3.1)</td>
</tr>
<tr>
<td>Single-agent taxane</td>
<td>13 (8.2)</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>9 (5.7)</td>
</tr>
<tr>
<td>Other&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59 (37.1)</td>
</tr>
<tr>
<td><strong>ECOG score of third-line patient</strong></td>
<td></td>
</tr>
<tr>
<td>0: Asymptomatic</td>
<td>5 (10.9)</td>
</tr>
<tr>
<td>1: Symptomatic but completely ambulatory</td>
<td>25 (54.3)</td>
</tr>
<tr>
<td>2: Symptomatic, &lt; 50% in bed during the day</td>
<td>16 (34.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Third-line regimen</strong></td>
<td></td>
</tr>
<tr>
<td>Single-agent fluoropyrimidine (+/- leucovorin)</td>
<td>12 (26.1)</td>
</tr>
<tr>
<td>Fluoropyrimidine+platinum agent (+/- leucovorin)</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>Single-agent taxane</td>
<td>9 (19.6)</td>
</tr>
<tr>
<td>Irinotecan+platinum and/or fluoropyrimide (+/- leucovorin)</td>
<td>7 (15.2)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (15.2)</td>
</tr>
</tbody>
</table>

MGC, metastatic and/or locally recurrent, unresectable gastric cancer; ECOG PS, Eastern Cooperative Oncology Group performance status; 5-FU, 5-fluorouracil.<sup>a</sup>Karnofsky scores were converted to ECOG PS scores (100 [ECOG PS 0], 80-90 [ECOG PS 1], 60-70 [ECOG PS 2], 40-50 [ECOG PS 3], and 10-30 [ECOG PS 4]).<sup>b</sup>A patient could have received a maximum of four therapeutic agents.<sup>c</sup>Other regimens included: irinotecan or oxaliplatin (6.9%), leucovorin/irinotecan (6.3%), cisplatin (5.7%), cisplatin/docetaxel (3.1%), and various other agents received by fewer than 2% of patients.

3. Disease and tumor characteristics

Upon initial gastric cancer diagnosis, 76.8% of patients had stage IV disease according to the American Joint Committee on Cancer TNM (tumor size, lymph nodes affected, metastases) system [6]. At MGC diagnosis, 97.5% of patients had stage IV disease, with diffuse histology (by the Laurén system [7]) being the most frequently reported type (33.3%). There were no patients with stage III disease, and the staging for the remaining 2.5% of patients was unknown/other. Histology information was missing for 32.8% of patients. The antrum and pylorus were the primary tumor locations in the majority of patients (51.5%). The most frequently reported metastatic sites were the peritoneum (53.5%), lymph nodes (47.5%), and liver (38.9%) (Table 1). On average, MGC diagnosis occurred 4.5 months (SD, 14.3) after initial gastric cancer diagnosis.

Only 84 of the 198 patients were tested for HER2 positivity, of which 9.5% had positive status (Table 1). Variability in HER2 positivity rates of testing over a 5-year period including 2009 to 2013 ranged from 0% in 2009 to 52.0% in 2012, with an overall rate of testing of 42.4% for patients in this sample. A higher percentage of patients were tested for HER2 status at major cancer centers (68.0%) than patients treated in other centers (30.0%).

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4. Treatment patterns

1) First-line therapy

By design, all patients in the study were required to have first-line therapy for MGC. At therapy initiation, most patients (76.3%) were symptomatic but completely ambulatory (Eastern Cooperative Oncology Group performance status [ECOG PS], 1), while some remained asymptomatic (ECOG PS, 0; 12.6%).

In this sample of 198 patient charts, the most frequent first-line regimen type was a fluoropyrimidine with a platinum agent (+/- leucovorin) (60.6%), which consisted primarily of 5-fluorouracil with a platinum agent (40.9%), capectabine with a platinum agent (14.6%), or S-1 with a platinum agent (5.1%). Single-agent fluoropyrimidine (+/- leucovorin) was prescribed for 19.2% of patients, and in fewer patients, irinotecan plus platinum and/or fluoropyrimidine (+/- leucovorin) (4.5%) was prescribed (Table 2). For 88.9% of patients, physicians reported selecting first-line treatment based on national guidelines. For 22.7% of patients, physician experience was a factor in selection of the first-line treatment.

### Table 3. Patient demographic characteristics stratified by BSC or second-line therapy after first-line therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>First-line followed by BSC (n=39)</th>
<th>First-line followed by second-line (n=159)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at MGC diagnosis (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years</td>
<td>64.5±9.3</td>
<td>60.5±9.8</td>
<td>0.012</td>
</tr>
<tr>
<td>Median (Q1-Q3)</td>
<td>66 (60-71)</td>
<td>62 (55-67)</td>
<td>0.012</td>
</tr>
<tr>
<td>Distribution (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>1 (2.6)</td>
<td>3 (1.9)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>35-44</td>
<td>0</td>
<td>8 (5.0)</td>
<td>0.360</td>
</tr>
<tr>
<td>45-54</td>
<td>5 (12.8)</td>
<td>26 (16.4)</td>
<td>0.587</td>
</tr>
<tr>
<td>55-64</td>
<td>11 (28.2)</td>
<td>62 (39.0)</td>
<td>0.211</td>
</tr>
<tr>
<td>≥ 65</td>
<td>22 (56.4)</td>
<td>60 (37.7)</td>
<td>0.034</td>
</tr>
<tr>
<td>Male</td>
<td>31 (79.5)</td>
<td>115 (72.3)</td>
<td>0.363</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Asian</td>
<td>39 (100)</td>
<td>159 (100)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8±2.8</td>
<td>21.3±2.5</td>
<td>0.121</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>18 (46.2)</td>
<td>65 (40.9)</td>
<td>0.550</td>
</tr>
<tr>
<td>Current smoker</td>
<td>9 (23.1)</td>
<td>29 (18.2)</td>
<td>0.492</td>
</tr>
<tr>
<td>Former smoker</td>
<td>11 (28.2)</td>
<td>44 (27.7)</td>
<td>0.947</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2.6)</td>
<td>21 (13.2)</td>
<td>0.084</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No alcohol use</td>
<td>11 (28.2)</td>
<td>57 (35.8)</td>
<td>0.368</td>
</tr>
<tr>
<td>Light to moderate</td>
<td>19 (48.7)</td>
<td>75 (47.2)</td>
<td>0.862</td>
</tr>
<tr>
<td>Heavy</td>
<td>6 (15.4)</td>
<td>12 (7.5)</td>
<td>0.131</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (7.7)</td>
<td>15 (9.4)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>Performance status (ECOG score)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0: Asymptomatic</td>
<td>1 (2.6)</td>
<td>24 (15.1)</td>
<td>0.033</td>
</tr>
<tr>
<td>1: Symptomatic but completely ambulatory</td>
<td>31 (79.5)</td>
<td>120 (75.5)</td>
<td>0.597</td>
</tr>
<tr>
<td>2: Symptomatic, &lt; 50% in bed during the day</td>
<td>5 (12.8)</td>
<td>14 (8.8)</td>
<td>0.542</td>
</tr>
<tr>
<td>3: Symptomatic, &gt; 50% in bed, but not bedbound</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4: Bedbound</td>
<td>2 (5.1)</td>
<td>0</td>
<td>0.038*</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>1 (0.6)</td>
<td>&gt; 0.990</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%) unless otherwise indicated. BSC, best supportive care; MGC, metastatic and/or locally recurrent, unresectable gastric cancer; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group. *Chi-squared or Fisher exact tests for categorical variables, Wilcoxon rank sum tests for continuous variables. p-values of < 0.05 are indicated by an asterisk (*). †Patients weighing less than 20 kg (n=1) were assumed to have a population average weight of 58 kg. ‡Performance status was assessed at the beginning of first-line treatment.
regimen.

First-line treatment lasted a median of 84 days (interquartile range [IQR], 49 to 155 days). Response to treatment included stable disease (52.0%), partial response (25.3%), disease progression (18.2%), complete response (3.0%), and unknown (1.5%). In 75.8% of patients, first-line therapy was discontinued due to disease progression, while it was discontinued in 12.6% of patients because of an adverse event (AE) or toxicity. Other reasons for discontinuation included patient preference, completion of protocol, lack of benefit, and unknown.

2) Second-line therapy and BSC

In this study, 39 patients (19.7%) received BSC only following first-line therapy, while 159 (80.3%) received second-line therapy.

Second-line therapy was initiated due to tumor progression in 95.6% of patients. When qualitatively compared with first-line regimens, second-line regimens were more heterogeneous. Physicians reported treating 22.0% of patients with a single agent fluoropyrimidine (+/- leucovorin), 19.5% with irinotecan with a platinum agent and/or fluoropyrimidine (+/- leucovorin), 13.2% with fluoropyrimidine with a platinum agent (+/- leucovorin), and 8.2% with single-agent taxane. Other types of regimens were used in 37.1% of patients (Table 2). For 80.5% of patients, physicians reported selecting second-line therapy based on national guidelines, while in 34.6%, selection was guided at least in part by experience.

Second-line treatment lasted a median of 64 days (IQR, 37 to 105 days). The most frequent best response to therapy was stable disease (45.9%). Partial response was observed in 15.1% of patients, complete response in 1.3%, progression occurred in 22.6%, and response was unknown for 15.1%. Second-line therapy was discontinued because of disease progression in 61.6% of patients, patient refusal in 17.6%, AEs or toxicity in 11.3%, lack of benefit in 2.5%, other in 1.3%, end of protocol in 0.6%, and for unknown reasons in 13.2% of patients. When we stratified patients between those who received BSC and those who received second-line therapy, we found differences only in age and ECOG PS. Patients who received second-line therapy after first-line treatment were younger and more likely to be asymptomatic (Table 3).

3) Third-line therapy

Third-line therapy was administered in 23.2% of patients (46 out of 198). Due to the small number of patients who received third-line therapy, treatment patterns were not evaluated in detail.
Table 4. Patient supportive care and hospitalization stratified by line of therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>First-line (n=198)</th>
<th>Second-line (n=159)</th>
<th>BSC, no second-line(^a) (n=39)</th>
<th>Third-line (n=46)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supportive care</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiemetics</td>
<td>103 (52.0)</td>
<td>71 (44.7)</td>
<td>7 (17.9)</td>
<td>21 (45.7)</td>
</tr>
<tr>
<td>Analgesics</td>
<td>74 (37.4)</td>
<td>58 (36.5)</td>
<td>9 (23.1)</td>
<td>13 (28.3)</td>
</tr>
<tr>
<td>Granulocyte-colony stimulating factors</td>
<td>11 (5.6)</td>
<td>13 (8.2)</td>
<td>0</td>
<td>3 (6.5)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>9 (4.5)</td>
<td>6 (3.8)</td>
<td>1 (2.6)</td>
<td>1 (2.2)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>6 (3.0)</td>
<td>4 (2.5)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>Erythropoiesis stimulating agents</td>
<td>4 (2.0)</td>
<td>2 (1.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GM-colony stimulating factors</td>
<td>2 (1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Narcotics</td>
<td>0</td>
<td>1 (0.6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Nutritional support</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one stay</td>
<td>27 (13.6)</td>
<td>18 (11.3)</td>
<td>4 (10.3)</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>No. of visits/patient</td>
<td>71 (35.9)</td>
<td>48 (30.2)</td>
<td>13 (33.3)</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>Length of stay/hospitalization (day)</td>
<td>8.2±9.4</td>
<td>9.1±11.3</td>
<td>14.3±15.6</td>
<td>10.2±12.5</td>
</tr>
<tr>
<td><strong>Main reasons for visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemotherapy infusion</td>
<td>182 (73.4)</td>
<td>68 (68.7)</td>
<td>17 (53.1)(^b)</td>
<td>23 (56.1)</td>
</tr>
<tr>
<td>Disease symptom management</td>
<td>38 (15.3)</td>
<td>21 (21.2)</td>
<td>9 (28.1)</td>
<td>11 (26.8)</td>
</tr>
<tr>
<td>Adverse events/toxicity</td>
<td>19 (7.7)</td>
<td>6 (6.1)</td>
<td>2 (6.3)</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td>Pain management</td>
<td>2 (0.8)</td>
<td>2 (2.0)</td>
<td>3 (9.4)</td>
<td>3 (7.3)</td>
</tr>
<tr>
<td>Gastric cancer-related surgery</td>
<td>6 (2.4)</td>
<td>0</td>
<td>1 (3.1)</td>
<td>0</td>
</tr>
<tr>
<td>Regular monitoring</td>
<td>0</td>
<td>2 (2.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Outpatient hospitalization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one visit</td>
<td>59 (64.1)</td>
<td>25 (31.6)</td>
<td>4 (30.8)</td>
<td>7 (33.3)</td>
</tr>
<tr>
<td>Mean visits/patient</td>
<td>3.2±4.3</td>
<td>2.5±3.3</td>
<td>1±0.0</td>
<td>2.7±2.5</td>
</tr>
<tr>
<td><strong>Hospice unit (patients with information available)</strong></td>
<td>34</td>
<td>29</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>At least one stay</td>
<td>1 (2.9)</td>
<td>0</td>
<td>1 (20)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td><strong>Oncologist clinic (patients with information available)</strong></td>
<td>127</td>
<td>102</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>At least one visit</td>
<td>86 (67.7)</td>
<td>60 (58.8)</td>
<td>9 (36)</td>
<td>10 (35.7)</td>
</tr>
<tr>
<td>No. of visits/patient</td>
<td>3.9±3.3</td>
<td>3.0±3.6</td>
<td>5.1±5.2</td>
<td>2.8±1.9</td>
</tr>
</tbody>
</table>

Values are presented as number (%) or mean±standard deviation unless otherwise indicated. BSC, best supportive care; GM, granulocyte-macrophage. \(^b\)Hospitizations were classified as occurring in a particular line of therapy if there was overlap in the dates of hospitalization and the line of therapy. Hospitalizations for chemotherapy infusion that overlapped between first-line therapy and BSC were counted toward both lines of treatment.

5. Physician-reported patient symptoms, supportive care, and healthcare resource use

For each line of therapy, physicians reported symptoms in patients related to either cancer or cancer treatments. The most common cancer-related symptoms or complications reported were pain in 26.3%, 27.7%, and 30.4%, and nausea/vomiting in 13.6%, 10.7%, and 10.9% of patients during first-, second-, and third-line therapy, respectively. Gastric obstruction was recorded in 5.6%, 6.3%, and 13.0%, ascites in 3.5%, 6.3%, and 6.5%, and bleeding in 3.0%, 1.9%, and 0% of patients undergoing first-, second-, and third-line therapy, respectively (Fig. 1).

Symptoms related to cancer treatment were similar to those recorded for cancer. Nausea/vomiting was reported in 43.9%, 44.7%, and 19.6%, pain in 10.6%, 11.3%, and 23.9%, and ascites in 5.5%, 2.5%, and 2.2% of patients undergoing first-, second-, and third-line therapies, respectively. Treatment-related symptoms were similar during second-line treatment, with nausea/vomiting (44.7%) and pain (11.3%) being the most common. Fewer than 1% of patients experienced gastric obstruction or bleeding during any line of treat-
ment.

Supportive care needs were common during first-, second-, and third-line therapies as well as BSC, and most frequently consisted of antiemetics (52.0%, 44.7% and 45.7%, 17.9%), analgesics (37.4%, 36.5% and 28.3%, 23.1%), and granulocyte colony-stimulating factors (5.6%, 8.2% and 6.5%, 0%). Nutritional support was provided to 13.6%, 11.3% and 8.7% of patients undergoing first-, second-, and third-line treatment and 4% of patients during BSC (Table 4). The most commonly performed procedure was endoscopy, which was conducted in 18.2%, 7.5%, and 4.3% of patients during first-, second-, and third-line treatment, respectively, and in 10.3% of patients in BSC.

Inpatient hospitalizations were reported in 35.9% of patients during first-line treatment, 33.3% of patients receiving BSC only, and 30.2% of patients during second-line treatment. Chemotherapy infusions and disease symptom management were the most commonly cited reasons for inpatient hospitalizations across all groups (Table 4).

Outpatient hospitalizations, which were most frequently for disease symptom management or AE/toxicity, occurred in 64.1% of patients during first-line treatment, 31.6% of patients in second-line treatment, 33.3% of patients during third-line treatment, and 30.8% of patients receiving BSC. Visits to oncology clinics, which were most commonly associated with pain management, were reported in 67.7%, 58.8%, 35.7%, and 36.0% of patients receiving first-, second-, and third-line treatment, and BSC, respectively (Table 4).

6. Survival and disease progression

Overall, the median survival time was 26.8 months (IQR, 9.9 to 41.6 months) from MGC diagnosis with 72.7% of patients censored. Patients who received second-line therapy had a median survival of 28.1 months (IQR, 10.5 to 36.6 months) with 74.2% of patients censored. Patients who received BSC only following first-line therapy had a median survival of 20.1 months (IQR, 8.5 to 41.6 months), with 66.7% of patients censored.

Among patients who received second-line therapy, median survival from initiation of second-line treatment was 13.0 months (IQR, 4.5 to 24.7 months) with 59.6% of patients censored.

Discussion

Our observational study supplements the information currently available regarding treatment of MGC in South Korea. We found variations in treatment patterns in both first- and second-line treatment regimens of patients with MGC. These findings are consistent with the results reported for a REGATE study in which 96% of patients received adjuvant chemotherapy consisting of varying agents, combinations and routes of administration [4]. Recently published guidelines for the diagnosis and treatment of gastric cancer in Korea [8] recommend fluoropyrimidines, platinum, taxanes, irinotecan, and anthracyclines as first-line chemotherapy and state no standard for second-line has been established, while these guidelines were published prior to recent approvals the second line [9-11]. In the present study, the selected patients were required to have P/F first-line therapy (with or without other drugs; i.e., therapy with trastuzumab) after MGC diagnosis, but these criteria were not strictly adhered to and impacted the treatment regimens observed during first-line therapy. Following first-line treatment, 80.3% of the studied patients received second-line treatment, while 19.7% of patients received BSC. The large proportion of patients receiving second-line treatment rather than BSC only may have been due to recent reports of the clinical benefits of second-line chemotherapy to overall survival and quality of life [12-14]. In the REAL-2 study, which was a randomized, controlled clinical trial, only 14% of patients received second-line treatment [15]. More recently, in the AVAGAST trial, 66% of Asian patients received second-line treatment [16]. Patients in our study were required to have had either second-line therapy or BSC after first-line therapy. These criteria may impact survival estimates following MGC diagnosis. Patients in our study who received second-line treatment were younger and had higher ECOG PS than those who received BSC. Performance status was reported to be a predictor of response to chemotherapy [17]. A recent meta-analysis suggests that patients with PS 0 have better survival after chemotherapy than those with PS 1 [18]. Among patients who received second-line therapy in the present study, 61.6% discontinued treatment because of disease progression and 46 patients (23.2%) received third-line treatment, highlighting the need for more clinical trials in advanced gastric cancer.

Overall, 42% of patients in this sample were tested for HER2 positivity, with a larger proportion of patients tested annually during the more recent years of the study. Of those tested, 9.5% tested positive for HER2. This rate of HER2 expression is consistent with the 6%-35% rate reported for gastric cancers [12,19]. HER2 testing is recommended in Korean gastric cancer treatment guidelines and in the ESMO and National Comprehensive Cancer Network guidelines [8,20,21]. Current guidelines recommend the addition of trastuzumab to chemotherapy in HER2+ patients [22].

Physician recommendations for MGC first-line therapy were most influenced by national guidelines and publications in top clinical journals. While the majority of first-line
regimens administered to patients in this study consisted of a platinum agent and fluoropyrimidine as per eligibility criteria, second-line therapies varied widely, and include regimens that consisted of various single-agent fluoropyrimidines, doublet and triplet regimens, and single-agent taxanes. In a large, international prospective study of gastric cancer treatment (REGATE I), the use of a taxane such as paclitaxel, docetaxel, or irinotecan was reportedly the suggested second-line treatment for gastric cancer in Asia, though fewer than 10% of patients in the present sample were prescribed a taxane [1]. Other recent studies have reported a survival advantage associated with treatment with a second-line taxane such as docetaxel, or with irinotecan for cancers refractory to fluoropyrimidine and platinum treatment when compared to BSC [13,14]. In addition, no single agent option has been shown to be better than another in the second-line setting [18,23]. In our data, there appeared to be a trend in the increased use of taxanes as second-line therapy in 2012 relative to earlier years of the study (2009-2011); however, taxanes were prescribed to less than 20% of patients.

Best response to therapy and reasons for discontinuation of first- and second-line therapies revealed a significant unmet need in the treatment of MGC. Most patients achieved at most partial response or stable disease while on first-line therapy, and most discontinued this treatment due to disease progression. Similarly, the reason for initiating second-line therapy was tumor progression in 96% of patients. Responses to second-line therapy were less favorable than those to the first-line. In addition to disease progression, a major reason for discontinuing second-line therapy was patient refusal.

Healthcare resource use was driven both by chemotherapy administration and symptom management. The most commonly reported cancer treatment-related symptoms experienced by patients were nausea/vomiting and pain, and the most commonly used supportive care agents were antiemetics and analgesics. In addition to inpatient hospitalization visits for chemotherapy administration, patients were often seen in outpatient hospitals and oncology clinics for management of disease symptoms, AEs, toxicities, and pain.

It should be noted that this study is subject to the limitations of physician-administered chart abstractions. The completeness and accuracy of collected patient-level information depended on the accuracy of the physician recording the medical history information and treatment information, as well as the availability of a complete medical history in patient charts. Automated quality control checks for survey questions helped minimize possible inconsistencies in the recording of information. Patient-reported information documented in medical records and abstracted in this study may have been subject to self-report bias, including histories of smoking and alcohol use. Moreover, physicians may not have had full access to records documenting medical care administered to the patients over the course of MGC treatment, or to medical history prior to MGC diagnosis. Because a physician agreement was needed to participate, selection bias may play a role and treatment pattern information may not be representative of the treatment practice of all physicians or the treatments for all MGC patients in South Korea. It is important to interpret these results in light of the fact that the timeframe of this study was prior to more recent evidence supporting new therapies to guide practice in this space [9-11]. In addition, more than 70% of the study population was censored for survival, which is relatively high and may limit interpretation of the survival data.

Conclusion

The present study documents the high disease burden of gastric cancer and the significant unmet need that exists, particularly in the second-line setting. This study may help inform clinical practice and future research to ultimately improve patient outcomes.

Conflicts of Interest

This study was supported by Eli Lilly and Company. The following authors are employees of Eli Lilly and Company and may own company stock: G.C.C., A.M.L., N.R., R.C., and J.S.K.

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Survey of Medical Oncology Status in Korea (SOMOS-K): A National Survey of Medical Oncologists in the Korean Association for Clinical Oncology (KACO)

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Purpose
This study was conducted to investigate the current role of medical oncologists in cancer care with a focus on increasing the recognition of medical oncology as an independent specialty.

Materials and Methods
Questionnaires modified from the Medical Oncology Status in Europe Survey dealing with oncology structure, resources, research, and patterns of care given by medical oncologists were selected. Several modifications were made to the questionnaire after feedback from the insurance and policy committee of the Korean Association for Clinical Oncology (KACO). The online survey was then sent to KACO members.

Results
A total of 214 medical oncologists (45.8% of the total inquiries), including 71 directors of medical oncology institutions, took the survey. Most institutions had various resources, including a medical oncology department (94.1%) and a department of radiation oncology (82.4%). There was an average of four medical oncologists at each institution. Medical oncologists were involved in various treatments from diagnosis to end-of-life care. They were also chemotherapy providers from a wide range of institutions that treated many types of solid cancers. In addition, 86.2% of the institutions conducted research.

Conclusion
This is the first national survey in Korea to show that medical oncologists are involved in a wide range of cancer treatments and care. This survey emphasizes the contributions and proper roles of medical oncologists in the evolving health care environment in Korea.

Key words
Medical oncology, Surveys and questionnaires, Korea

Introduction

Medical oncologists are core members of multidisciplinary teams that mediate the evidence-based, safe, and cost-effective use of cancer drugs while preserving the quality of life of patients. A recent position paper by the European Society for Medical Oncology (ESMO) emphasized the role of medical oncologists in the care of cancer patients in clinical and translational research, and it described the active political involvement of medical oncologists [1,2].

The ESMO published two reports on the status of medical oncology based on the results of the Medical Oncology Status in Europe Survey (MOSES) [3,4]. The study described the status of medical oncology in Europe, which mainly involved teaching the discipline to undergraduate students and post-medical graduates, and a multidisciplinary approach to cancer treatment. Phase III MOSES stated that medical oncology could be improved by providing a greater number of medical oncology courses to undergraduate students and greater...
postgraduate specialization and/or subspecialization, although this differed between countries. The roles of medical oncologists in overall care and multidisciplinary collaboration were emphasized and showed improvement over time, while organ specialists still played a significant role.

In Korea, medical oncology has been recognized as a separate specialty since 1997, and 504 physicians were board-certified in medical oncology as of July 2015 [5]. Cancer treatment is becoming more specialized and complex; therefore, the need for multidisciplinary approaches to cancer treatment is increasing. The Korean Association for Clinical Oncology (KACO) was established in 2005 to enhance knowledge and facilitate multidisciplinary care for cancer patients. Although medical oncologists have played an increasing role in cancer patient care in Korea, their current status in clinical practice has not been adequately evaluated.

Therefore, this survey was conducted to provide an overall analysis of the status of medical oncologists and to improve the recognition of medical oncology as an independent specialty in Korea. This is the first national survey of medical oncologists accredited by the KACO.

Materials and Methods

This was a prospective study evaluating the current status of medical oncologists in Korea. Questionnaires modified from the MOSES were first reviewed by select representatives of the insurance and policy committee of the KACO. Feedback and reviews were exchanged, and 53 questions to be answered by directors of departments of medical oncology and 27 questions to be answered by individuals working at each institution were finally selected. The major components included are listed below.

(1) General information: demographics and an overview of medical oncology facilities; (2) clinical practice: the types of multidisciplinary collaborations and the proportion of work performed by medical oncologists; (3) clinical research: the numbers of clinical studies performed and protocols proposed to the Korean Cancer Study Group (KCSG).

Questions involving an overview of facilities, the pattern of multidisciplinary collaboration, and clinical research were answered by the director of each institution. We used an electronic questionnaire. The online survey was sent to full members of the KACO in August 2015 via email, and those who did not respond were sent follow-up emails requesting responses by October 2015. Our local Institutional Review Board approved this study (Dongguk University Ilsan Hospital protocol number 2015-104).

Results

A total of 214 of 467 individuals (45.8%) who were sent the survey completed it. Overall, 60% of the directors of departments of medical oncology replied (71/119), while 41% of individuals replied (143/348). The answers from the directors of medical oncology departments were as follows.

1. General information

   1) Characteristics

   Males comprised 59% of the respondents, with a mean age of 42 years (range, 29 to 70 years). Overall, 98% reported using chemotherapy in clinical practice. Most responses were sent by individuals in Seoul and the surrounding metropolitan area (64%).

2) Overview of medical oncology facilities

   Overall, 47.7% of surveyed institutions (32/68) were cancer centers. The distribution of facilities related to medical oncology is shown in Fig. 1. Most institutions were equipped with a department of medical oncology (94.1%) and a department of radiation oncology (82.4%). A clinical trial center and palliative care center were present in less than 50% of the institutions.

   The mean number of medical oncologists in an institution was four (range, 1 to 21). Specialists who were fully responsible for palliative care were present in 32.4% of the institutions (22/68). Subspecialties of medical oncologists were present at 16 institutions, and family medical doctors at six institutions.

2. Clinical practice

   1) Percentages of multidisciplinary collaborations for the four major types of solid cancers

   Fig. 2 shows the average percentage of multidisciplinary collaborations at the institutions for the four major solid cancers (lung cancer, breast cancer, stomach cancer, and colorectal cancer). Although multidisciplinary care was provided from diagnosis to end-of-life, the percentage of multidisciplinary care was less than 10%. Major decisions were made via multidisciplinary collaboration in the following proportion of cases: lung cancer, 26.6%; breast cancer, 18.3%; stomach cancer, 21%; and colorectal cancer, 30.2%.
2) The role of medical oncologists in patient care for the four major solid cancers

Fig. 3 shows the average percentages of medical oncologists involved in treating the four major solid cancers. Organ specialists (e.g., surgical oncologists, pulmonologists, and gastroenterologists) were mainly involved in diagnoses. Medical oncologists contributed significantly to the administration of chemotherapy, which was the most common palliative chemotherapy given to patients with stomach cancer (84.5%), while it was the least common adjuvant chemotherapy for breast cancer (33.3%). Medical oncologists provided adjuvant chemotherapy in cases of lung cancer (50%), stomach cancer (50%), and colorectal cancer (45.8%), and were involved in palliative care/end-of-life care in approximately 60.0%-77.6% of the cases.

3) The role of medical oncologists as chemotherapy providers in the treatment of other solid cancers

Among the 62 respondents, the average percentage of medical oncologists who were chemotherapy providers was 42.0% for central nervous system cancer, 84.0% for head and neck cancer, 41.9% for hepatobiliary cancer, and 44.0% for genitourinary cancer.

3. Research

Overall, 86.2% of institutions were performing clinical research.

1) Research facilities

An institutional review board was present at all 71 institutions. There were clinical research nurses, clinical trial centers, animal laboratories, and cellular laboratories at 84.0%, 59.0%, 59.0%, and 57.1% of the institutions, respectively.

2) Number of clinical trials performed

We surveyed the number of clinical trials performed during the previous year (Fig. 4). More than 10 clinical trials were conducted at 11 institutions (11/49, 22.4%) involved in global trials, and seven institutions involved in domestic trials (7/53, 13.2%). A range of one to five of the institutions (44.7%) performed sponsor-initiated clinical trials (SITs), while 71.2% conducted investigator-initiated clinical trials (IITs). Phase I, phase II, and phase III trials were performed at 40.6%, 80%, and 83.3% of the institutions, respectively. Most (37/56, 66.1%) of the institutions participated in clinical trials involving the KCSG.
Fig. 2. The average percentage of multidisciplinary cancer care at institutions that provided care for the four major cancer types of cancer: lung cancer (A), breast cancer (B), stomach cancer (C), and colorectal cancer (D). Adj CTx, adjuvant chemotherapy; Palliat CTx, palliative chemotherapy; Neoadj CTx, neoadjuvant chemotherapy; MDT, multidisciplinary team; MO, medical oncologist; TS, thoracic surgeon; PUL, pulmonologist; PS, palliative care specialist; FM, family medicine doctor; BS, breast surgeon; SS, stomach surgeon; GE, gastroenterologist; CS, colorectal surgeon.

Discussion

This is the first survey to conduct an overall analysis of the current status of medical oncologists in Korea. The results showed that medical oncologists are involved in various phases of cancer treatment to different degrees, and that they actively contribute to cancer research.

As of 2015, 504 physicians were accredited with a specialty in medical oncology and/or hematology in Korea. The status of medical oncologists differs between countries. We analyzed the status of medical oncologists in Korea using a European survey (MOSES) prepared by the ESMO MOSES Task Force in 2008 [4]. The survey measured items such as
the teaching of oncology to undergraduate students, postgraduate specialization and/or subspecialization in oncology, continuing medical education, national internal certification, oncology facilities, types of cancer care, multi-disciplinary collaboration, and prescription/administration of cytotoxic therapy. The current study, Survey of Medical Oncology Status in Korea (SOMOS-K), mainly asked questions regarding clinical practice.

MOSES III comprehensively reported the types of cancer care and multi-disciplinary collaboration [4]. The results showed that the role of medical oncologists in multi-disciplinary teams is important and improving over time. However, organ specialists (e.g., gastroenterologists, pulmonologists, urologists, and gynecologists) also played an important role, consistent with our findings. Because there are few data describing the percentage of medical oncologists that contribute to the treatment of various types of cancer, it is not possible to compare our data directly with those from other national medical societies, and this percentage is most likely dependent on each nation’s health care infrastructure, health

Fig. 3. Average percentage of medical oncologists involved in the care of cancer patients with the four major types of solid cancers.
policies, and the number of active oncology professionals. According to MOSES III, nonmedical oncology professionals were mainly involved in hormone-related therapies. Moreover, European cancer patients experienced more difficulties receiving reimbursement for treatments prescribed by specialists other than medical oncologists because of legal issues regarding the handling and administration of cytotoxic drugs. Korea should emphasize the position of the ESMO, which states that medical oncologists are cancer specialists trained to provide treatment with drugs, ranging from chemotherapy to newly targeted agents and immunotherapies. That paper also stated that only specialized medical oncology training could prepare physicians to provide optimal care because of the increasingly complex scientific advances [2].

We found that clinical cancer trials are actively conducted in Korea, and that an impressive number of Korean medical oncologists are involved in global cancer clinical trials, with 5 institutions engaged in more than 20 global clinical trials. In addition, the number of IITs was higher than that of SITs, and five institutions (5/47 SITs, 10.6% and 5/52 IITs, 9.6%) performed more than 10 clinical trial SITs and/or IITs during the previous year. Furthermore, a recent analysis of clinical cancer trials in Korea reported that the number of such trials is rapidly increasing [6]. The authors suggested that the rapid growth of IITs is due to increased interest in clinical trials by Korean physicians, and the effects of multicenter clinical trial organizations such as the KCSG. However, they also mentioned the lack of funding for IITs, as well as a need for more financial support from government agencies and public donations. Nevertheless, medical oncologists should actively establish their roles as clinical researchers in this innovative area of cancer medicine.

The political involvement of medical oncologists also included anticancer drug approval, reimbursement systems, participation in risk-benefit assessments of new drugs, and plans for potential future workforce shortages. The medical oncologist workforce was another important issue. Although the United Kingdom recommended 1.25 whole time equivalent medical oncologists / 200,000-250,000 persons in 2000, there are no international recommendations regarding the optimal proportion of medical oncologists for the general population [7]. Accordingly, increasing numbers of studies of the workforce necessary to fulfill the needs of cancer services are being conducted. For example, the American Society of Clinical Oncology began a workforce survey about 10 years ago and recently reported a projected shortage of oncologists (overall demand will grow by 40%, whereas supply will grow by only 25%) by the year 2025. A rapidly aging population and increasing medical insurance were considered main factors in this discrepancy [8,9]. Although there are no exact numbers regarding the medical oncologist workforce in Korea, there is no reason to suspect that the situation in Korea is different. The World Health Organization also has concerns about the workforce available to meet the need for cancer services. Korean medical oncologists, including the KACO group, must therefore be proactive in solving this problem [10,11].

It should be noted that our study has some limitations. First, questionnaires are inherently not fully reliable. Second,
the percentage of medical oncologists involved in cancer care and as chemotherapy providers was based primarily on the reports of directors at each institution. Thus, this information may be insufficient to determine the current status of medical oncologists in Korea. Further investigation using data extracted from health insurance records and assessments of records from other government agencies that deal with health policies will be necessary to fully characterize the roles of medical oncologists in the treatment of cancer.

**Conclusion**

The data presented by the SOMOS-K should be used to enhance the contribution of medical oncologists to cancer care and to emphasize the vital contributions that medical oncologists make to the currently evolving health care environment.

**Conflicts of Interest**

Conflict of interest relevant to this article was not reported.

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Proton Pump Inhibition Enhances the Cytotoxicity of Paclitaxel in Cervical Cancer

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Purpose
This study was conducted to investigate whether a proton pump inhibitor (PPI) could enhance chemosensitivity via the inhibition of vacuolar-type H+ ATPase (V-ATPase) in cervical cancer.

Materials and Methods
The expression of V-ATPase was evaluated in 351 formalin-fixed, paraffin-embedded human cervical cancer tissues using immunohistochemistry and compared with clinicopathologic risk factors for disease prognosis. The influence of cell proliferation and apoptosis following V-ATPase siRNA transfection or esomeprazole pretreatment was assessed in cervical cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide and enzyme-linked immunosorbent assay, respectively.

Results
Immunohistochemical analysis revealed that V-ATPase was expressed in about 60% of cervical cancer tissue samples (211/351), and the expression was predominantly found in adenocarcinoma histology (p=0.016). Among patients with initially bulky cervical cancer (n=89), those with V-ATPase expression had shorter disease-free survival (p=0.005) and overall survival (p=0.023). Co-treatment with V-ATPase siRNA or esomeprazole with paclitaxel significantly decreased the cell proliferation of cervical cancer cell lines, including HeLa and INT407, compared to cell lines treated with paclitaxel alone (p < 0.01). Moreover, V-ATPase siRNA or esomeprazole followed by paclitaxel significantly increased the expression of active caspase-3 in these cells compared to cells treated with paclitaxel alone (both, p < 0.05).

Conclusion
V-ATPase was predominantly expressed in cervical adenocarcinoma, and the expression of V-ATPases was associated with poor prognosis. The inhibition of V-ATPase via siRNA or PPI (esomeprazole) might enhance the chemosensitivity of paclitaxel in cervical cancer cells.

Key words
Uterine cervical neoplasms,
Vacuolar proton-translocating ATPases,
Proton pump inhibitors, Esomeprazole, Small interfering RNA, Antineoplastic agents

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Introduction

Cancer of the uterine cervix is the second most common malignancy in women worldwide [1]. Although it is accepted that early-stage cervical cancer is curable with radical surgery, radiotherapy, or chemoradiation, the prognosis in patients with bulky tumors or adenocarcinoma histology is relatively poor [2,3]. Moreover, although chemotherapy for advanced-stage or recurrent cervical cancer has been applied, it is not effective and has merely a palliative purpose because of chemoresistance [4]. Therefore, a new therapeutic strategy for these patients to overcome chemoresistance in such patients is urgently needed.

One of major mechanisms of chemoresistance is increased acidification of the tumor microenvironment. Proton pumps such as vacuolar type H+-ATPase (V-ATPase) are major regulators of cellular pH [5]. V-ATPases pump protons across the plasma membrane and across the membranes of a wide array of intracellular compartments [5]. Some human tumor cells, particularly those selected for multidrug resistance, exhibit enhanced V-ATPase activity [6]. Therefore, treatment with proton pump inhibitor (PPI; esomeprazole), which directly inhibits V-ATPase at the cellular level [7,8], may be an option for reversing multidrug resistance. Here, we investigated whether PPI increased the sensitivity of tumor cells to cytotoxic agents via inhibition of V-ATPase in cervical cancer.

Materials and Methods

1. Tumor samples

This study was reviewed and approved by the Institutional Review Board at Samsung Medical Center, Seoul, Republic of Korea. A total of 351 patients with cervical cancer who underwent type I-III radical hysterectomy with or without pelvic/paraaortic lymph node dissection at Samsung Medical Center between February 2002 and May 2009 were included in this study. Patient inclusion criteria were (1) availability of tissue samples for tissue microarray construction and (2) early-stage cervical cancer that were candidates for surgery because pathological findings provide the most accurate information about tumor volume, depth of tumor invasion, and lymph node metastasis. Cervical cancer patients with the pathology of sarcoma, malignant melanoma, or neuroendocrine carcinoma, including small cell carcinoma, were excluded. After surgery, patients received adjuvant treatment according to their pathological findings. Patients then underwent follow-up examinations approximately every three months for the first 2 years, every 6 months for the next 3 years, and every year thereafter. Disease-free survival was defined as the period between initial treatment and relapse. Overall survival was measured from the time of initiation of therapy to the time of death or the date of final contact. Data for patients who had not experienced relapse or death were censored as of the date of the final observation.

2. Immunohistochemical analysis

All hematoxylin and eosin–stained slides were reviewed and representative tumor tissue samples were selected. The corresponding formalin-fixed paraffin-embedded tissue blocks were retrieved and the selected area was circled on the slide with a marker pen for tissue microarray construction. Each 6.0-mm tissue core was selected from the representative region of each paraffin block using the AccuMax (IshuAbxis, Seoul, Korea) as described previously [9]. For immunohistochemical studies, tissue microarray blocks were sectioned into 4-μm-thick slices. The primary antibody used was rabbit polyclonal V-ATPase subunit C1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Tissue sections were deparaffinized three times in xylene for a total of 15 minutes and subsequently rehydrated. Immunostaining for V-ATPase was performed using a Bond-max automated immunostainer (Leica Biosystems, Melbourne, Australia) and the Bond Polymer Refine Detection Kit (Vision Biosystems, Melbourne, Australia). Briefly, antigen retrieval was conducted at 97°C for 20 minutes in ER1 buffer. After blocking the endogenous peroxidase activity with 3% hydrogen peroxide for 10 minutes, primary antibody incubation was conducted for 15 minutes at room temperature at an antibody dilution of 1:200. Negative controls (substitution of primary antibody for Tris-buffered saline [TBS]) were run simultaneously. The immunostaining was evaluated independently by two pathologists who were blinded to patient outcome. A previously described scoring method [10,11] was used for the evaluation of V-ATPase expression. A case was considered positive when more than 1% of the epithelial cells in 10 random, high-power fields were positively stained.

3. Cell lines

Human cervical cancer cell lines (HeLa, SiHa, ME180, MS751, and CaSki) were obtained from the American Type Culture Collection (Manassas, VA). The INT407 cell line was obtained from the European Collection of Cell Culture (Salisbury, UK). The HeLa cell line was cultured in Dulbecco’s modified Eagle’s medium (Gibco BRL, Rockville, MD), the INT407, SiHa, ME180, and MS751 cell lines were cultured in
MEM (Gibco BRL); and the CaSki cell line was cultured in RPMI (Gibco BRL). All media were supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO) and 0.1% penicillin-streptomycin (Sigma-Aldrich). All cells were grown at 37°C in a humidified 5% CO₂ atmosphere.

4. Western blot analysis

For analysis of V-ATPase expression, cells were lysed in PRO-PRE Protein Extraction Solution (Intron Biotechnology, Seongnam, Korea). Protein concentration was determined using a BCA protein kit (Thermo Scientific, Rockford, IL). Cell lysates (50 μg of total protein) were separated in 12% acrylamide gels by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to Immobilon PVDF membrane filter paper (Millipore, Billerica, MA). Membranes were blocked with 5% skim milk in TBS containing 0.1% Tween-20 for 1 hour at room temperature. Protein bands were probed with V-ATPase subunit C1 antibody at a 1:1,000 dilution (Santa Cruz Biotechnology), tubulin and glyceraldehyde-3-phosphate dehydrogenase antibody at a 1:3,000 dilution (Epitomics, Burlingame, CA), and then labeled with hors eradish peroxidase-conjugated anti-rabbit antibody (GE Healthcare, Piscataway, NJ) and anti-goat antibody (Santa Cruz Biotechnology). Bands were visualized by enhanced chemiluminescence using an ECL kit (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer’s protocol.

5. Small interfering RNA transfection and drug treatment

V-ATPase V1C1 small interfering RNA (siRNA) and negative control siRNA were obtained from Santa Cruz Biotechnology. Cells were seeded at 5×10⁵ cells/well in a 96-well microplate in RPMI 1640 with 10% FBS. All siRNAs were transfected into the cells using Lipofectamine 2000 (Invitrogen, San Diego, CA) according to the manufacturer’s protocol. After 24 hours of siRNA transfection, cells were treated with various concentrations of paclitaxel (Sigma-Aldrich) and then incubated at 37°C for 48 hours. Esomeprazole (AstraZeneca, Mölndal, Sweden) was resuspended in normal saline at a concentration of 5 mg/mL. Cells were seeded at 3×10⁵ cells/well in a 96-well microplate in RPMI 1640 with 10% FBS. Cells were pretreated or not with esomeprazole (20-30 mg/mL) based on the protocols in previous reports [12,13]. After 24 hours of treatment, cells were treated with various concentrations of paclitaxel and incubated at 37°C for 48 hours.

6. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay is based on the conversion of MTT to insoluble MTT-formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of living cells. MTT solution (Amresco, Solon, OH) was subsequently added to each well. After 4 hours of additional incubation, the medium was discarded, 100 μL of acidic isopropanol (0.1 N HCl in absolute isopropanol) was added, and the plate was shaken gently. The absorbance at a wavelength of 540 nm was recorded by using a Falcon microplate reader (Becton Dickinson Labware, Franklin Lakes, NJ) and the percentage viability was calculated as follows: (optical density [OD] of drug-treated sample/control OD)×100. Additionally, the mean percent viability was calculated from cytotoxicity experiments at different paclitaxel concentrations that showed a significant difference from the control (p < 0.05). Each sample was assayed in triplicate, and the experiment was repeated three times.

7. Enzyme-linked immunosorbent assay

For analysis of active caspase-3 expression, cells were lysed in PRO-PRE Protein Extraction Solution (Intron Biotechnology). The protein concentration was determined using a BCA protein kit (Thermo Scientific). Enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer’s instructions to measure concentrations of active caspase-3 (Invitrogen). All samples were measured in triplicate.

8. Measurement of intracellular pH change

The change in intracellular pH was measured as previously described [14]. Briefly, cells grown as a monolayer on a 35 mm confocal dish (#200350, SPL Lifescience, Pocheon, Korea) were loaded with 1 μg/mL BCECF-AM (2’,7’-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein; Invitrogen) solution. The distribution of fluorescence was determined using a confocal microscope (LSM700, Zeiss, Jena, Germany) with excitation and emission wavelengths set to 488 nm and 525 nm, respectively [15]. The intensity of the fluorescence signal was measured using a VICTOR2 plate reader (PerkinElmer, Boston, MA), and the values were corrected to cell numbers by MTT assay [16]. Each sample was assayed in triplicate, and the experiment was repeated three times.
9. Statistical analysis

After using the Shapiro-Wilk test to confirm that the data were normally distributed, the Wilcoxon rank sum test was used to compare the median values, and the two-sample t test was used to compare the mean values. The chi-square test or Fisher exact test was used to compare the frequency distributions between categorical variables. Disease-free survival and overall survival curves were evaluated with the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards model was used to calculate the hazard ratio and the 95% confidential interval (CI). The SPSS software ver. 17.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses. All p-values were two-sided and were considered statistically significant at p < 0.05.

Results

1. Immunohistochemistry and its clinical significance

We assessed V-ATPase expression using immunohistochemistry in tissue microarrays composed of 351 human cervical cancer tissues. V-ATPase was predominantly expressed in the cell membrane and cytoplasm, and representative results of immunohistochemical staining for V-ATPase are shown in Fig. 1A. Immunohistochemical analysis demonstrated that V-ATPase was expressed in 60.1% of cervical cancer tissue samples (211/351), and the expression rate of V-ATPase in cervical adenocarcinomas was 73.4% (Fig. 1B), which is significantly higher than that of squamous cell cervical cancer (57.1%, p=0.016). During the median follow-up period of 65.5 months, a total of 351 patients included in this study had a disease-free survival of 84.4% and overall survival of 95.0%. When the disease-free survival of all 351 patients was analyzed based on the V-ATPase expression (S1 Table), V-ATPase was not correlated with disease-free survival. However, when the analysis was confined to 89 patients with bulky cervical tumor (defined as tumor diameter > 4 cm), patients with V-ATPase expression had shorter disease-free survival (p=0.005) and overall survival (p=0.023) than those without V-ATPase expression (Fig. 1C, S2 Table). Moreover, when confined to 55 patients with bulky V-ATPase positive tumors, patients with adenocarcinoma had shorter disease-free survival than those with squamous cell carcinoma (p=0.005) (Fig. 1D).

2. V-ATPase V1C1 siRNA transfection significantly enhanced cytotoxicity of paclitaxel in cervical cancer cells

We measured the basal expression of V-ATPase in various cervical cancer cell lines of HeLa, SiHa, MS751, INT407, and CaSki using Western blot. V-ATPase protein was expressed in most cervical cancer cell lines, with the exception of ME180 cells (Fig. 2A). Subsequently, in vitro experiments were performed to assess whether blocking V-ATPase by specific siRNA transfection enhanced the sensitivity to chemotherapeutic agents in cervical cancer cell lines. Based on our immunohistochemical results of V-ATPase expression, we used HeLa and INT407 cells, which were originally derived from cervical adenocarcinoma, for subsequent analyses. After HeLa and INT407 cells were transfected with V-ATPase siRNA or control siRNA, the expression of V-ATPase was determined by western blot analysis. V-ATPase expression was decreased at 48 hours after V-ATPase siRNA transfection in comparison to that in the controls (Fig. 2B), suggesting that V-ATPase expression was effectively down-regulated by the V-ATPase siRNA. We then assessed the effects of V-ATPase siRNA transfection on cell survival after treatment with cytotoxic drugs. As shown in Fig. 2C, pretreatment with V-ATPase siRNA significantly enhanced the cytotoxicity of paclitaxel in HeLa and INT407 cells compared with paclitaxel treatment alone. However, V-ATPase siRNA transfection did not enhance the cytotoxicity of cisplatin in HeLa cells or the cytotoxicity of paclitaxel in SiHa cells originating from squamous cervical cancer (S3 Fig.). To assess cellular apoptosis, active caspase-3 was measured using ELISA in HeLa and INT407 cells following treatment with paclitaxel with or without V-ATPase siRNA pretreatment. The results showed that V-ATPase siRNA transfection significantly enhanced the apoptotic activity of chemotherapy in HeLa and INT407 cells (Fig. 3).

3. Esomeprazole pretreatment significantly increased the cytotoxicity of paclitaxel in cervical cancer cells

Subsequently, in vitro studies using esomeprazole were repeated to assess whether esomeprazole pretreatment, such as blocking V-ATPase by specific siRNA transfection, enhanced cytotoxicity and apoptosis. The results showed that esomeprazole pretreatment significantly enhanced the cytotoxicity of paclitaxel in HeLa and INT407 cells compared with paclitaxel treatment alone (Fig. 4A). Interestingly, the effects of esomeprazole pretreatment in HeLa (37%) and INT407 (47%) cell lines derived from adenocarcinoma were more remarkable than those in SiHa (11%) and MS751 (18%) derived from non-adenocarcinomas such as squamous cell carcinoma (S4 Fig.). The effects of esomeprazole pretreatment (20 μg/mL concentration) on apoptosis also signifi-
vacuolar-type H\(^+\) ATPase (V-ATPase) was predominantly expressed in cervical adenocarcinoma, which was associated with poor prognosis. (A) Representative V-ATPase staining from cervical cancer (a, no staining; b, weak staining; c, moderate staining; d, strong staining, ×400). (B) Expression of V-ATPase in cervical cancer according to histology based on immunohistochemistry (IHC) (n=351). (C) Kaplan-Meier curves showed disease-free survival and overall survival according to V-ATPase expression in 89 patients with bulky cervical cancers (tumor diameter > 4 cm). (Continued to the next page)

4. Measurement of intracellular pH change

To verify that esomeprazole treatment induces cytosolic acidification through inhibition of V-ATPase activity, we measured the changes in intracellular pH following esomeprazole treatment based on the intensity and distribu-
tion of BCECF using a confocal microscope (LSM700). We used HeLa cells that showed high expression of V-ATPase and were originally derived from cervical adenocarcinoma based on the results of earlier cell survival and apoptosis studies. Live-cell imaging of the BCECF distribution within the cells revealed that immunofluorescence staining after esomeprazole treatment was weak compared with that of no treatment, suggesting that esomeprazole treatment induced a decrease in intracellular pH (Fig. 5A). As shown in Fig. 5B, the fluorescence intensity ratio showed that intracellular pH decreased significantly after esomeprazole treatment in HeLa and INT407 cells treated with esomeprazole for 3 hours (both, p < 0.01).

**Discussion**

In this study, we found that the V-ATPase expression in cervical cancer was significantly increased in patients with

![Graph](image1.png)

**Fig. 1.** (Continued from the previous page) (D) Kaplan-Meier curves showed disease-free survival according to cell type in 55 patients with bulky V-ATPase positive tumors.

![Image](image2.png)

**Fig. 2.** (A) Expression of vacuolar-type H+ ATPase (V-ATPase) in various cervical cancer cell lines. (B) Expression of V-ATPase was decreased by V-ATPase siRNA in HeLa and INT407 cells. (C) The effects of V-ATPase siRNA transfection on cell viability by paclitaxel in HeLa and INT407 cells. GAPDH, glyceraldehyde 3-phosphate dehydrogenase. *p < 0.05, **p < 0.01.
adenocarcinoma histology and was correlated with poor disease-free survival and overall survival in patients with bulky cervical tumor. Moreover, the inhibition of V-ATPase via PPI (esomeprazole) or its specific siRNA enhanced the cytotoxicity of paclitaxel in cervical cancer cells. To the best of our knowledge, this is the first study of V-ATPase in cervical cancer.

Bulky cervical tumors in early cervical cancer are known as prognostic factors for survival after surgery, and patients with bulky cervical tumors often receive additional adjuvant therapy or neoadjuvant chemotherapy [17]. In the present study, we also found that patients with bulky cervical tumors had a 1.21 hazard ratio for recurrence (95% CI, 1.06 to 1.37) (S1 Table), and V-ATPase expression was correlated with

Fig. 3. Effects of vacuolar-type H⁺ ATPase (V-ATPase) siRNA transfection on cell apoptosis with paclitaxel in HeLa and INT407 cells. (A) Cell death was observed by light microscopy in HeLa and INT407 cells (×100). (B) Expression of active caspase-3 was measured by enzyme-linked immunosorbent assay in HeLa and INT407 cells. *p < 0.05.
Fig. 4. Effects of esomeprazole (ESOM) pretreatment on cell survival and apoptosis with paclitaxel in HeLa and INT407 cells. (A) ESOM pretreatment significantly enhanced the cytotoxicity of paclitaxel in HeLa (37%, *p < 0.01) and INT407 (47%, *p < 0.01) cells when compared with paclitaxel treatment alone. (B) Cell death was observed by light microscopy in HeLa and INT407 cells (×100). (C) Expression of active caspase-3 was measured by enzyme-linked immunosorbent assay in HeLa and INT407 cells. *p < 0.05, **p < 0.01.
poor prognosis in patients with bulky cervical tumor. Bulky solid tumors contain poorly perfused dense tumor tissue [18,19], such as hypoxic tumors, in which protons and lactic acid are inefficiently removed from the extracellular space, creating an acidic extracellular microenvironment [20]. Therefore, proton pumps including V-ATPase are overexpressed to compensate for alkalization of the cytoplasm in the acidic microenvironment of the bulky hypoxic tumor [21,22]. However, when the activity of V-ATPase is inhibited using esomeprazole or its specific siRNA, the acidic pH of the tumor microenvironment is dramatically neutralized, which induces chemosensitization, inhibition of tumor proliferation, and apoptosis [22].

The results of the immunohistochemical analysis showed that the expression of V-ATPase in cervical adenocarcinomas was significantly higher than that of squamous cell cervical cancer (73.4% vs. 57.1%, p=0.016). This finding is consistent with those observed for non-small cell lung cancer [11]. The
expression rate of V-ATPase was 83.7% in lung adenocarcinoma, which was significantly higher than that of squamous cell lung cancer (71.4%, p < 0.001) [11]. In clinical practice, cervical adenocarcinoma is commonly more resistant to chemotherapy drugs than squamous cell cervical cancer [2,3]. Furthermore, our in vitro study assessing the effects of esomeprazole pretreatment on cell survival after treatment with cytotoxic drugs demonstrated that the effects of esomeprazole pretreatment (37%-47%) in HeLa and INT407 cells derived from adenocarcinoma were more remarkable than those (11%-18%) in SiHa and MS751 cells derived from non-adenocarcinomas such as squamous cell carcinoma (S4 Fig.). Together, these findings indicate that V-ATPase expression is potentially related to drug resistance in cervical adenocarcinoma.

PPIs such as esomeprazole and omeprazole, which are used clinically to suppress gastric acidity in peptic disease, are activated by acidic conditions. These inhibitors tend to decrease intracellular pH and increase extracellular pH via inhibition of V-ATPases through a covalent interaction [23]. PPIs have been shown to have anti-proliferative and pro-apoptotic effects on certain cancer cell lines, including B-cell leukemia, hepatoblastoma, melanoma, stomach cancer, sarcoma, and pancreatic cancer [24-27]. In the present study, while esomeprazole itself did not directly affect cell viability, it functioned as a chemosensitizer in cervical cancer cells. It remains unclear whether these findings are tumor specific or depend on tumor aggressiveness itself, regardless of tumor histology [28,29]. However, it is clear that modification of tumor acidity by blocking V-ATPase is a promising strategy for enhancing the sensitivity of the current cytotoxic treatments in cervical cancer.

Although the inhibition of V-ATPase via siRNA or PPI did not enhance the chemosensitivity of cisplatin in the present study, the results of a study performed by De Milito et al. [30] evaluating the effects of PPI pretreatment on human melanoma and osteosarcoma growth and cisplatin sensitivity in xenografted SCID/SCID mice differed. Specifically, they evaluated the effects of PPI pretreatment on human melanoma and osteosarcoma growth and cisplatin sensitivity in xenografted SCID/SCID mice and found this treatment sensitized tumor cell lines to the effects of cisplatin, resulting in decreases in the IC50 value by up to two-fold. Based on these findings, they concluded that oral pretreatment with PPI was able to induce/increase sensitivity of human solid tumors to cisplatin.

A search of clinical trial registries on April 8, 2015 yielded two phase II trials of PPI as a chemosensitizer in cancer patients undergoing chemotherapy registered at ClinicalTrials.gov. One was a completed trial on metastatic breast cancer (NCT01069081) that had not yet been published and another was an ongoing trial on castration-resistant prostate cancer (NCT01748500). Accordingly, further clinical trials investigating PPI in cervical cancer are needed.

It should be noted that this study had several limitations. Specifically, we did not validate the results of our study based on analysis of other PPIs such as omeprazole, lanoprazole, pantoprazole, or rabeprazole. We also did not perform an in vivo study to assess the potential clinical relevance of the in vitro results. However, positive aspects of this study include use of a large number of cancer tissue samples (n=351) with a long median follow-up period (65 months).

**Conclusion**

In conclusion, V-ATPase expression in cervical cancer was predominantly found in adenocarcinoma and was correlated with poor prognosis, especially in patients with bulky cervical tumor. Moreover, inhibition of the V-ATPase via PPI (esomeprazole) and its specific siRNA enhanced the sensitivity of chemotherapeutic agents in cervical cancer cell lines. These data suggest that a PPI may be useful as a chemosensitizer in treatment of patients with cervical cancer.

**Electronic Supplementary Material**

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

**Conflicts of Interest**

Conflict of interest relevant to this article was not reported.

**Acknowledgments**

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References


Current Trends in the Incidence and Survival Rate of Urological Cancers in Korea

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Purpose
This descriptive study assessed the current trends in the incidence of urological cancers and patient survival in Korea.

Materials and Methods
In this nationwide retrospective observational study based on the data from the Korea National Cancer Incidence Database (KNICIDB), this study analyzed the age-standardized incidence rates (ASRs) and annual percentage changes (APCs) of kidney, bladder, prostate, testicular, and penile cancers as well as cancer of the renal pelvis and ureter between 1999 and 2012. The relative survival rates (RSRs) were calculated for urological cancer patients diagnosed between 1993 and 2012 from the KNICIDB data.

Results
Prostate cancer was diagnosed in 66,812 individuals followed by bladder (41,549) and kidney (36,836) cancers. The overall ASR (18.26 per 100,000) increased with age because of the higher ASRs of bladder and prostate cancers in the elderly. The ASR for kidney cancer was highest in the 40-59-year-old group, whereas testicular cancer occurred most frequently before the age of 40. The incidence of most urological cancers increased (overall APC, 6.39%; p < 0.001), except for penile (APC, −2.01%; p = 0.05) and bladder (APC, −0.40%; p = 0.25) cancers. The overall survival increased steadily (5-year RSR, 66.4% in 1993-1995 vs. 84.2% in 2008-2012; p < 0.001), particularly for prostate (by 34.10%) and kidney (by 16.30%) cancers, but not for renal pelvis and ureter cancers (−7.20%).

Conclusion
The most common urological cancer in Korea was prostate cancer followed by bladder and kidney cancers. The incidence of most urological cancers, except for penile and bladder cancers, increased. Survival also increased, particularly for prostate and kidney cancers.

Key words
Urologic neoplasms, Incidence, Survival, Korea

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http://www.e-crt.org
Introduction

Cancer is a major public health problem worldwide [1,2]. In Korea, more than 200,000 new cancers are diagnosed each year, and cancer is responsible for one in four deaths [3]. In general, the incidence of urological cancers is higher in Western countries compared with that in Asian countries. For example, the incidence of prostate cancer is three to four times higher in Western countries than in Korea [4]. On the other hand, the rapidly increasing elderly population in eastern Asian countries, including Korea, might induce an increase in the incidence of urological cancers in the future. Over the last few years, the rates of prostate cancer in Korea appear to have risen steadily, with an up to 28.2% increase during 1996-1998 and 1999-2001 [5]. To the best of the authors’ knowledge, there is limited data concerning the incidence of urological cancers in Korea. Furthermore, these data are not based on nationwide samples and concern specific cancer types such as prostate cancer [5,6].

Improved knowledge regarding the incidence of urological cancers and patient survival rates can help assess diagnostic measures and the need for the continued promotion of urological cancer screening programs. We hypothesized that the overall incidence of urological cancer has increased in Korea, and that the patterns of each cancer have started to resemble those observed in Western countries. Using the Korea Central Cancer Registry (KCCR) data, this study aimed to assess trends in the incidence rate of urological cancers between 1999 and 2012 as well as the relative survival rate (RSR) of patients diagnosed with urological cancers from 1993 to 2012 in Korea.

Materials and Methods

1. Data sources

The KCCR was started as a nationwide, hospital-based cancer registry in 1980 by the Ministry of Health and Welfare and has covered the entire population since 1999. The Korea National Cancer Incidence Database (KNICIDB) is composed of the KCCR data and includes information on the cancer patient demographics, primary cancer site, morphology, diagnostic date, and initial treatment. For this study, the incidence and survival data were obtained from the KNICIDB. The urological cancer cases were classified according to the International Classification of Diseases for Oncology, third edition [7] and converted according to the International Classification of Diseases, 10th edition (ICD-10) [8]. In this study kidney cancer (C64), bladder cancer (C67), prostate cancer (C61), cancer of the renal pelvis or ureter (C65, C66), testis cancer (C62), and penile cancer (C60) were included as urological cancers.

The Institutional Review Board of the National Cancer Center approved this study (IRB No. NCC2015-0249). The ethics committee waived the need for participants’ consent because this study employed collected data that were anonymized for statistical analysis.

2. Analysis

The age-standardized incidence rates (ASRs) were calculated for each cancer site in patients diagnosed with cancer between 1999 and 2012 according to sex and age group (<40, 40-59, 60-69, and ≥70 years) using the world standard population. ASRs were expressed per 100,000 persons. The incidence trends for urological cancers were analyzed by the annual percentage change (APC) for each sex from 1999 to 2012. The male/female ratio was calculated as the ratio of the ASR in men to that in women.

The survival data were retrieved using the KNICIDB for individuals newly diagnosed with cancer from 1993 to 2012 and followed until December 31, 2013. The 5-year RSRs were calculated according to 5 periods of diagnosis (1993-1995, 1996-2000, 2001-2005, 2006-2010, and 2008-2012). The RSRs were calculated by comparing the observed survival with the expected survival using the Ederer II method. All analyses were conducted using the SAS ver. 9.2 (SAS Institute Inc., Cary, NC), and p < 0.05 was considered statistically significant.

Results

1. Incidence

During 1999-2012, urological cancer occurred in 155,991 patients (Table 1). The most common cancer was prostate cancer (66,812 patients) followed by bladder cancer (41,549 patients), kidney cancer (36,836 patients), renal pelvis and ureter cancer (7,537 patients), testis cancer (2,439 patients), and penile cancer (818 patients). The overall ASR for urological cancer was 18.26 per 100,000 persons. The ASR was 37.00 for men and 4.72 for women. The higher ASR observed among men was due most likely to prostate cancer. On the other hand, a similar difference between sexes was also found for bladder and kidney cancer.

Table 2 shows the ASRs per 100,000 persons by age group. The overall ASR of urological cancer increased with age.
### Table 1. Age-standardized rates (per 100,000) and male/female ratios of urologic cancer by sex in Korea, 1999-2012

<table>
<thead>
<tr>
<th>Site (ICD-10)</th>
<th>Overall</th>
<th>Male</th>
<th>Female</th>
<th>Male/female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases (%)</td>
<td>ASR</td>
<td>APC</td>
<td>No. of cases (%)</td>
</tr>
<tr>
<td>Kidney (C64)</td>
<td>36,836 (23.61)</td>
<td>4.40</td>
<td>5.80*</td>
<td>25,252 (18.90)</td>
</tr>
<tr>
<td>Bladder (C67)</td>
<td>41,549 (26.64)</td>
<td>4.79</td>
<td>-0.40</td>
<td>33,180 (24.84)</td>
</tr>
<tr>
<td>Prostate (C61)</td>
<td>66,812 (42.83)</td>
<td>7.75</td>
<td>12.96*</td>
<td>66,812 (50.01)</td>
</tr>
<tr>
<td>Renal pelvis, ureter (C65, C66)</td>
<td>7,537 (4.83)</td>
<td>0.88</td>
<td>3.76*</td>
<td>5,088 (3.81)</td>
</tr>
<tr>
<td>Testis (C62)</td>
<td>2,439 (1.56)</td>
<td>0.35</td>
<td>4.49*</td>
<td>2,439 (1.83)</td>
</tr>
<tr>
<td>Penis (C60)</td>
<td>818 (0.52)</td>
<td>0.09</td>
<td>-2.01</td>
<td>818 (0.61)</td>
</tr>
<tr>
<td>Total</td>
<td>155,991 (100)</td>
<td>18.26</td>
<td>6.39*</td>
<td>133,389 (100)</td>
</tr>
</tbody>
</table>

ICD-10, International Classification of Diseases, 10th edition; ASR, age-standardized rate (world standard population); APC, annual percentage change. * is significant at 0.05 significance level.

### Table 2. Age-standardized rates (per 100,000) of urologic cancer according to the age group in Korea, 1999-2012

<table>
<thead>
<tr>
<th>Site (ICD-10)</th>
<th>&lt; 40 yr</th>
<th>40-59 yr</th>
<th>60-69 yr</th>
<th>≥70 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases (%)</td>
<td>ASR</td>
<td>APC</td>
<td>No. of cases (%)</td>
</tr>
<tr>
<td>Kidney (C64)</td>
<td>3,587 (52.21)</td>
<td>0.50</td>
<td>5.75*</td>
<td>15,487 (44.99)</td>
</tr>
<tr>
<td>Bladder (C67)</td>
<td>1,118 (16.27)</td>
<td>0.13</td>
<td>-2.08</td>
<td>9,543 (27.72)</td>
</tr>
<tr>
<td>Prostate (C61)</td>
<td>93 (1.35)</td>
<td>0.01</td>
<td>9.24*</td>
<td>7,075 (20.55)</td>
</tr>
<tr>
<td>Renal pelvis, ureter (C65, C66)</td>
<td>123 (1.79)</td>
<td>0.01</td>
<td>-1.62</td>
<td>1,697 (4.93)</td>
</tr>
<tr>
<td>Testis (C62)</td>
<td>1,918 (27.92)</td>
<td>0.29</td>
<td>4.94*</td>
<td>399 (1.16)</td>
</tr>
<tr>
<td>Penis (C60)</td>
<td>31 (0.45)</td>
<td>0.00</td>
<td>3.82</td>
<td>225 (0.65)</td>
</tr>
<tr>
<td>Total</td>
<td>6,870 (100)</td>
<td>0.95</td>
<td>4.39*</td>
<td>34,426 (100)</td>
</tr>
</tbody>
</table>

ICD-10, International Classification of Diseases, 10th edition; ASR, age-standardized rate (world standard population); APC, annual percentage change. * is significant at 0.05 significance level.
Fig. 1. The change of age-standardized incidence rate (ASR) of urologic cancer per 100,000 populations, 1999-2012. (A) Both. (B) Male. (C) Female.
Table 3. 5-year relative survival rates (%) for urologic cancers according to the time period in Korea, 1993-2012a

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Case</td>
<td>RSR</td>
<td>Case</td>
<td>RSR</td>
<td>Case</td>
<td>RSR</td>
<td>RSR</td>
<td></td>
<td>Case</td>
</tr>
<tr>
<td>Kidney (C64)</td>
<td>2,112</td>
<td>63.6</td>
<td>5,676</td>
<td>66.8</td>
<td>8,998</td>
<td>73.4</td>
<td>14,383</td>
<td>78.2</td>
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<td>Bladder (C67)</td>
<td>3,757</td>
<td>70.2</td>
<td>9,168</td>
<td>73.5</td>
<td>12,719</td>
<td>75.5</td>
<td>14,812</td>
<td>76.4</td>
</tr>
<tr>
<td>Prostate (C61)</td>
<td>1,649</td>
<td>58.2</td>
<td>5,363</td>
<td>68.8</td>
<td>12,754</td>
<td>80.2</td>
<td>29,456</td>
<td>91.0</td>
</tr>
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<td>Renal pelvis, ureter (C65, C66)</td>
<td>447</td>
<td>64.1</td>
<td>1,313</td>
<td>61.0</td>
<td>1,879</td>
<td>59.6</td>
<td>2,812</td>
<td>59.0</td>
</tr>
<tr>
<td>Testis (C62)</td>
<td>224</td>
<td>86.7</td>
<td>573</td>
<td>90.4</td>
<td>747</td>
<td>90.6</td>
<td>911</td>
<td>93.1</td>
</tr>
<tr>
<td>Penis (C60)</td>
<td>131</td>
<td>58.2</td>
<td>252</td>
<td>70.4</td>
<td>255</td>
<td>74.4</td>
<td>274</td>
<td>70.1</td>
</tr>
<tr>
<td>Total</td>
<td>8,320</td>
<td>66.4</td>
<td>22,345</td>
<td>70.5</td>
<td>37,352</td>
<td>76.1</td>
<td>62,648</td>
<td>83.1</td>
</tr>
</tbody>
</table>

Fig. 2. Annual percentage change (APC) of urologic cancer by sex in Korea, 1999-2012.

Table 4. Five-year relative survival rate (%) of urologic cancer according to the age group in Korea, 1993 to 2012$^a$

<table>
<thead>
<tr>
<th>Site (ICD-10)</th>
<th>&lt; 40</th>
<th>40-59</th>
<th>60-69</th>
<th>≥ 70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney (C64)</td>
<td>87.5</td>
<td>81.4</td>
<td>71.2</td>
<td>55.5</td>
</tr>
<tr>
<td>Bladder (C67)</td>
<td>91.6</td>
<td>85.6</td>
<td>79.0</td>
<td>62.5</td>
</tr>
<tr>
<td>Prostate (C61)</td>
<td>44.3</td>
<td>86.1</td>
<td>88.7</td>
<td>82.7</td>
</tr>
<tr>
<td>Renal pelvis, ureter (C65, C66)</td>
<td>78.9</td>
<td>67.1</td>
<td>62.6</td>
<td>50.0</td>
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<td>Testis (C62)</td>
<td>93.3</td>
<td>88.4</td>
<td>76.5</td>
<td>45.1</td>
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<tr>
<td>Penis (C60)</td>
<td>75.8</td>
<td>69.0</td>
<td>71.7</td>
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</tr>
<tr>
<td>Total</td>
<td>89.1</td>
<td>82.9</td>
<td>81.1</td>
<td>71.7</td>
</tr>
</tbody>
</table>


Discussion

In Korea, cancer registration began officially in 1980 with the formation of the KCCR, and reporting of cancer cases has been fairly complete since 1999 [9]. Analyzing the incidence of urological cancer may assist in its early detection and prevention as well as promote a better understanding of the urological cancer patterns in Korea.

In 2002, the Korean Urological Cancer Society examined the incidence of urological cancer in Korea based on the survey data of 46 hospitals between 1985 and 1999. During that period, bladder cancer was the most common urological cancer; the incidence of prostate cancer and renal cancer was similar, and male patients outnumbered female patients by a ratio of 5.4:1 [6]. Recent reports concerning the incidence of urologic cancers, including kidney, bladder, and prostate cancer, between 1999 and 2011 revealed prostate cancer to be the most common urological cancer, which are similar to those of the current study [10].

1. Prostate cancer

Generally, prostate cancer is not a common cancer in Asian countries, including Korea, compared to Western countries (Fig. 1, S2 Table); however, the incidence of prostate cancer has begun to increase recently [3,5,6]. Interestingly, Western countries have higher incidence rates of prostate cancer than Asian countries. On the other hand, the incidence of prostate cancer differ according to the socioeconomic status across
Asian countries. High-income Asian countries, including Korea, Japan, and Singapore, have higher incidences of prostate cancer than low-income Asian countries, such as Philippines, Malaysia, and Thailand (S3 Fig.). Increasing age, high dietary fat intake, and cigarette smoking, as well as pre-disposing genetic factors, are considered common risk factors [11,12]. The rapid increase in the incidence of prostate cancer may be due to the widespread use of a prostate-specific antigen (PSA), which also induced stage migration of newly diagnosed prostate cancers to localized disease at diagnosis. For example, a previous study including 46 hospitals found that prostate cancer had been diagnosed in 2,417 patients comprising 18.3% of all urological cancers cases during 1985-1999 [6]. In another study, although the incidence of prostate cancer was low (7.9 per 100,000 man-years), it increased by 28.2% between 1996-1998 and 1999-2001 [5]. Furthermore, in the present study, 66,812 patients in the KCCR were diagnosed with prostate cancer between 1999 and 2012, a much higher number than previous studies examining survey data [6].

Based on the KCCR annual report, prostate cancer comprised 4.1% of all newly diagnosed cancers in 2012 with a crude incidence rate of 18.4 per 100,000 persons and an ASR of 11.6 per 100,000 persons, making prostate cancer the seventh most common cancer among the total population and the fifth most common cancer among men [3]. The ASR for prostate cancer was highest among those ≥70 years old, but the APC for prostate cancer was highest among those 60-69 years old (15.4%). These results are in accordance with general knowledge about prostate cancer development. In addition, the APC among those 40-59 years old was also high (14.5%) and comparable to the rate among those in their 60s. Considering the longer estimated survival observed in the younger age group, there should be more concern about a prostate cancer diagnosis for those in their 50s and 60s. In addition, the APC of prostate cancer among the total Korean population was 12.96 between 1999 and 2012, an APC that is much higher than in Japan (4.8) between 1985 and 2000 [13]. On the other hand, prostate cancer survival has increased remarkably, which may be related to the PSA test commonly used since the late 1990s that resulted in an increased rate of localized prostate cancer among newly diagnosed prostate cancer patients. However, the role of PSA-based screening in a prostate cancer diagnosis is still controversial because screening implementation has not decreased the prostate cancer mortality significantly [14]. In Korea, prostate cancer is not included in the national cancer screening program. Prostate cancer in Korean men has different biological characteristics than in Western populations. For Korean men, the incidence of high-grade or advanced-stage prostate cancer is higher [15,16], even when the levels of PSA are low, which may be due to late detection or differences specific to the Asian population. This emphasizes the need to detect prostate cancer at its early stages in Korean men.

2. Bladder cancer

The ASR of bladder cancer was similar in Japan and Korea (5.6 and 5.2, respectively) (S2 Table). On the other hand, despite the lower incidence of bladder cancer in Korea than in Western countries, bladder cancer remained the most common urological cancer in Korea before 2004. In addition, the incidence of bladder cancer gradually increased until 2003 and generally affected those ≥50 years old, peaking among those older than 70.

In the United States, the incidence of bladder cancer is increasing among men and declining slightly among women. In Japan, the incidence rate has slowly increased with an APC of 0.9 between 1985 and 2003 [13]. Therefore, compared to the APC of other urological cancers, including prostate and kidney cancer, the change in bladder cancer was not remarkable in Korea. In general, bladder cancer is prevalent in men, and in this study, the male to female ASR ratio was 5.68 between 1999 and 2012, which is similar to a previous study (ASR 5.50 between 1985 and 1999) [6]. Since 2008, the ASR of bladder cancer has decreased, and in 2012, the ASR was 4.30, which is the lowest value compared to other urological cancers (prostate cancer, 11.65; kidney cancer, 5.60). This may be related to cigarette smoking, which is considered the most important risk factor in bladder cancer [17]. For example, cigarette smoking increased rapidly in Korean society until the 1990s. However, since 1999, the rate of cigarette smoking in Korean adults has decreased gradually, which might be related to the decreasing incidence of bladder cancer.

3. Kidney cancer

The incidence of kidney cancer is higher in North America and Europe than in Asia and South America [18]. A previous report showed that male patients outnumbered female patients by a ratio of 2.3:1 between 1985 and 1999 [6]. In the United States, the ASR increased from 10.6 in 2001 to 12.4 in 2010, and the incidence in men was almost double that in women [19]. As shown in S2 Table, most Western countries have a higher ASR than Asian countries, such as Korea and Japan. Interestingly, Korea tended to have a higher ASR of kidney cancer than Japan among both men and women in 2012. In Japan, the APC in kidney cancer was 4.8 between 1985 and 1996 based on population-based data [13]. In Korea, the incidence of kidney cancer increased steadily during the study period (APC, 5.80). This increase may be related to early or incidentally detected kidney cancer derived from the prevalent health check-ups, including radiologic examina-
tions, such as ultrasonography and computed tomography, since the 1990s in Korea. In addition, kidney cancer showed the highest ASR (1.75) in the 40-59 years age group, the period when Korean adults usually undergo health screening supported by the National Health Insurance system for the first time in their life. Accordingly, the survival of kidney cancer is currently increasing in Korea due to incidental and early detection of kidney cancer.

On the contrary, when compared with other urological cancers that usually develop in old age, kidney cancer is more prevalent in the middle-aged group, and the highest ASR is found among those aged 40-59 years. In the United States, however, kidney cancer is the most prevalent among an even younger age group (20 to 39 years old) [19]. This may be because of the association between renal cell carcinoma and obesity [20]. Therefore, since obesity in younger age groups has been increasing for several decades in the United States, childhood obesity may be at least partially responsible for the higher APC in 20-39 years age group. Furthermore, childhood obesity is currently an emerging health issue in Korea because of the high fat diet and Westernized food intake. In Korea, the prevalence of obesity has increased markedly since 1998 [21], indicating that the incidence of kidney cancer may increase in the future.

4. Testis and penile cancer

Testicular cancer is rare urological cancers; however, the incidence rate has been steadily increasing in Western countries, which is more common than Asian countries, including Korea. The incidence of testicular cancer in Western countries is approximately 5-9 times of that in Korea [4]. In Korean men, middle-aged men had the highest incidence of testicular cancer because of the commonly diagnosed seminoma. In general, testicular cancer is largely a disease of young and middle-aged men, but approximately 7% of cases occur in children and teens, and approximately 7% of cases occur in men over the age of 55 [22].

Penile cancer occurs in approximately one of 100,000 men in the United States [23]. Although penile cancer is more common in some parts of Asia, it is rare among Koreans and Israeli Jews (ASR, 0.22 in Koreans and 0.10 in Israeli Jews), which might be related to improvements in hygiene or commonly performed circumcisions [24,25].

Despite the use of a population-based registry, there were some limitations to this study. First, because the KCCCR does not collect data regarding the tumor characteristics, such as tumor stage, histological grade, or specific treatments, we could only examine relative survival and not cancer-specific survival or overall survival. Moreover, the KCCCR does not record the cancer risk factors, such as tobacco/alcohol use, exposure to carcinogenic arsenic, dietary habits, and lifestyle, and this lack of data makes it more difficult to determine the real relationship between these etiological factors and the urinary tract cancer risk. Second, because of the very low incidence in Korea, some urological cancers, such as testicular cancer and penile cancer, were not analyzed comprehensively. Third, a study of cancer trends is mainly affected by the completeness of cancer registration. The completeness of cancer registration in the early period might be lower than that in recent years. These differences in completeness according to the time period might cause an overestimation or underestimation of the cancer trends. In addition, many Korean insurance companies do not currently cover patients with Ta bladder tumors or carcinoma in-situ (CIS) lesions. Therefore, several cases of Ta tumors or CIS lesions may not have been counted as new instances of cancer, which may have contributed to the apparent decrease in the incidence of bladder and renal pelvis or ureter tumors. Finally, this study was conducted in the Korean population, which consists almost entirely of ethnic Koreans, whereas the Korean health care system is markedly different from those of other countries. These factors should be taken into consideration when interpreting the present findings. Despite these shortcomings, this nationwide registry-based study included the largest numbers of cases of these rare diseases and contained information that is more than 97% complete and encompasses entire South Korea. Therefore, the results are likely to be the most accurate and can allow for an analysis of the internal validity and generalizability.

Conclusion

In Korea, prostate cancer was the most common urological cancer followed by bladder cancer and kidney cancer. The ASR of urological cancer was 18.26 per 100,000 persons, and men had a higher incidence than women. The incidence of urological cancers generally increases with age; however, kidney cancer and testicular cancer were diagnosed more frequently in the younger age groups. In addition, the urological cancer survival rates are currently increasing. In particular, the 5-year RSR for prostate cancer and kidney cancer has increased remarkably.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).
Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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References

Lung Cancer Epidemiology in Korea

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Purpose
The current study was undertaken to examine the trends in the lung cancer incidence, mortality, and survival after a diagnosis in Korea.

Materials and Methods
Lung cancer incidence data according to the histologic type and mortality data were obtained from the Korea Central Cancer Registry and the Statistics Korea, respectively. The age-standardized incidence and mortality rates were calculated, and the Joinpoint model and age-period-cohort analyses were used to describe the trends in the rates. The 5-year relative survival rates of lung cancer were also calculated.

Results
Although the number of new lung cancer cases increased between 1999 and 2012, the age-standardized incidence rate decreased by 0.9% per year in men, whereas the incidence in women increased by 1.7% per year over the same time. Until 2010, the most common histologic type in men was squamous cell carcinoma, then adenocarcinoma prevailed thereafter. Since 1999, the most frequent histological type in women was adenocarcinoma. The lung cancer mortality started to decrease in 2002, with a more apparent decline for the younger age groups in both men and women. Overall, the 5-year relative survival rates have improved significantly from 11.2% for men and 14.7% for women among patients diagnosed between 1993 and 1997 to 19.3% for men and 28.2% for women among patients diagnosed between 2008 and 2012, respectively. An improvement in survival rate was observed for all major histology groups.

Conclusion
The epidemiology of lung cancer in Korea has changed over a short time span, with decreasing mortality and improving survival rates. Further study is warranted to determine the cause of these changes.

Key words
Lung neoplasms, Epidemiology, Incidence, Mortality, Survival, Adenocarcinoma

Introduction

Lung cancer has been the most common cancer in both incidence and mortality worldwide [1]. However, a decrease in lung cancer incidence and mortality has been observed in men in many developed countries, whereas an increase or no significant changes have been observed in women. When the histological types of lung cancer are considered, the incidence of squamous cell carcinoma and small cell carcinomas has been decreasing, whereas the incidence of adenocarcinomas has been increasing in both men and women [2-5]. This may be explained by changes in the prevalence of tobacco smoking, which is the most important risk factor for lung cancer and accounts for 70%-90% of the lung cancer burden [6,7]. The strongest association between smoking and lung cancer is observed for squamous cell carcinoma and small cell carcinomas (odds ratio, 63 to 111 for smokers > 30 ciga-
rettes/day), but this association is still quite high for adenocarcinomas (odds ratio, 19 to 21 for smokers > 30 cigarettes/day) [8].

In Korea, the lung cancer mortality in both sexes as well as the incidence in men have decreased, whereas the incidence in women has increased over the last few decades [9]. In addition, early detection by screening and the introduction of new chemotherapy and molecular targeted-agents may improve the survival of lung cancer patients. The objectives of the current study were to examine the trends in the lung cancer incidence, mortality, and survival in Korea. For the incidence and survival, the trends for each histological type were also assessed.

**Materials and Methods**

1. **Lung cancer incidence**

The Korea Central Cancer Registry (KCCR), a nation-wide, hospital-based cancer registry, was initiated by the Ministry of Health and Welfare, Korea in 1980. Since 1999, the KCCR expanded the cancer registration to cover the entire Korean population under the Population-Based Regional Cancer Registry program. The age- (5-year intervals) and sex-specific incidence rates and the number of cases for lung cancer patients between 1999 and 2012 were obtained from the Korea National Cancer Incidence Database. The histological subtypes of lung cancer were classified as follows: carcinomas (International Classification of Diseases for Oncology third edition morphological codes 8010-8576), sarcoma (8800-8811, 8830, 8840-8921, 8990-8991, 9040, 9044, 9120-9133, 9150, and 9540-9581), other specified cancers, including pulmonary blastoma, and unspecified (8000-8005). Carcinomas were further classified as follows: squamous cell carcinoma (8050-8078 and 8083-8084), adenocarcinoma (8140, 8211, 8230-8231, 8250-8260, 8323, 8480-8490, 8550-8551, 8570-8574, and 8576), small-cell carcinoma (8041-8045 and 8246), large-cell carcinoma (8010-8012, 8014-8031, 8035, and 8310), and other specified carcinomas.

The age-standardized rates (ASRs) were calculated using the mid-year population of 2000 as the standard population. The annual percent changes (APC) for the incidence rates were calculated based on a linear model using the following formula: $(\exp(b)-1)\times100$, where $b$ is the slope of the regression of the natural logarithm of the ASR in a calendar year [10]. The 95% confidence intervals were obtained with a standard error from the fit of the regression and the t-distribution function [11]. All analyses were stratified according to sex.

2. **Lung cancer mortality**

The lung cancer mortality data were obtained from the Statistics Korea for the years 1983-2013 (http://kosis.kr/). The ASRs for mortality, as well as the truncated rates for the four age groups (0-39, 40-59, 60-69, and ≥ 70 years) were estimated using the mid-year population of 2000 as the standard population. The trends in lung cancer mortality were tested using Joinpoint regression models, using Joinpoint software ver. 3.5.3 [12]. A maximum of four Joinpoints were allowed, and the default settings were used.

To evaluate the birth cohort effects on lung cancer mortality, 5-year age groups starting at age 20 years were categorized. The age-specific mortality rates were illustrated by the birth cohort. To evaluate the birth-cohort effects after adjusting for age and period effects, a log-linear model using the intrinsic estimator method was performed on the assumption that the number of deaths in each age group followed a Poisson distribution [13].

3. **Lung cancer survival**

The survival duration for registered lung cancer patients was determined as the interval between the date of the initial diagnosis and the date of death, date of loss to follow-up, or closing date for follow-up. The 5-year relative survival rates were calculated using the Ederer II method [14] based on an algorithm written in SAS by Dickman [15] with minor modifications. The 5-year relative survival rates of 1993-1997 and 2008-2012 were compared by the percentage changes. In addition, the excess risk model with a Poisson error structure was used to determine the difference in survival between 1993-1997 and 2008-2012 [16]. Statistical analysis was performed using Stata/SE 12.0 for Windows (StataCorp LP, College Station, TX) and SAS ver. 9.3 software (SAS Institute Inc., Cary, NC).

**Results**

1. **Lung cancer incidence**

In men, despite the increase in the crude incidence rates for lung cancer from 41.1/100,000 in 1999 to 60.9/100,000 in 2012 with an APC of 3.4%, the age-standardized incidence rate decreased from 51.8/100,000 in 1999 to 44.9/100,000 in 2012 with an APC of −0.9% (Table 1). The most frequent histological type was squamous cell carcinoma until 2010; however, since 2011, adenocarcinoma has been the most commonly diagnosed cancer. The number of new lung cancer
<p>| Table 1. CR and ASR per 100,000(^d) for lung cancer and APC by sex and histological subtypes, the Korea Central Cancer Registry, 1999-2012 |
|-----------------------------|-------------|-----------|-------------|-------------|-------------|-------------|-----------|-------------|-------------|-------------|-------------|
| <strong>Histological group</strong>      | <strong>Rate</strong>    | <strong>1999</strong>  | <strong>2000</strong>    | <strong>2001</strong>    | <strong>2002</strong>    | <strong>2003</strong>    | <strong>2004</strong>   | <strong>2005</strong>    | <strong>2006</strong>    | <strong>2007</strong>    | <strong>2008</strong>    | <strong>2009</strong>    | <strong>2010</strong>    | <strong>2011</strong>    | <strong>2012</strong>    | <strong>APC</strong>     | <strong>95% CI</strong>  | <strong>p-value</strong> |
| <strong>Men</strong>                     |             |           |             |             |             |             |           |             |             |             |             |             |             |             |             |             |
| Overall                     | Cases       | 9,722     | 9,788       | 10,473      | 10,956      | 11,187      | 11,961     | 12,497      | 12,682      | 13,182      | 13,593      | 14,240      | 14,914      | 15,319      | 15,350      | 3.4         | 3.1 to 3.6  | &lt;0.01       |
| Carcinoma                   | Cases       | 6,867     | 6,947       | 7,916       | 8,262       | 8,735       | 9,373      | 9,861       | 10,361      | 10,907      | 11,354      | 12,148      | 12,741      | 13,173      | 13,365      | 4.6         | 4.6 to 5.4  | &lt;0.01       |
| Squamous cell carcinoma     | Cases       | 3,394     | 3,256       | 3,572       | 3,746       | 3,724       | 4,000      | 4,034       | 4,172       | 4,312       | 4,097       | 4,575       | 4,627       | 4,707       | 4,737       | 2.4         | 2.0 to 2.9  | &lt;0.01       |
| Adenocarcinoma              | Cases       | 1,731     | 1,838       | 2,205       | 2,177       | 2,404       | 2,561      | 2,859       | 3,041       | 3,323       | 3,780       | 4,024       | 4,414       | 4,900       | 5,045       | 8.3         | 7.8 to 8.8  | &lt;0.01       |
| Small cell carcinoma        | Cases       | 1,260     | 1,238       | 1,355       | 1,408       | 1,460       | 1,530      | 1,474       | 1,457       | 1,721       | 1,733       | 1,839       | 1,969       | 1,971       | 2,045       | 3.5         | 3.1 to 4.0  | &lt;0.01       |
| Large cell carcinoma        | Cases       | 483       | 490         | 643         | 572         | 325         | 267        | 312         | 340         | 269         | 275         | 264         | 281         | 263         | 233         | 5.9         | 0.9 to 0.1  | 0.01        |
| Other specified carcinomas  | Cases       | 99        | 125         | 141         | 359         | 822         | 1,015      | 1,182       | 1,251       | 1,373       | 1,469       | 1,446       | 1,450       | 1,332       | 1,305       | 22.4        | 13.0 to 32.7| &lt;0.01       |
| Sarcoma                     | Cases       | 17        | 16          | 28          | 24          | 25          | 16         | 21          | 27          | 30          | 24          | 38          | 21          | 28          | 30          | 4.0         | 0.5 to 7.7  | 0.03        |
| Other specified cancer      | Cases       | 9         | 10          | 8           | 9           | 10          | 10         | 16          | 11          | 11          | 9           | 6           | 11          | 10          |              |             |             |             |
| Unspecified cancer          | Cases       | 2,729     | 2,815       | 2,521       | 2,671       | 2,417       | 2,562      | 2,605       | 2,278       | 2,234       | 2,204       | 2,045       | 2,146       | 2,107       | 1,945       | 7.7         | 3.6 to 2.4  | &lt;0.01       |</p>
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<th>Table 1. Continued</th>
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<td><strong>Histological group</strong></td>
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CRs and ASRs were expressed per 100,000 people. CR, crude rate; ASR, age-standardized rate; APC, annual percent change; CI, confidence interval. <sup>a</sup>The Korean population in 2000 was used as standard population. <sup>b</sup>Large cell carcinoma includes giant cell, clear cell, and large cell undifferentiated carcinoma.
cases increased from 9,722 in 1999 to 15,350 in 2012 (Table 1). The proportion of adenocarcinomas also increased during the same period (17.8% in 1999 and 32.9% in 2012), whereas the proportion of squamous cell carcinomas decreased (34.9% in 1999 and 30.8% in 2012) (Fig. 1). A significant decrease in ASRs for the incidence was observed for squamous cell carcinomas, small cell carcinomas, and large cell carcinomas, whereas the ASR for the adenocarcinoma incidence increased significantly (APC, 4.1%) between 1999 and 2012 (Table 1, Fig. 2A).

In women, both the crude rates and ASRs for the lung cancer incidence increased between 1999 and 2012 with an APC of 5.2% and 1.7% for the crude rate and incidence, respectively (Table 1). Adenocarcinoma was the most frequent histological subtype during this period. The proportion of adenocarcinomas increased from 34.5% in 1999 to 61.7% in 2012 (Fig. 1). Similar to men, both the crude rate and the ASR for adenocarcinoma incidence increased between 1999 and 2012, with an APC of 10% for the crude rate and 6.6% for ASR, whereas the ASRs for the incidence of squamous cell carcinomas, small cell carcinomas, and large cell carcinomas decreased (Table 1, Fig. 2B).

2. Lung cancer mortality

In both men and women, the number of deaths from lung cancer increased between 1983 and 2013 (S1 Table). In 2013, a total of 12,512 men and 4,653 women died from lung cancer, which places lung cancer as the most common cancer site for death in both sexes [9]. The lung cancer mortality rapidly increased between 1983 and 1994, with an APC of 9.4% for men and 7.6% for women (Table 2). From 1994, the slope of the increase began to stabilize, and since 2002, it started to decrease (Table 2). In stratified analysis according to age groups, there was a decline in mortality for the younger age groups in both men and women. For men, there was a significant decline in mortality from 1992 for the 40-59-year age group, from 2001 for the 60-69-year age group, and from 2002 for the ≥70 year age group. Similarly, for women, there was a decline in mortality from 1983 for the 0-39-year age group, from 1993 for the 40-59-year age group, from 1994 for the 60-69-year age group, and from 2002 for the ≥70 year age group.

Among elderly individuals aged ≥65 years, the ASR for the lung cancer mortality was highest among those born in the 1920s. When adjusting for age and period effects, the risk
Fig. 2. Age-standardized incidence rates of lung cancer according to the histologic types in men (A) and women (B), based on the Korea Central Cancer Registry, 1999-2012.
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<th>Table 2. Joinpoint analysis for lung cancer mortality at all ages and at age 0-39, 40-59, 60-69, and ≥ 70, in the Republic of Korea, 1983-2013</th>
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Joinpoint regression models were used to detect significant changes in trends. APC, annual percent change; CI, confidence interval.

**Discussion**

Despite the increase in the number of lung cancer patients, the ASK for the lung cancer incidence, decreased between 1999 and 2012 in men in Korea. Until 2010, squamous cell carcinoma was the most frequently diagnosed histological type in men, but adenocarcinoma was the most frequent histological type in women. In Korea, adenocarcinoma has been followed by a decrease in squamous cell carcinoma and an increase in cancer deaths, possibly due to the introduction of tobacco use. However, no significant changes in smoking habits were noted. The smoking rate has increased from 72% in 1992 to 75% in 2013 in adult men [17]. Despite the increase in adult women smoking rates, the percentage of women who smoke has been stable in the past 20 years. Overall, the smoking rate in Korea has improved significantly from 11.2% among patients diagnosed between 1993 and 1997 to 12.3% among patients diagnosed between 1997 and 2008. An improved survival rate was observed for squamous cell carcinoma and adenocarcinoma, whereas survival rates for small cell carcinoma and lung cancer as a whole have not improved significantly. The survival rate was observed for squamous cell carcinoma and adenocarcinoma, whereas survival rates for small cell carcinoma and lung cancer as a whole have not improved significantly. The survival rate was observed for squamous cell carcinoma and adenocarcinoma, whereas survival rates for small cell carcinoma and lung cancer as a whole have not improved significantly.
Fig. 3. Age-period-cohort analysis for lung cancer mortality in men (A) and women (B) in Korea.

sively than non-filtered higher-yield cigarettes to satisfy their nicotine needs; this leads to a more peripheral distribution of tobacco smoke in the lung and promotes peripheral tumors, such as adenocarcinomas [20,21].

A decrease in mortality in male lung cancer has been observed in many developed countries [4,22,23]. Lung cancer mortality is strongly influenced by tobacco consumption, peaking 20-30 years after the peak in tobacco consumption [23,24]. Price and non-price tobacco control policies were introduced as early as 1986; these policies restricted advertising and included health warnings on cigarette packages in Korea [18]. In addition, in 1995, the Korean government passed the National Health Promotion Act and strengthened tobacco control policies [18]. The consequent decrease in lung
Table 3. Five-year relative survival rates of lung cancer patient by sex and histological subtypes, the Korea Central Cancer Registry, 1993-2012

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<td><strong>Overall total</strong></td>
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<td>Carcinoma</td>
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| **Men**                     | 28,695    | 11.2      | 44,342    | 12.9      | 53,690    | 15.9        |          |
| Total                       | 23,575    | 11.1      | 35,235    | 13.5      | 45,080    | 17.0        |          |
| Carcinoma                   | 12,099    | 13.7      | 16,493    | 16.5      | 18,705    | 20.0        |          |
| Squamous cell carcinoma     | 5,366     | 9.0       | 9,263     | 12.4      | 12,747    | 19.9        |          |
| Adenocarcinoma              | 4,299     | 5.6       | 6,197     | 7.5       | 7,298     | 7.4         |          |
| Small-cell carcinoma        | 1,450     | 9.4       | 2,500     | 8.9       | 1,424     | 10.5        |          |
| Large-cell carcinoma        | 361       | 28.6      | 782       | 25.0      | 4,906     | 14.0        |          |
| Other specified carcinomas  | 45        | 16.8      | 82        | 15.6      | 95        | 7.0         |          |
| Sarcoma                     | 44        | 28.2      | 36        | 25.2      | 44        | 37.4        |          |
| Other specified cancer      | 5,031     | 11.2      | 8,989     | 10.3      | 8,471     | 10.0        |          |
| Unspecified cancer          | 14,070    | 11.2      | 21,243    | 12.9      | 21,206    | 17.4        |          |

| **Women**                   | 8,450     | 14.7      | 14,937    | 18.3      | 19,426    | 21.7        |          |
| Total                       | 6,223     | 12.7      | 10,293    | 16.8      | 14,771    | 22.6        |          |
| Carcinoma                   | 1,332     | 12.3      | 1,716     | 15.2      | 1,926     | 15.1        |          |
| Squamous cell carcinoma     | 3,475     | 12.6      | 6,227     | 17.8      | 9,401     | 27.5        |          |
| Adenocarcinoma              | 880       | 9.1       | 1,270     | 10.5      | 1,441     | 8.3         |          |
| Small-cell carcinoma        | 384       | 10.8      | 729       | 10.8      | 400       | 10.7        |          |
| Large-cell carcinoma        | 152       | 44.1      | 351       | 42.1      | 1,603     | 18.8        |          |
| Other specified carcinomas  | 26        | 20.1      | 36        | 31.6      | 58        | 21.2        |          |
| Sarcoma                     | 11        | 27.5      | 18        | 45.3      | 15        | 76.0        |          |
| Other specified cancer      | 2,190     | 20.5      | 4,590     | 22.0      | 4,582     | 18.9        |          |

aChange (%) in the 5-year relative survival rates from 1993-1997 to 2008-2012. b)p-values for changes in survival from 1993-1997 and 2008-2012 were derived by relative excess risk model.
cancer mortality since 2002 in Korea is consistent with previous reports [25].

The improvement in the lung cancer survival rate may be related to several factors, including the introduction of the target agents [26], earlier diagnosis [27], and a decrease in surgical mortality [27]. The introduction of (neo)adjuvant chemotherapy contributed to the improvement of stages I-III resectable non-small cell lung cancer patients [28]. Currently, adjuvant chemotherapy is applied to patients with resected stage II or III non-small cell lung cancer, and in patients with stage IB with a resected lesion of 4 cm or larger [29]. These findings appear to contribute to improved survival in adenocarcinoma. In addition, patients are now more likely to have access to health insurance and receive a timely diagnosis as well as high quality treatment and supportive care.

The limitations of the current study include a relatively high proportion of unspecified histology. The proportion of unspecified cancer was 31.6% in 1999, which improved to 14.2% in 2012. This can be explained in part by the improvements in diagnostic methods and in the quality of characterization, which may lead to more specific diagnoses. On the other hand, the substantial increase in adenocarcinoma cases cannot be explained by a misclassification of other histologic types to unspecified cancers. Another limitation regarding survival analysis was due to the limited information in stage information. Among the 88,655 lung cancer patients diagnosed between 2008 and 2012, the Surveillance, Epidemiology, and End Results stage information for 9,753 patients (11.0%) was missing [30]. The 5-year relative survival was 53.9% for localized cancer, 29.9% for regional cancer, 5.1% for distant cancer, and 15.8% for unknown stage cancers. Therefore, we cannot rule out the possibility that improved stage information might contribute to improvements in overall survival.

Despite these limitations, the KCCCR provides reasonably high quality data (S2 Table). For instance, for lung cancer, the proportion of cases with a death certificate only was 10.8% in 1999 and improved to 3.3% in 2012. Moreover, the mortality/incidence ratio and the proportion of microscopic verification were in the acceptable ranges.

**Conclusion**

The lung cancer mortality began to decrease in both men and women in 2002. Since 1999, the of incidence lung cancer has decreased among men, whereas it has increased among women. Adenocarcinomas have become the most frequent histological type in both men and women. Finally, there has been an improvement in the 5-year relative survival of lung cancer patients.

**Electronic Supplementary Material**

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

**Conflicts of Interest**

Conflict of interest relevant to this article was not reported.

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**References**


Clinically Significant Unclassified Variants in BRCA1 and BRCA2 Genes among Korean Breast Cancer Patients

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Purpose
Unclassified variants (UVs) of BRCA1 and BRCA2 genes are not defined as pathogenic for breast cancer, and their clinical significance currently remains undefined. Therefore, this study was conducted to identify potentially pathogenic UVs by comparing their prevalence between breast cancer patients and controls.

Materials and Methods
A total of 328 breast cancer patients underwent BRCA1/2 genetic screening at the National Cancer Center of Korea. Genetic variants of BRCA genes that were categorized as unclassified according to the Breast Cancer Information Core database were selected based on allelic frequency, after which candidate variants were genotyped in 421 healthy controls. We also examined family members of the study participants. Finally, the effects of amino acid substitutions on protein structure and function were predicted in silico.

Results
Genetic tests revealed 33 UVs in BRCA1 and 47 in BRCA2. Among 15 candidates genotyped in healthy controls, c.5339T>C in BRCA1 and c.6029T>G, c.7522G>A in BRCA2 were not detected. Moreover, the c.5339T>C variant in the BRCA1 gene was detected in four patients with a family history of breast cancer. This nonsynonymous variant (Leu1780Pro) in the BRCA1 C-terminal domain was predicted to have an effect on BRCA1 protein structure/function.

Conclusion
This study showed that comparison of genotype frequency between cases and controls could help identify UVs of BRCA genes that are potentially pathogenic. Moreover, our findings suggest that c.5339T>C in BRCA1 might be a pathogenic variant for patients and their families.

Key words
Familial breast cancer, BRCA1, BRCA2, Unclassified variants
Introduction

Breast cancer is the second-most common cancer among women in Korea, with an estimated incidence of 65.7 per 100,000 women per year [1]. Moreover, the incidence of breast cancer in Korea has been increasing annually, with relatively younger-aged women increasingly being affected.

Germline mutations of the BRCA1 and BRCA2 genes that encode truncated proteins are associated with a significantly increased risk of cancer in carriers [2-4]. The Korean Hereditary Breast Cancer (KOHBCRA) study reported that 15.7% of patients with breast cancer who were tested for genetic mutation carried pathogenic mutations in BRCA genes. Additionally, breast cancer patients with a family history of breast or ovarian cancers showed a prevalence of BRCA mutations as high as 22.3% [5,6].

Mutation screening for BRCA genes has become a widely applied genetic test for cancer predisposition. Currently, the clinical significance of BRCA1/2 sequence variations can be interpreted according to several databases of genetic mutation, including the Breast Cancer Information Core (BIC; http://research.nhgri.nih.gov/bic/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) [7-9]. However, a large portion of genetic variants of BRCA1 and BRCA2 genes are nontruncating, such as missense or potential splice site changes. Nevertheless, the contribution of these variants to cancer risk currently remains undefined. These unclassified variants (UVs) of BRCA genes have become a critical issue for carriers because of their unknown clinical significance [10,11]. Some UVs that are found in highly conserved domains or splice sites have been predicted to be deleterious by in silico analyses. Moreover, many UVs are classified as neutral polymorphisms, and some are considered potentially deleterious. The effects of variants on biological function are difficult to assign based on functional assays of BRCA genes. To define the clinical significance of UVs, researchers have suggested various approaches and algorithms to determine whether UVs are deleterious or neutral for the biological function of proteins encoded by BRCA genes [12,13]. Many models based on statistical methods that combine clinical features or predicted gene function with informatics tools, such as PolyPhen Phenotyping (PolyPhen, http://genetics.bwh.harvard.edu/pph/) or Sorting Intolerant from Tolerant (SIFT, http://blocks.fhcrc.org/sift/SIFT.html), have been suggested [14,15].

In this study, we investigated the prevalence of UVs in BRCA genes in a Korean population. To address the clinical significance of unclassified BRCA gene variants, we collected BRCA gene sequencing data from 328 breast cancer patients. Additionally, six selected UVs of the BRCA1 gene and nine of the BRCA2 gene were genotyped in 421 controls. We also examined the family history of variant carriers and tested BRCA genes of family members. This is the first report comparing the frequency of BRCA UVs in Korean breast cancer patients and healthy controls.

Materials and Methods

1. Study population

Patients with histologically confirmed breast cancer were enrolled in this study from the genetic counseling clinic and underwent BRCA1/2 mutation testing between April 2008 and June 2015 at the National Cancer Center in Korea. A total of 328 patients who underwent genetic testing for BRCA1 and BRCA2 genes voluntarily participated in this study and agreed to provide the results of genetic testing. As control group, 421 healthy controls were recruited from individuals who visited the National Cancer Center as part of a cancer-screening program. All individuals who participated in this study signed an informed consent form that was approved by the Institutional Review Board of National Cancer Center Korea (IRB No. NCCNCS 13717).

2. Sequencing and genotyping of variants

Genomic DNA was extracted from the peripheral blood of participants using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA) or a Chemagic DNA Blood 200 Kit (Chemagen, Baesweiler, Germany) according to the manufacturers’ instructions. Genetic testing of BRCA1 and BRCA2 genes was conducted by the Green Cross Company (Yongin, Korea) using a direct sequencing method. Briefly, amplified products were sequenced on an ABI 3500xl Analyzer (Applied Biosystems, Foster City, CA) using Bigdyde Terminator v3.1 Cycle Sequencing Kits, and sequences were analyzed using the Sequencer v5.0 software. All genetic variants in BRCA1 and BRCA2 genes were categorized as pathogenic, unclassified, or polymorphic according to the BIC database. All mutations are described according to HUGO-approved systematic nomenclature (http://www.hgvs.org/mutnomen/). GenBank accession numbers NM_007294.3 for BRCA1 and NM_000059.3 for BRCA2 were used as reference sequences.

Large genomic rearrangements of BRCA1/2 genes were also tested using a multiplex ligation-dependent probe amplification (MLPA) assay for patients without pathogenic mutations of the BRCA genes.

Candidate variants were selected for further genotyping in healthy controls based on the frequency of variants of BRCA1/2 genes among cases. Variants were identified by
TaqMan probe genotyping (Applied Biosystems) using a QuantaStudio 7 Flex real-time PCR system. The reproducibility of genotyping results was confirmed by genotyping 10% of the samples in duplicate.

3. In silico analysis of UVs

The effects of amino acid substitutions on protein structure and function were predicted using PolyPhen [16] and SIFT [17]. The tolerance score from SIFT and damaging score from PolyPhen-2 were used to predict the potential effects of UVs on the function of proteins encoded by BRCA genes. The structure of variant proteins was predicted using SWISS-MODEL (http://swissmodel.expasy.org/) [18]. The BRCA1 C-terminal (BRCT) domain structure of BRCA1 (PDB entry: 4U4A) was used as a template for modeling.

Results

1. Patient characteristics

Table 1 shows the demographic features of breast cancer patients and controls. All patients were female and were diagnosed with histologically confirmed breast cancer, with the exception of four patients who were diagnosed with ovarian cancer. Patients in our study population were predominantly stage I, with only 11 patients categorized as stage IV. Additionally, more than 76% of patients had a family history of breast or ovarian cancer, including 11 with a family history of both breast and ovarian cancer.

All patients underwent genetic testing of BRCA1 and BRCA2 genes by the direct sequencing method. As shown in Tables 1 and 2, a total of 47 patients (14.3%) harbored 35 different deleterious mutations (frameshift or nonsense) of BRCA genes. To examine large genomic rearrangement of the BRCA genes, we performed MLPA assays in 196 patients. Only four patients showed deletions of a large genomic region.

2. UVs of BRCA1/2 genes

Sequencing results showed that a total of 181 patients (55.2%) harbored UVs of BRCA genes. Among these, 33 kinds of UVs in the BRCA1 gene were detected in 127 patients, while 47 UVs in the BRCA2 gene were identified from 113 patients. Although these variants were already classified as having uncertain clinical importance, such a high frequency of variants among the population could weaken the significance of the association with cancer. Therefore, we excluded

| Table 1. Demographic characteristics of breast cancer patients tested for BRCA1/2 genes |
|-----------------------------------------------|-----------------|
| Characteristic                               | No. (%)         |
| Female                                       | 328             |
| Current age, median (range, yr)              | 44 (25-76)      |
| Age at cancer diagnosis, median (range, yr)  | 43 (25-73)      |
| Classification of cancer type                |                 |
| Invasive ductal carcinoma                    | 236 (72.0)      |
| Ductal carcinoma in situ                     | 36 (11.0)       |
| Invasive lobular carcinoma                   | 18 (5.5)        |
| Lobular carcinoma in situ                    | 4 (1.2)         |
| Others                                       | 34 (10.4)       |
| Stage of breast cancer                       |                 |
| Stage 0                                      | 40 (12.2)       |
| Stage I                                      | 124 (37.8)      |
| Stage II                                     | 102 (31.1)      |
| Stage III                                    | 47 (14.3)       |
| Stage IV                                     | 11 (3.4)        |
| Unknown                                      | 4 (1.2)         |
| Family history                               |                 |
| Breast cancer                                | 216 (65.9)      |
| Ovarian cancer                               | 25 (7.6)        |
| Breast and ovarian cancer                    | 11 (3.4)        |
| Without family history                       | 76 (23.2)       |
| Pathogenic mutation carrier                  |                 |
| BRCA1 pathogenic variant                     | 20 (6.1)        |
| BRCA2 pathogenic variant                     | 27 (8.2)        |
| Large genomic rearrangement                  |                 |
| MLPA tested patients                         | 196 (59.7)      |
| BRCA1 rearrangement carrier                  | 3 (0.9)         |
| BRCA2 rearrangement carrier                  | 0               |
| Unclassified variants                        |                 |
| Patients with BRCA1 unclassified variant     | 127 (38.7)      |
| Patients with BRCA2 unclassified variant     | 113 (34.5)      |

Control (n=421): current age, 45 (27-71) years. MLPA, multiplex ligation-dependent probe amplification assay.
nine in BRCA2 were further genotyped in 421 age-matched female controls. Among these, c.5339T>C in BRCA1 and c.6029T>G, c.7522G>A in BRCA2 were not detected in healthy controls (Table 3). The c.5339T>C were detected in four patients and c.6029T>G, c.7522G>A were detected in three patients with breast cancer. All three variants caused a substitution of amino acid sequence and c.5339T>C (Leu1780Pro) in BRCA1, and c.7522G>A (Gly2508Ser) in BRCA2 were predicted as damaging variants. In contrast, c.4883T>C and c.2566T>C in BRCA1 and c.2350A>G and c.8187G>T in BRCA2 showed a genotype frequency greater than 2% in the control group.

3. Potential risk of c.5339T>C variant in the BRCA1 gene

We examined the potential risk of three UVs that were not detected in 421 healthy controls. Fortunately, we were able to recruit family members of the proband harboring the c.5339T>C variant in the BRCA1 gene. As shown in the pedigree in Fig. 1A, two breast cancer patients in this family and the proband were also diagnosed with ovarian cancer 2 years after being diagnosed with breast cancer. The father of the proband also carried the same UV, and his sister died of breast cancer at the age of 46. Another patient who harbored the same variant was diagnosed with breast cancer at the age of 33, as shown in Fig. 1B. Her mother suffered from ovarian cancer and could not participate in this study. The c.5339T>C variant results in an amino acid change from leucine to proline at position 1780. The predicted structure shows that the mutation site is in the middle of a helix in the BRCT domain of BRCA1, forming a hydrophobic patch with its surrounding residues (Fig. 1C). The BRCT domain is known to recognize and bind phosphorylated pSXXF motifs of FAM175A/ Abraxas to recruit BRCA1 to regions of DNA damage [19-21].

Discussion

Interpreting UVs in BRCA1 and BRCA2 genes has become a particularly important issue for genetic counseling of cancer patients because of the clinical importance of germline mutations in BRCA genes. Here, we sought to define potentially pathogenic variants by comparing the prevalence of BRCA UVs in 421 healthy controls and 328 breast cancer patients in a Korean population by genotyping. Among the 80 UVs that were found in our patients, 15 were identified in controls while three were detected only in patients with breast cancer, not in controls. Some of these latter variants were predicted to be “probably damaging” based on a high score in PolyPhen-2, and were classified as “intolerant” variants by the SIFT tool. Additionally, the non synonymous variant c.5339T>C, which causes an amino acid substitution of proline for leucine (Leu1780Pro) in the BRCT domain, was detected in the BRCA1 gene of four patients with breast cancer. The BRCT domain in the C-terminal, which is known to be essential for BRCA1 to function as a tumor suppressor [19], contributes to binding to target proteins with specificity for phosphorylated pSer-X-X-Pho motifs [20,21]. The substitution of proline for leucine may weaken the hydrophobic patch structure of the BRCT domain, potentially influencing the protein-protein interactions needed for the proper function of BRCA1.

The average age at diagnosis of four patients harboring the c.5339T>C variant was 34, and the youngest patient was diagnosed at the age of 25. One breast cancer patient harboring the same variant was subsequently diagnosed with ovarian cancer following breast cancer, and the mother of one patient suffered from ovarian cancer. Based on these findings, it is plausible to suggest c.5339T>C as a potentially pathogenic variant.

Interestingly, one candidate UV in the BRCA2 gene, c.7522G>A, has been reported as a risk factor for breast cancer in a case-control study. This variant is a nonsynonymous single-nucleotide polymorphism known as rs80359878 that causes an amino acid substitution (Gly2508Ser) in BRCA2. Zhang et al. [22] showed that this missense variant was associated with a 16.5-fold increase in the risk of breast cancer among Chinese women, with an allele frequency of this variant of 0.0023 in cases and 0.0001 in controls.

To define rare variants with potential pathogenicity, we compared the frequency of UVs of BRCA genes among healthy controls with that in breast cancer patients. Our results suggest the potentially deleterious variants, c.5339T>C (Leu1780Pro) in BRCA1 and c.6029T>G (Val2010Gly), c.7522G>A (Gly2508Ser) in BRCA2, which were detected only in cases. This strategy could be strengthened using a large number of cases-controls to select signifi-
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<td>c.6325G&gt;A</td>
<td>p.Val2109Ile</td>
<td>rs79456940</td>
<td>3</td>
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<td>32,354,905</td>
<td>13</td>
<td>7280C&gt;G</td>
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<td>rs80358932</td>
<td>3</td>
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<td>0.007</td>
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<td>32,356,514</td>
<td>15</td>
<td>7750G&gt;A</td>
<td>c.7522G&gt;A</td>
<td>p.Gly2508Ser</td>
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<td>0</td>
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<tr>
<td></td>
<td>32,363,389</td>
<td>18</td>
<td>8415G&gt;T</td>
<td>c.8187G&gt;T</td>
<td>p.Lys2729Asn</td>
<td>rs80359065</td>
<td>10</td>
<td>0.003</td>
<td>0.012</td>
<td>10</td>
<td>0.024</td>
</tr>
</tbody>
</table>

BIC, Breast Cancer Information Core; 1000G ALL, minor allele frequency from all population in the 1000 Genomes Phase 3 database; 1000G EAS, minor allele frequency from East-Asian population in the 1000 Genomes Phase 3 database.
Fig. 1. Unclassified variant c.5339T>C in BRCA1. The candidate UV, c.5339T>C, was tested in breast cancer patients and family members (A, B). Red in each pedigree indicates a carrier of the variant genotype, while green indicates family members without the variant. The proband of each family is indicated by a black arrow. (Continued to the next page)

cant variants among previously UVs. Biological experiments should be performed to validate the effects of the variants. pathogenicity by affecting the function of the BRCT domain of BRCA1. The information provided herein will be useful for individuals carrying these variants, who should be carefully monitored for potential cancer risk.

Conclusion

In conclusion, the c.5339T>C variant in BRCA1 that was detected in four patients may be involved in breast cancer
Fig. 1. (Continued from the previous page) (C) Predicted structure of BRCA1 variant (Leu1780Pro) in the BRCA1 C-terminal (BRCT) domain. Left, overall structure of the BRCT domain of BRCA1; right, detailed view of the region surrounding the variant. Hydrophobic residues around Leu1780 are shown and labeled in red.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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Nomograms Predicting Platinum Sensitivity, Progression-Free Survival, and Overall Survival Using Pretreatment Complete Blood Cell Counts in Epithelial Ovarian Cancer

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Purpose
This study was conducted to evaluate the prognostic significance of pretreatment complete blood cell count (CBC), including white blood cell (WBC) differential, in epithelial ovarian cancer (EOC) patients with primary debulking surgery (PDS) and to develop nomograms for platinum sensitivity, progression-free survival (PFS), and overall survival (OS).

Materials and Methods
We retrospectively reviewed the records of 757 patients with EOC whose primary treatment consisted of surgical debulking and chemotherapy at Samsung Medical Center from 2002 to 2012. We subsequently created nomograms for platinum sensitivity, 3-year PFS, and 5-year OS as prediction models for prognostic variables including age, stage, grade, cancer antigen 125 level, residual disease after PDS, and pre-treatment WBC differential counts. The models were then validated by 10-fold cross-validation (CV).

Results
In addition to stage and residual disease after PDS, which are known predictors, lymphocyte and monocyte count were found to be significant prognostic factors for platinum-sensitivity, platelet count for PFS, and neutrophil count for OS on multivariate analysis. The area under the curves of platinum sensitivity, 3-year PFS, and 5-year OS calculated by the 10-fold CV procedure were 0.7405, 0.8159, and 0.815, respectively.

Conclusion
Prognostic factors including pretreatment CBC were used to develop nomograms for platinum sensitivity, 3-year PFS, and 5-year OS of patients with EOC. These nomograms can be used to better estimate individual outcomes.

Key words
Nomograms, Prognosis, Ovarian neoplasms
Introduction

Epithelial ovarian cancer (EOC) is one of the leading causes of death in females with gynecological malignancies [1]. Most patients respond to primary treatment, and 75% of patients reach complete response. However, 40%-60% of all patients with EOC and 75% of those with advanced stage disease will eventually experience recurrence [2,3]. Accurate estimation of survival for patients with EOC is important because prognosis is a determinate of treatment aggressiveness tailored to the individual situation. Patients who experience recurrence after 6 months from the end of primary chemotherapy are classified as platinum sensitive, and currently, platinum sensitivity is considered an important factor for predicting survival outcomes [4]. Predicting platinum sensitivity in patients with EOC may play an important role in establishing treatment plans.

Previous studies demonstrated several biological markers as significant prognostic factors for oncological outcomes after treatment. Laboratory systemic inflammatory response markers have been studied as prognostic factors in a variety of cancers [5-7]. Paraneoplastic lymphocytopenia, leukocytosis, and thrombocytosis are significant prognostic factors in many solid tumors. However, the level of contribution of each biological marker to oncological outcomes such as platinum sensitivity and survival in EOC is not fully understood.

This study was conducted to evaluate the clinical impact of pre-treatment complete blood cell count (CBC) including white blood cell (WBC) differential components as prognostic factors for platinum-sensitivity on EOC patients with primary debulking surgery (PDS) and to develop nomograms for platinum sensitivity, 3-year progression-free survival (PFS), and 5-year overall survival (OS) with prognostic CBC components and known prognostic clinical parameters.

Materials and Methods

1. Patients

After obtaining Institutional Review Board approval (IRB file No. 2015-06-092), data were collected from Samsung Medical Center for patients with EOC who were treated from January 2002 to December 2012. We identified 757 patients whose primary treatment consisted of PDS and adjuvant chemotherapy. Patients who underwent neo-adjuvant chemotherapy and interval debulking surgery, those who had a transfusion within 2 weeks prior to PDS, and patients with concurrent cancer other than ovarian cancer were excluded from the study. Patients who received intraperitoneal or dose-dense chemotherapy were not included in this study.

2. Treatment and follow-up

Standard primary surgical treatment consisted of hysterectomy, bilateral salpingo-ophorectomy, omentectomy, retroperitoneal (pelvic and para-aortic) lymphadenectomy, and any tumorectomy of metastatic lesions, if applicable. Peritoneal washing was routinely conducted. If any abnormalities were identified, peritoneal biopsies from different sites were performed. Early stage EOC patients who wanted fertility saving received fertility saving surgery with/without chemotherapy as primary treatment.

Within 2 weeks prior to PDS, patients were routinely required to undergo basic preoperative evaluation including complete blood count. After PDS, patients started the first cycle of platinum-based combination chemotherapy, which was repeated every 3 weeks for six cycles. Abdominopelvic computed tomography (CT) scan was routinely performed after first three cycles of chemotherapy and after six cycles of first-line treatment. Following primary treatment, patients were assessed by physical examination, CBC, and chemistry with serum tumor markers, including cancer antigen 125 (CA-125) measurements, every 3 months for the first 2 years and twice per year thereafter. Chest radiography and abdominopelvic CT scan (or alternatively abdominopelvic magnetic resonance imaging) were performed every 6 months for the first 3 years and every 12 months thereafter. Additional diagnostic procedures were performed according to specific clinical suspicions. If recurrence was suspected with symptoms or CA-125 elevation, additional imaging studies were performed. Recurrence may have been detected by imaging studies with or without CA-125 elevation. Response to chemotherapy was assessed and recorded according to the Response Evaluation Criteria in Solid Tumors (RECIST).

OS was defined as the time from diagnosis to the date of the patient’s death or loss to follow-up. PFS was defined as the time from diagnosis to the date of recurrence or loss to follow-up. Disease-free interval (DFI) was defined as the time from the end of primary treatment to the date of recurrence.

3. Statistical analysis

A multivariate logistic regression model with stepwise variable selection using Akaike’s information criterion (AIC) was employed to identify factors predictive of platinum sensitivity. A multivariate Cox regression model with stepwise variable selection using AIC was used to identify prognostic factors for OS and PFS. The proportional hazards assumption was assessed using the method proposed by Grambsch and
Therneau [8] and the linearity assumption was checked using a penalized smoothing spline method. Two assumptions for neutrophil, lymphocyte, monocyte, platelet, and age were satisfied (S1 and S2 Tables). Prognostic factors identified by multivariate analysis were used to create a nomogram to predict platinum sensitivity, 3-year PFS, and 5-year OS. We validated each nomogram using 10-fold cross-validation (CV) [9]. We then constructed a receiver operating characteristic (ROC) curve for platinum-sensitivity and calculated the area under the curve (AUC). The optimal cut-off point for predicted probability was determined by maximizing the Youden index [10]. Time-dependent ROC curves were constructed using the Nearest Neighbor Estimation method for 3-year PFS and 5-year OS [11] and AUCs were calculated. Statistical analyses were performed using R 3.0.3 (Vienna, Austria; http://www.R-project.org).

Prognostic variables including age, histology, stage, tumor grade, residual disease after PDS, and preoperative CBC (hemoglobin, WBC differential [neutrophil count, lymphocyte count, monocyte count], and platelet count) were used in the analysis. Platinum sensitivity was included in analysis of the 5-year OS. Patients with non-serous carcinoma were combined and compared to those with serous carcinoma. Residual disease after PDS was categorized based on size (no residual, ≤ 1 cm, and > 1 cm). Platinum sensitivity was classified as platinum-resistant or platinum-sensitive (DFI ≤ 6 months, DFI > 6 months), or unknown for platinum sensitivity. Patients with insufficient observation time to determine platinum sensitivity, as well as those who did not receive platinum-based chemotherapy as primary treatment were classified as unknown for platinum sensitivity.

Table 1. Patient demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) (n=757)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range, yr)</td>
<td>52 (15-84)</td>
</tr>
<tr>
<td>CA-125, median (range, U/mL)</td>
<td>522.75 (1.0-100,080.0)</td>
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<tr>
<td>Stage (%)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>162 (21.4)</td>
</tr>
<tr>
<td>II</td>
<td>78 (10.3)</td>
</tr>
<tr>
<td>III</td>
<td>428 (56.5)</td>
</tr>
<tr>
<td>IV</td>
<td>89 (11.8)</td>
</tr>
<tr>
<td>Grade (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>61 (8.1)</td>
</tr>
<tr>
<td>2</td>
<td>159 (21.0)</td>
</tr>
<tr>
<td>3</td>
<td>537 (70.9)</td>
</tr>
<tr>
<td>Histology (%)</td>
<td></td>
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<tr>
<td>Serous</td>
<td>483 (63.8)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>80 (10.6)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>62 (8.2)</td>
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<tr>
<td>Clear cell</td>
<td>59 (7.8)</td>
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<tr>
<td>Transitional</td>
<td>28 (3.7)</td>
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<tr>
<td>Mixed</td>
<td>31 (4.1)</td>
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<tr>
<td>Others</td>
<td>14 (1.8)</td>
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<tr>
<td>Residual disease after PDS (%)</td>
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<tr>
<td>No residual</td>
<td>277 (36.6)</td>
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<tr>
<td>≤ 1 cm</td>
<td>284 (37.5)</td>
</tr>
<tr>
<td>&gt; 1 cm</td>
<td>196 (25.9)</td>
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<tr>
<td>Platinum-sensitivity (%)</td>
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<tr>
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<td>110 (14.5)</td>
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<tr>
<td>Platinum sensitive</td>
<td>616 (81.4)</td>
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<tr>
<td>Unknown</td>
<td>31 (4.1)</td>
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<td>Pretreatment complete blood count,</td>
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<tr>
<td>median (range)</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.2 (7.6-15.6)</td>
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<tr>
<td>Platelet count (×10^9/μL)</td>
<td>304 (63-764)</td>
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<tr>
<td>Neutrophil count (×10^9/μL)</td>
<td>4.355 (0.730-27.250)</td>
</tr>
<tr>
<td>Lymphocyte count (×10^9/μL)</td>
<td>1.584 (0.401-3.883)</td>
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<tr>
<td>Monocyte count (×10^9/μL)</td>
<td>0.427 (0.089-1.756)</td>
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CA-125, cancer antigen 125; PDS, primary debulking surgery.

Table 1. Patient demographic and clinical characteristics

Results

Data from the records of a total of 757 patients with EOC who were treated at Samsung Medical Center were analyzed. Patient demographic and clinical characteristics are listed in Table 1. There were 430 cases of cancer recurrence and 276 cases of cancer-specific death with a median follow-up of 51 months (range, 4 to 156 months). The majority of patients had stage III (56.5%), grade 3 disease (70.9%) of serous type (63.8%). There was no residual disease in 277 patients (36.6%) in the whole cohort, nor in 22.2% (115/517) of those in advanced stage. Of all patients, 647 patients (85.5%) were platinum-sensitive.

Multivariate logistic regression analysis for platinum sensitivity and multivariate Cox modeling for PFS and OS were used to evaluate independent prognostic factors and estimate their effects for all patients. Stage and residual disease after PDS were significant predictors of OS, PFS, and platinum sensitivity upon multivariate analysis. In addition, some of the preoperative CBC components were found to be significant prognostic factors.

Analyses for platinum sensitivity are shown in S3 Table. Among the significant variables identified upon univariate analysis, preoperative lymphocyte count and monocyte count, stage, histology, and residual disease after PDS were significant predictors of platinum sensitivity. Platelet count, stage, grade, and residual disease after PDS appeared to be significant predictors of 3-year PFS upon multivariate analy-
sis (S4 Table). Pretreatment neutrophil count, stage, residual disease after PDS, and platinum sensitivity were significant prognostic factors for 5-year OS (S5 Table).

Nomograms were developed to account for the importance of each clinical prognostic variable. Nomograms were created using the multivariate logistic regression model for platinum sensitivity and multivariate Cox regression models for 3-year PFS and 5-year OS (Figs. 1-3). Use of the nomograms is described in the figure captions. For example, in the nomogram for platinum sensitivity, the user should find the patient’s stage on the Stage axis, then draw a straight line upward to the Points axis to determine how many points toward progression the patient receives for stage. This should be done again for the other axes, with the user drawing a straight line upward toward the Points axis each time. The points received for each predictor are then summed and the sum is found on the total points axis. The user should then draw a straight line down to the platinum sensitivity probability axis to find the patient’s probability of platinum sensitivity.

The AUCs of platinum sensitivity, 3-year PFS, and 5-year OS calculated using the 10-fold CV procedures were 0.7405,
0.8159, and 0.815, respectively. The optimal cut-off value of predicted probability for platinum sensitivity was 0.8594, and the sensitivity and specificity were 0.6318 and 0.8073, respectively. Calibration curves for the platinum sensitivity, 3-year PFS and 5-year OS nomograms are shown in Fig. 4. The nomograms for 3-year PFS and 5-year OS were well calibrated.

Discussion

In this study, we evaluated pre-treatment CBC as a prognostic factor for EOC treated with PDS and adjuvant chemotherapy and its impact on prognosis. We found that platelet count, neutrophil count, monocyte count, and lymphocyte count were significantly associated with platinum sensitivity, PFS, and OS. Other common prognostic factors identified in our study included stage and residual disease after PDS. Therefore, we sought to develop nomograms that would include these prognostic factors for platinum sensitivity, PFS, and OS. The nomograms were validated for platinum sensitivity, 3-year PFS, and 5-year OS.

There are number of risk factors associated with survival in EOC. Most of the previous risk analyses only provide information regarding individual risk factors based on univariate and multivariate analyses. Traditional risk stratification strategies assign all risk factors the same weight, which may cause bias. In contrast, a nomogram provides parametric information for end-point prediction by integrating multiple weighted risk factors. After summing all of the points for each factor, results can be translated into information regarding survival. This information can facilitate discussion between the physician and the patient and guide clinical care. Nomograms have been constructed to predict various clinical end points for patients with different types of cancer [12-14]. A nomogram should theoretically be more specific to each individual patient and thus able to predict specific clinical endpoints more accurately.

Paraneoplastic lymphocytopenia, leukocytosis, and thrombocytosis are well-known prognostic factors for many solid tumors. Various studies explaining the prognostic significance of inflammatory markers from peripheral blood including components from pretreatment CBCs have been reported [15-17]. Cancer cells secrete cytokines such as interleukin 6, which directly and indirectly stimulate platelet production in tumor cells, thereby enhancing proliferation and metastasis [17]. Tumors are known to induce neutrophilic differentiation and stimulate angiogenesis and cell proliferation by producing chemokines, cytokines, and prostaglandins [18]. Previous reports demonstrated that a higher neutrophil count and lower lymphocyte count predict poorer survival in EOC [7,16]. Lymphocytes showed greater decreases in patients with higher stage, ascites, and residual cancer after PDS in EOC [19]. Moreover, significantly lower lymphocyte counts and higher neutrophil counts were associated with greater tumor grade, advanced stage, and presence of ascites [20]. These associations between WBC differential counts and tumor aggressiveness may partially explain the correlation with survival outcomes. A recent study demonstrated that elevated peripheral blood mono-
Fig. 4. Calibration plot for platinum sensitivity (A), progression-free survival (PFS) nomogram model (B), and overall survival (C) nomogram model.

cyte count was associated with worse OS, and that monocytes are associated with increased adrenergic signaling via monocyte chemotactic protein 1, which facilitates tumor progression in EOC [21,22]. Despite evidence that factors from WBC differential counts are predictors of prognosis for different cancers including EOC, larger studies with greater detail on patient profiles, tumor features, and treatment will be necessary to demonstrate that these factors are truly independent predictors.

To the best of our knowledge, this is the first study presenting prognostic nomograms for the endpoint of platinum sensitivity with pre-treatment CBC including WBC differential counts. Previous reports of survival models in EOC have been based on variables such as stage, residual disease, grade, histology, age, and performance status [23-25]. Furthermore, a number of studies demonstrated laboratory markers from routine testing, such as CBC, as prognostic variables [13,26-28]. Our study demonstrated that, in addi-
tion to the previously published prognostic variables for EOC, WBC differential counts (lymphocyte, monocyte, and neutrophil counts) of pre-treatment CBC may be considered prognostic factors for platinum sensitivity and survival in EOC. We expected minimal bias associated with using pre-treatment CBC since it is commonly performed in a majority of institutions and gives objective values. Based on patient clinicopathologic information and components of pretreatment CBC, the nomogram could be used to estimate platinum sensitivity, 3-year PFS, and 5-year OS rates. With nomograms applied to clinical management, clinicians will be able to apply different strategies for subsequent systemic anti-tumor therapy and follow-up interval based on the platinum sensitivity in EOC patients with PDS.

It should be noted that our study had several limitations. Specifically, its retrospective, single-center nature may have resulted in unmeasured confounding factors. When analyzing variables, not all known prognostic factors identified in other prognostic models, such as the volume of ascites and performance status [13,23,25,29], were included because of a substantial lack of data in the medical records. We also did not have sufficient data describing specific circumstances for each patient included in the unknown platinum sensitivity category to explain the reason for the lower hazard ratio. Moreover, the fact that systemic conditions other than cancer (e.g., inflammatory disease or infection status) may have affected the pretreatment CBC status should not be overlooked. Finally, preoperative comorbidities were not assessed in this analysis.

Although previous studies have shown that factors from WBC differential counts are predictors of prognosis for EOC, the mechanism of results cannot be clearly explained. In addition, our finding that grade reversely affected the survival outcome is difficult to explain. In multivariate analysis for 3-year PFS, grade 3 showed a lower hazard ratio than grade 2, contrary to univariate analysis. In a previous study, no difference was found in clinical outcomes between grade 2 and 3 of serous ovarian cancer, and in serous ovarian cancer, a 2-tier grade system (high grade vs. low grade) was reportedly more accurate for predicting clinical outcomes than a International Federation of Gynecology and Obstetrics (FIGO) 3-tier (grade 1, 2, and 3) system [30]. In our study, we used a FIGO 3-tier system because we included not only the results of serous type, but also other histologic types. It is possible that the lack of a survival difference between grade 2 and 3 serous types might have affected the results of the hazard ratio for grade upon multivariate analysis.

In our study, information regarding dose, schedule, and toxicities of the adjuvant chemotherapy or surgical complications, which may be associated with survival, is lacking because of the deficit of associated information owing to this study’s retrospective nature. A larger number of patient pro-files and detailed information will be needed for definite results in the future. Although internally validated, the nomogram needs to be externally validated before it can be generally accepted for clinical application.

Conclusion

In summary, our multivariate analysis identified WBC differential counts on pre-treatment CBC (neutrophil, monocyte, and lymphocyte counts) as prognostic factors in addition to stage and residual disease after PDS in patients with EOC that was primarily treated with PDS and adjuvant chemotherapy. These prognostic factors allowed development of nomograms predicting platinum sensitivity, 3-year PFS, and 5-year OS. Such nomograms could be used to better estimate outcomes for individual patients.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References

Antitumor Effect of KX-01 through Inhibiting Src Family Kinases and Mitosis

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Purpose
KX-01 is a novel dual inhibitor of Src and tubulin. Unlike previous Src inhibitors that failed to show clinical benefit during treatment of breast cancer, KX-01 can potentially overcome the therapeutic limitations of current Src inhibitors through inhibition of both Src and tubulin. The present study further evaluates the activity and mechanism of KX-01 in vitro and in vivo.

Materials and Methods
The antitumor effect of KX-01 in triple negative breast cancer (TNBC) cell lines was determined by MTT assay. Wound healing and immunofluorescence assays were performed to evaluate the action mechanisms of KX-01. Changes in the cell cycle and molecular changes induced by KX-01 were also evaluated. A MDA-MB-231 mouse xenograft model was used to demonstrate the in vivo effects.

Results
KX-01 effectively inhibited the growth of breast cancer cell lines. The expression of phospho-Src and proliferative-signaling molecules were down-regulated in KX-01–sensitive TNBC cell lines. In addition, migration inhibition was observed by wound healing assay. KX-01–induced G2/M cell cycle arrest and increased the aneuploid cell population in KX-01–sensitive cell lines. Multi-nucleated cells were significantly increased after KX-01 treatment. Furthermore, KX-01 effectively delayed tumor growth in a MDA-MB-231 mouse xenograft model.

Conclusion
KX-01 effectively inhibited cell growth and migration of TNBC cells. Moreover, this study demonstrated that KX-01 showed antitumor effects through the inhibition of Src signaling and the induction of mitotic catastrophe. The antitumor effects of KX-01 were also demonstrated in vivo using a mouse xenograft model.

Key words
Src kinase inhibitor, Mitotic catastrophe, Microtubules, KX-01, Triple negative breast neoplasms
Introduction

Breast cancer is classified into several subtypes based on the expression patterns of estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor type 2 (HER2) [1]. When all three receptors are absent, they are classified as triple negative breast cancer (TNBC). This type of cancer is normally associated with a very poor prognosis because, unlike other subtypes, no known therapeutic agents can specifically target TNBC [1]. Patients with this disease have been treated with cytotoxic chemotherapy alone; however, chemo-resistance develops easily, resulting in treatment failure. Therefore, new therapeutic strategies for TNBC are urgently needed.

One of the reasonable targets of treatment is the Src family kinases (SFKs). SFKs are non-receptor tyrosine kinases that are activated in various solid tumors, including breast cancer [2-5]. Activation of Src signaling can induce cell proliferation, invasion, and metastasis, which are hallmarks of aggressive tumors; therefore, Src has been an attractive therapeutic target, especially for TNBC [4]. A previous study also confirmed that cytoplasmic Src expression was much higher in TNBC patient samples than non-TNBC patient samples [2]. Although several Src inhibitors have been developed, none have produced remarkable responses as mono-therapeutic agents [6-8]. Therefore, a new Src inhibitor is needed for TNBC treatment.

Microtubule dynamics are precisely regulated for accurate cell division during mitosis. Several drugs target microtubule dynamics, and their application can lead to mitotic catastrophe in cancer cells. Mitotic catastrophe is a type of cell death induced by either aberrant mitosis or the accumulation of damaged chromosomes [9-13]. Tumor cell populations are usually composed of high numbers of tetraploid cells that are more prone to mitotic aberrations and more sensitive to mitotic catastrophe-inducing agent [13]. Moreover, mitotic catastrophe can be exploited to eliminate apoptosis-resistant cancer cells [11]. Therefore, the induction of mitotic catastrophe may be an alternative method of overcoming chemo-resistance caused by apoptosis-resistant cells.

KX-01 is a novel, non-ATP-competitive Src inhibitor that also inhibits tubulin polymerization [14-18]. KX-01 has shown activity against various types of cancers, including TNBC, ER-positive breast cancer, and mucinous ovarian cancer, both in vitro and in vivo [14,15,18]. However, previous studies focused more on verifying the Src signaling inhibitory effects of KX-01 and only showed decreasing phosphorylated Src (p-Src) level in vivo. Moreover, the microtubule polymerization inhibitory effects of KX-01 have not been studied in depth. The present study evaluated the activity of KX-01 against a broad range of TNBC cell lines and investigated the mechanism of action in greater depth. We focused on verifying that KX-01 actually inhibits its primary target, Src, and confirmed it down-regulates the expression of p-Src in vitro. Moreover, we showed that the tubulin inhibitory effect of KX-01 causes induction of mitotic catastrophe. We also evaluated the overall effect of KX-01 in an established mouse model. Our results provide further insight into the mechanistic activity of KX-01, which may guide the development of better therapeutic strategies based on KX-01 for the treatment of breast cancer.

Materials and Methods

1. Antibodies and reagents

KX-01 was kindly provided by Kinex Pharmaceutical (Buffalo, NY). The compound was initially dissolved in dimethyl sulfoxide (DMSO) and stored at ~80°C. Paclitaxel was obtained from Samyang Co., Ltd. (Seoul, Korea). Antibodies against phosphorylated (p)-Src (Y416), FAK, p-p130cas, p-ERK (T202/Y204), ERK (p44/p42), p-AKT (S473), AKT, p-STAT3 (Y705), and STAT3 were purchased from Cell Signaling Technology (Danvers, MA), p-FAK (Y397) and p130cas were obtained from BD Biosciences (San Jose, CA), actin was acquired from Sigma Aldrich (St. Louis, MO), and Src and p-FAK (Y861) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

2. Cell lines and cell culture

Human breast cancer cell lines (BT-474, BT-549, HCC1937, HS578T, MCF7, MDA-MB-231, MDA-MB-468, SK-BR-3, and T47D) verified using short tandem repeat analysis were purchased from the American Type Culture Collection (ATCC; Manassas, VA). The cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, CA) and 10 μg/mL gentamicin (Cellgro, Manassas, VA) at 37°C under 5% CO2.

3. Cell growth inhibitory assay

Cells were seeded in 96-well plates and incubated overnight at 37°C under 5% CO2. The cells were exposed to increasing concentrations of KX-01 or paclitaxel for 3 days. After drug treatment, 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution (Sigma Aldrich) was added to each well and the plates were incubated for 4 hours at 37°C. After dissolving the formazan crystals with
150 μL of DMSO, the absorbance of each well was measured at 540 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA). The absorbance and IC_{50} of KX-01 were analyzed using the SigmaPlot software (SPSS Inc., Chicago, IL). Six replicates were included in each dose and at least three independent experiments were conducted.

4. Western blot analysis

The Western blot analytic method was previously reported [19]. Proteins were extracted, after which equal amount were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking with buffer, the blots were incubated with primary antibodies, followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies. Antibody binding was detected using an enhanced chemiluminescence system according to the manufacturer’s protocol (Amersham Biosciences, Piscataway, NJ).

5. Wound healing assay

Cells were seeded in 6-well plates and incubated overnight at 37°C under 5% CO₂. The cell monolayer was then scratched with a pipette tip and the cells were incubated with medium alone or containing 20 nmol/L KX-01. After 48 hours, the plates were examined by light microscopy (Olympus, Tokyo, Japan) to monitor restoration of the cell monolayer. The percentage of the filled gap was analyzed using the SigmaPlot software.

6. Cell cycle analysis

KX-01 treated cells were harvested with trypsin, fixed with cold 70% ethanol, and stored at -20°C for at least 24 hours. The cells were then washed in phosphate buffered saline (PBS) and incubated with 10 μg/mL RNase A (Sigma Aldrich) at 37°C for 20 minutes. Next, the cells were stained with 20 μg/mL propidium iodide (Sigma Aldrich), after which the DNA contents were quantified using a FACS Calibur flow cytometer (BD Biosciences).

7. Immunofluorescence assay

This experiment was conducted following a previously reported method [20]. Briefly, cells were plated on coverslips and incubated with KX-01 for 24 hours or 48 hours. The primary antibody ratio was 1:50 to 1:200. Immunofluorescence was visualized using a Zeiss LSM 510 laser scanning microscope (Zeiss, Jena, Germany).

8. In vivo studies

All animal experiments were carried out at the animal facility of Seoul National University (Seoul, Korea) in accordance with institutional guidelines. To measure the in vivo activity of KX-01, 5-week-old female BALB/c athymic nude mice were purchased from Central Lab Animal, Inc. (Seoul, Korea). The mice were allowed to acclimatize for 1 week before receiving a subcutaneous injection of MDA-MB-231 cancer cells (5.0×10⁶) in 200 μL of PBS. When tumors reached a volume of 150 mm³, the mice were randomly divided into two groups, a control group that received vehicle (10% 2-hydroxy-β-cyclodextrine [Sigma Aldrich] diluted in PBS solution), and a treatment group that received 5 mg/kg KX-01 in vehicle solution twice daily for 4 weeks. The vehicle solution and KX-01 were administered orally. The tumor was measured every other day using calipers and the volume was calculated with the following formula: [(width)²×(height)] / 2. At the end of the measurement period, the mice were euthanized with CO₂. The tumors were then excised and fixed in neutral-buffered formalin for routine histological examination and immunohistochemical staining. Total proteins were extracted from fresh tissue samples to assess the protein expression and Src activity.

9. Immunohistochemistry

Sections from individual paraffin-embedded xenograft tumor tissues were deparaffinized and rehydrated. Immunohistochemical detection of proliferating cells was determined using an anti–Ki-67 antibody (GeneTex, Irvine, CA). A terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assay was performed to detect apoptosis using an ApopTag In Situ Apoptosis Detection Kit (Chemicon International, Temecula, CA) according to the manufacturer’s protocol.

10. Statistical analysis

Statistical analyses were conducted using SigmaPlot ver. 9.0. A two-sided Student’s t test was performed when appropriate. Results are expressed as the mean±standard deviations or standard errors. A p-value of < 0.1 was considered statistically significant. All experiments were conducted in duplicate or triplicate and repeated at least twice.
Results

1. KX-01 effectively inhibits the growth of breast cancer cells and regulates SFK phosphorylation

To verify the growth inhibitory effects of KX-01 on breast cancer cells, nine breast cancer cell lines were treated with KX-01 in vitro, and their inhibitory effects were evaluated using an MTT assay (data not shown). The luminal ER+ cell lines MCF7 and T47D, the HER2+ cell line SK-BR-3, and the TNBC cell lines MDA-MB-231, MDA-MB-468, and BT-549 were sensitive to KX-01 with IC_{50} values lower than 0.1 μmol/L (Table 1), whereas TNBC cell lines Hs578T and HCC1937 were resistant to KX-01. Four TNBC cell lines (MDA-MB-231, MDA-MB-468, BT-549, and Hs578T) were selected for further in vitro studies.

To determine if KX-01 directly inhibits the activity of Src and FAK, Western blotting was performed to measure the levels of total and phosphorylated proteins after treatment. p-Src in BT-549 significantly decreased following exposure to KX-01 (Fig. 1A). Moreover, FAK and p130cas phosphorylation, which are known to be regulated by Src, also decreased. Other sensitive cell lines, MDA-MB-231 and MDA-MB-468, showed similar responses (Fig. 1A, S1 Fig.). Therefore, these results indicate that KX-01 indeed inhibits Src activity and its downstream proteins in vitro.

Src signaling regulates invasion and metastasis [3]; therefore, we investigated whether the inhibition of Src signaling would lead to inhibited cell migration and invasion. Consistent with the Western blot results and a previous study using MDA-MB-231 cells [14], the wound healing assay showed that cell migration was inhibited by treatment in KX-01-sensitive BT-549 cells (Fig. 1B). In comparison, Hs578T cells, which were less sensitive to KX-01, showed no difference between the control and KX-01 treatment at 20 nmol/L concentration. This concentration is known to only inhibit Src signaling, so these results infer that cell migration was inhibited by KX-01-sensitive cell lines because Src phosphorylation was suppressed.

To further explore the action mechanism of KX-01, we measured additional downstream signaling molecular-level changes after 24-hour KX-01 treatment. Src regulates survival and proliferation. Interestingly, while the p-Src recovered 24 hours after the treatment of KX-01, regardless of the sensitivity (data not shown), only the sensitive cell lines had low p-AKT, p-ERK, and p-STAT3 (Fig. 1C).

These data indicate that KX-01 has effective antitumor effects against a broad range of TNBC cell lines, and these effects are related, at least in part, to inhibition of Src signaling by KX-01 treatment. Moreover, inhibition of Src by KX-01 not only inhibits cell migration, but also cell proliferation and survival signaling molecules.

2. KX-01 treatment leads to G2/M arrest

The cell cycle of four cell lines was analyzed using flow cytometry. Other reported Src inhibitors in previous studies were found to cause G1 cell cycle arrest [21]. However, unlike these reported Src inhibitors, we were able to demonstrate that KX-01 caused cells to arrest at the G2/M cell phase in a dose-dependent manner (Fig. 2). TNBC cells sensitive to KX-01 displayed 2- to 4-fold increased G2/M cell phase population, while G1 and S cell phases decreased. Conversely, the KX-01-insensitive cell, Hs578T, did not show increased G2/M cell phase populations in response to a 100 nmol/L concentration of KX-01.

It is worth noting that G2/M cell cycle arrest was most prominent at 100 nM KX-01, suggesting that 100 nM is more effective at inhibiting microtubule polymerization than 50 nM.

Table 1. Growth inhibitory effect of KX-01

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Subtype</th>
<th>KX-01 IC_{50} (mean±SD, μmol/L)</th>
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<tbody>
<tr>
<td>MCF7</td>
<td>Luminal (ER+)</td>
<td>0.0418±0.0010</td>
</tr>
<tr>
<td>T47D</td>
<td>Luminal (ER+/PR+)</td>
<td>0.0435±0.0423</td>
</tr>
<tr>
<td>BT-474</td>
<td>HER2</td>
<td>0.1286±0.0076</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>HER2</td>
<td>0.0338±0.0010</td>
</tr>
<tr>
<td>BT-549</td>
<td>Triple negative</td>
<td>0.0467±0.0019</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Triple negative</td>
<td>0.0446±0.0009</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>Triple negative</td>
<td>0.0613±0.0017</td>
</tr>
<tr>
<td>HCC1937</td>
<td>Triple negative</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Hs578T</td>
<td>Triple negative</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>

The IC_{50} values of KX-01 determined using an MTT assay as described in "Materials and Methods" are shown. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor type 2.
3. **KX-01 increases aneuploidy and induces mitotic catastrophe**

KX-01 has microtubule polymerization inhibitor activity that leads to G2/M arrest. The substantial increase in the G2/M cell cycle population and expanded cell size with KX-01 treatment led us to consider the possibility of an increase in aneuploidy. Consequently, the DNA content was measured by flow cytometry analysis. First, we defined cells with DNA content of more than 6N as the aneuploidy population. The aneuploidy population of each cell line was subsequently analyzed by flow cytometry. Impressively, KX-01 treatment caused induction of aneuploidy 10 to 18 times higher than each control (Fig. 3A). This escalation was only observed in KX-01-sensitive cell lines. Hs578T cell did not show any induction of the aneuploidy population. These data suggested that KX-01 induces defective mitosis through microtubule polymerization inhibition.
Fig. 1. (Continued from the previous page) (B) BT-549 and Hs578T cells were incubated with dimethyl sulfoxide (control) or KX-01 for 48 hours. Wound healing assay results demonstrate the migration inhibitory effect of KX-01. The columns are shown with error bars (±standard error). *p < 0.05. (C) BT-549, MDA-MB-231, and Hs578T cells were exposed to KX-01 for 24 hours. Western blot results show molecular expression changes, which are related to Src signaling.
Mitotic catastrophe, which is cell death during mitosis caused as a result of premature entry into the mitotic cycle or irregular mitosis, can be induced by microtubule inhibitors [12]. One of the representative morphologic changes of mitotic catastrophe is increased multinuclei or micronuclei. Thus, we examined whether KX-01 could induce these morphological changes. Through immunofluorescence assays, we confirmed an increased number of multi-nucleated cells in KX-01–sensitive MDA-MB-231 cells. In contrast, the less sensitive Hs578T cells did not show any changes (Fig. 3B). The number of multi-nucleated cells was analyzed, and significant increases in multi-nucleated cell populations were only observed among KX-01–sensitive cells (Fig. 3C). Similar results were obtained with other KX-01–sensitive cell lines (S2 Fig.). Further investigations of microtubule arrangements were conducted to verify that the increase of multi-nucleated cells in KX-01–sensitive cell lines was caused by inhibition of microtubule polymerization by KX-01.

MDA-MB-231 cells were treated with KX-01 for 48 hours to identify any changes in microtubule polymerization. No significant changes were observed in cells treated with
Fig. 3. KX-01 increases aneuploidy and induces mitotic catastrophe by inhibiting microtubule polymerization. (A) BT-549, MDA-MB-231, MDA-MB-468, and Hs578T cells were treated with indicated concentrations of KX-01 for 48 hours. The percentages of cells that contained more than 6N were determined by flow cytometry analysis and compared to the control values. Each column is shown with error bars (±standard error). *p < 0.05, **p < 0.005. (B) MDA-MB-231 and Hs578T cells were incubated with 100 nmol/L of KX-01 or dimethyl sulfoxide (DMSO, control) for 24 hours. Confocal microscopy was used to observe the signal corresponding to α-tubulin (green) and DNA was counterstained with DAPI (blue). Arrows indicate multinucleated cells. (C) One hundred cells in each KX-01 treatment level indicated were counted and the number of multinucleated cells were represented by a percentage. The columns represent the means of three independent experiments and are shown with error bars (±standard error). **p < 0.005. (Continued to the next page)
DMSO control. However, in KX-01–treated cells, abnormal microtubule formations were observed and more cells were under the M phase (Fig. 3D). When observed in detail, only KX-01–sensitive cells failed to undergo cytokinesis due to KX-01 treatment (data not shown). Overall, these findings demonstrated that KX-01 induces mitotic catastrophe by inhibiting microtubule polymerization.

4. KX-01 inhibits in vivo tumor growth in mice

To confirm the antitumor effects of KX-01 observed in vitro, an in vivo mouse model was established using MDA-MB-231 cells. Briefly, 10 mice were divided into two groups and treated with vehicle or KX-01. After 4 weeks, the mice treated with KX-01 showed significantly delayed tumor growth (Fig. 4A). There were no significant weight changes in the mice treated with KX-01 (Fig. 4B). These results indicated that KX-01 had antitumor effects without obvious toxic effects on mice during the treatment period.

Tumor tissues from mice treated with KX-01 had lower levels of Ki-67 expression than the vehicle control tissues (Fig. 4C) [20,21], suggesting that KX-01 lowered the proliferation of the cancer cells. A TUNEL assay was used to measure the number of apoptotic cells. Tumor tissues from the KX-01 treatment group had significantly increased numbers of apoptotic cells relative to the vehicle control samples (Fig. 4C). Next, we determined whether trans-phosphorylation of Src was also inhibited by KX-01 treatment in vivo. Data from the Western blot assay demonstrated that phosphorylation levels were reduced, as were p-Src levels (Fig. 4D). These in vivo data demonstrated the antitumor effects of KX-01 in the human TNBC MDA-MB-231 xenograft model.

Discussion

KX-01 is a small molecule that inhibits Src and tubulin polymerization. The effects of this compound are currently being investigated in phase II clinical trials [15,16]. In a previous study, KX-01 produced promising inhibitory effects in breast cancer cell lines [14]; however, the underlying mechanism of KX-01 antitumor activity was not fully demonstrated. In this study, we explored the action mechanism of the KX-01 antitumor activity in vitro in greater depth using TNBC cell lines. We demonstrated that KX-01 effectively inhibits TNBC cell growth and migration in a broad range of tumor cell lines. Moreover, this effect was, at least partially, due to down-regulation of Src signaling by KX-01 treatment. Previous studies demonstrated the down-regulation of p-paxillin, p-p130cas, and p-AKT without showing evidence of p-Src down-regulation, which was the primary target for KX-01.

In the present study, we demonstrated the time course of inhibition of p-Src levels by KX-01 treatment in vitro. Interestingly, we also showed that Src signaling-related molecules were still down-regulated, even after the p-Src activity was restored. The reason for the different time courses for these inhibitory responses may have clinical implications and will therefore be the subject of further investigations. Unlike other Src inhibitors, KX-01 is a reversible inhibitor that does not bind to the ATP pocket of Src [14,15,18]; consequently, it may have important unique biological and clinical effects. We confirmed that KX-01 can effectively inhibit Src signaling, reduce cell growth, and prevent cell migration. Despite the Src inhibitory effect of KX-01, basal expression of Src or
Fig. 4. KX-01 inhibits in vivo tumor growth in MDA-MB-231 mouse xenograft model. (A) BALB/c nude mice were injected with 5×10⁶ MDA-MB-231 cells. The vehicle group received 10% (2-hydroxypropyl)-β-cyclodextrin solution in phosphate buffered saline and the other group was treated with 5 mg/kg of KX-01 administered by oral gavage twice daily for 4 weeks. Tumor volumes were recorded as mm³ and compared to the starting tumor sizes values. (B) Mouse weights were measured three times weekly. Each dot indicates the mean mouse weight. No significant differences in body weight were detected. Mean values are shown ±standard error. (C) The tumors were removed from the mice after KX-01 treatment ended, and pathologic examination was conducted using H&E slides (×200). Immunohistochemical staining for Ki-67 and terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assays showed decreased Ki-67 with increased apoptosis in KX-01 treatment tumors. (D) On the final day of treatment, total cell protein was extracted from mouse tissues for immunoblotting with the indicated antibodies.

p-Src levels was not associated with KX-01 sensitivity (S3 Fig.).

Another characteristic of KX-01 is the ability to inhibit microtubule polymerization [14,15,18]. This effect was observed when the KX-01 concentration exceeded 80 nmol/L, whereas Src and Src signaling inhibition was evident at concentrations as low as 20 nmol/L. In the present study, we detected increased G2/M phase arrest and aneuploidy when the cells were treated with 100 nmol/L of KX-01. Significantly increased populations of multi-nucleated cells and aneuploid cells indicated that KX-01 induced mitotic catastrophe in vitro [9]. Our data also demonstrated abnormal microtubule polymerization induced by KX-01 treatment. Usually, microtubule targeting agents are limited
in that normal cells may also be affected by the drug treatment. However, KX-01 does not appear to affect normal cells in an observable fashion. When MCF10A cells, a non-tumorigenic epithelial cell line, were treated with KX-01, G2/M cell phase arrest was not induced (data not shown). Thus, it seems that the antitumor effects of KX-01, inhibition of Src signaling and induction of mitotic catastrophe, are cancer specific phenomena.

Unlike sensitive cell lines (BT-549, MDA-MB-231, and MDA-MB-468), Hs578T cells were resistant to KX-01 treatment. Hs578T cells shows low levels of p-Src expression when compared with other sensitive cell lines (S3 Fig.). The expression of Src family members and additional downstream signaling does not appear to be affected by KX-01 treatment (Fig. 1C). Moreover, Hs578T cells tolerate microtubule aberrations by KX-01. Because Hs578T cells have hyper-tetraploid chromosome numbers, they appear to be less affected by deregulation of microtubule polymerization.

KX-01 showed the ability to overcome resistance induced by other anti-microtubule agents such as paclitaxel. In this study, KX-01 showed significant growth inhibitory activity in the paclitaxel resistant MCF7 cell line (S4 Fig.). Our data indicated that KX-01 could be an alternative to overcome resistance to paclitaxel or other anti-microtubule agents.

A relationship between Fyn (protein tyrosine kinase p59")( and microtubule polymerization during neuronal cell development has been reported [22]. A previous study demonstrated that Fyn has the potential to modulate membrane associated γ-tubulin activities, which are important to initiating the formation of microtubules. Recruitment of tyrosine-phosphorylated molecules during microtubule polymerization has also been described [23]. Recently, several studies demonstrated the possible involvement of Src and FAK in mitosis [24-27]. Therefore, we can hypothesize that Src inhibition activity could also contribute to the inhibitory effects on microtubule polymerization along with its direct binding and inhibitory effect of KX-01 on tubulin (data not yet published), although further investigation is needed to test this hypothesis.

Various inhibitors that target the ATP binding pocket of Src have been developed; however, the effects of these compounds in clinical trial have not been remarkable [7,8]. In clinical trials using dasatinib, which targets the ATP binding pocket of Src and other kinases, the compound did not show promising antitumor effects in solid tumor patients as a monotherapeutic agent [6,28]. Unlike previous Src inhibitors, KX-01 targets the non-ATP binding region. Therefore, this drug has less chance of blocking other tyrosine kinases that harbor the Src homology domain. A phase I clinical trial of KX-01 showed promising results against various solid tumors [17] and a phase Ib clinical trial is currently ongoing.

Here, we demonstrate the down-regulation of p-Src by KX-01 in vitro for the first time. Previous studies only demonstrated p-Src inhibitory effects of KX-01 in vivo and indirectly showed Src inhibition by demonstrating inactivation of downstream molecules, such as p-paxillin or p-p130cas, instead of the inhibited Src itself. Thus, this paper is noteworthy because it directly shows that KX-01 regulates Src signaling via its direct inhibition of p-Src. Moreover, we verified that treatment with KX-01 aggravates the burden on the G2/M cell phase and causes abnormal mitosis, which induces mitotic catastrophe in TNBC cells. Src and microtubule dual inhibitory effects are the key characteristics that distinguish KX-01 from other Src inhibitors, and therefore make this drug a more promising treatment for TNBC.

The first-line chemoagent used for treatment of TNBC, paclitaxel, is also a well-known anti-microtubule drug; however, there are many paclitaxel-resistant patients. Based on its two inhibitory effects, it is anticipated that KX-01 has the potential to treat paclitaxel-resistant patients. Taken together, the results presented herein provide a better understanding of the action mechanism of KX-01, which may help future clinical trial design.

**Conclusion**

The results of this study demonstrate that KX-01 inhibits TNBC growth by modulating Src signaling and microtubule polymerization. Furthermore, we confirmed that KX-01 inhibits Src phosphorylation in vitro. Moreover, our findings verify that KX-01 treatment induces mitotic catastrophe. Overall, our efforts will broaden the understanding of the action mechanism of KX-01.

**Electronic Supplementary Material**

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

**Conflicts of Interest**

Y.J.B. has advised or consulted for and received research funding from Bayer, Novartis, Boeringer-Ingeheim, Roche / Genentech, AstraZeneca, Merck Serano, MSD, Bristol-Myers Squibb, Eli Lilly, Pfizer, ONO, Taiho and GreenCross. S.A.I.
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Long Non-coding RNA HOXA11 Antisense Promotes Cell Proliferation and Invasion and Predicts Patient Prognosis in Serous Ovarian Cancer

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Introduction

Epithelial ovarian cancer has the highest mortality rate among cancers of the reproductive organs and is the fifth leading cause of female cancer death in the United States [1]. Despite advances in surgery and chemotherapeutic agents, the prognosis for ovarian cancer is poor, with a 5-year survival rate of less than 30% [2]. Serous ovarian cancer (SOC), which often presents at advanced stages and is characterized by metastases in the pelvic and abdominal cavity, is the most common histological type. The poor prognosis of SOC has been correlated with tumor metastasis and recurrence; therefore, it is crucial to understand the underlying molecular mechanisms involved in ovarian carcinogenesis and progression.
Long non-coding RNAs (lncRNAs) comprise a heterogeneous group of genomic transcripts longer than 200 nucleotides that have no protein coding functions [3]. Unlike short non-coding RNAs such as microRNAs, the functional role of lncRNAs have been underestimated since they were initially regarded as transcriptional noise in the genome [4]; however, recent data have demonstrated their importance in normal physiology, as well as in the modulation of various biological processes such as cell proliferation, apoptosis, invasion, and reprogramming of stem cell pluripotency [4]. Furthermore, emerging evidence indicates that lncRNA expression is altered in diverse human cancers, and that its expression pattern may be associated with cancer progression and metastasis [5-7].

Members of the homeobox (HOX) family of genes are known to contain transcription factors that contribute to embryogenesis and carcinogenesis [8]. Several studies have shown dysregulated HOX gene expression in breast, lung, prostate, and colon cancer [9-13]. In humans, HOX genes are located in four different chromosomes, organized into four clusters (A, B, C, and D) [14]. During development of the female reproductive tract, HOXA11 is expressed in the cervix and lower uterine segment; however, its inappropriate expression is believed to lead to epithelial ovarian neoplasia since it promotes aberrant epithelial differentiation [15,16]. Similarly, the HOXA cluster of protein-coding genes contributes to ovarian embryogenesis and carcinogenesis. This study focused on the ‘antisense’ strand of the HOXA gene cluster, which contains non-coding RNA genes. The 5′ region of the HOXA locus includes three protein-coding genes (HOXA9, HOXA10, and HOXA11) and three lncRNAs, HOT-TIP, HOXA10as (antisense), and HOXA11as. Since little is known about the function of locally residing lncRNAs, this study was conducted to investigate the role of HOXA11as in carcinogenesis.

In this study, the expression of HOXA11as in SOC was examined and its clinical significance and correlation to disease prognosis were analyzed. Functional assays were also conducted to explore the impact of HOXA11as on cancer cell invasion and migration in vitro. Finally, since epithelial-mesenchymal transition (EMT) is regarded as one of the major mechanisms inducing cancer metastasis, we investigated whether HOXA11as was involved in EMT and metastasis in SOC.

### Materials and Methods

#### 1. Patients and tissue samples

A total of 129 SOC tissue samples were obtained from patients who underwent surgery at the Department of

<table>
<thead>
<tr>
<th>Table 1. HOXA11as expression and clinicopathologic variables in serous ovarian cancer patients (n=129)</th>
</tr>
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<tbody>
<tr>
<td><strong>Factor</strong></td>
</tr>
<tr>
<td>Age, median±SD (yr)</td>
</tr>
<tr>
<td>FIGO stage, n (%)</td>
</tr>
<tr>
<td>Residual disease, n (%)</td>
</tr>
<tr>
<td>Lymph node metastasis, n (%)</td>
</tr>
<tr>
<td>CA125 level (U/mL)</td>
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</tbody>
</table>

**HOXA11as, HOXA11 antisense; SD, standard deviation; FIGO, International Federation of Gynecology and Obstetrics; CA125, cancer antigen 125.**
Obstetrics and Gynecology, Severance Hospital between April 2003 and December 2013. Patients with borderline ovarian tumor, concomitant gynecological or other primary cancer, as well as those who had received preoperative chemotherapy were excluded. Table 1 summarizes the clinical information describing the patients. The median duration of follow-up was 39 months (range, 2 to 116 months) for survivors. Progression-free survival (PFS) was defined as the interval between the date of surgery and the date of progression confirmed by imaging studies. Overall survival (OS) was defined as the date of surgery until the date of death. The control group consisted of 38 normal ovarian epithelial tissue samples obtained from patients that underwent simple hysterectomy or oophorectomy for benign uterine conditions.

All samples were immediately frozen in liquid nitrogen and stored at –80°C until RNA extraction. This research was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine (No. 4-2009-0738), and written informed consent was obtained from study participants.

2. Cell line and cell culture

The human epithelial ovarian cancer cell lines OVCAR3 and SKOV3 were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) and the A2780 cell line was purchased from the European Collection of Cell Cultures (ECACC, Sigma-Aldrich, St. Louis, MO). Ovarian cancer cell lines OVCA433, OVCA429, and TOV112D were provided by the Korea Gynecologic Cancer Bank through the Bio & Medical Technology Development Program of the Minister of Science, Information and Communication Technology and Future Planning (MSIP), Korea. Normal human ovarian surface epithelial (HOSE) cells were purchased from ScienCell Research Laboratories (San Diego, CA). OVCAR3, SKOV3, and A2780 cells were cultured in RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD), while OVCA433, OVCA429, and TOV112D cells were cultured in Dulbecco’s modified Eagle medium and the HOSE cell line was cultured in ovarian epithelial cell medium (OEpiCM, ScienCell). All culture media were supplemented with 10% (vol/vol) fetal bovine serum and 1% penicillin/streptomycin, and cell lines were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Culture medium was replaced with fresh medium every 2-3 days and cells were used between passages 5 and 10.

3. Quantitative real-time polymerase chain reaction

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA). For quantitative real-time polymerase chain reaction (qRT-PCR), total RNA was reverse transcribed to cDNA using a Reverse Transcription Reagent Kit (Invitrogen) according to the manufacturer’s protocols. Real-time PCR analyses were conducted using a SYBR Green Real-time PCR Kit (TOYOBO Co. Ltd., Osaka, Japan). Conditions for the amplification of HOXA11as IncRNA were as follows: initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 60 seconds, and elongation at 72°C for 60 seconds and then final elongation at 72°C for 5 minutes. qRT-PCR was performed using an ABI StepOne-Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). The results were normalized to the expression of U6. The polymerase chain reaction (PCR) primer sequences used for analyses were as follows: HOXA11as, 5’-GAGTTTGGAAGCCCGTGATGT-3’ (sense) and 5’-AGATGAGGGAGAGGTTGAT-3’ (antisense); E-cadherin, 5’-ATTCTGATTCTCTGTCGTCTTG-3’ (sense) and 5’- AGTATGCATATGCTGTCCT-3’ (antisense); N-cadherin, 5’-CCCAAGACAAAGACCCAG-3’ (sense) and 5’-GCCACTGTCCTACTGCCATTG-3’ (antisense); J-catenin, 5’-TGAGATGTCCTCCCTGTT-3’ (sense) and 5’-GGTCATCGTGATCGAGAAG-3’ (antisense); Snail, 5’-AGGCGGGTGCCAGACTAG-3’ (sense) and 5’-GACACATCGGTGACAGAG-3’ (antisense); Twist, 5’-CGGGAGTCCGAGCAGTCTTA-3’ (sense) and 5’-TGAGATGTCCTACGTTGC-3’ (antisense); U6, 5’-CTGCCTTCGCGACCAA-3’ (sense) and 5’-ACGCTTCCAGGAATTGCCG-3’ (antisense). The relative change in expression of mRNA was calculated by the 2-ΔΔCT method. All qRT-PCR experiments were replicated at least three times.

4. Small interfering RNA transfection

Homeobox A11 antisense IncRNA small interfering RNA (siRNA) (siHOXA11as) and negative control siRNA (siNC) were purchased from Genolution (Genolution Pharmaceuticals Inc., Seoul, Korea). Cells (5×10⁴ cells/well) were seeded into 6-well plates and transfected with 10 nM siRNA in OptiMEM I (Invitrogen) using the Lipofectamine RNAiMax (Invitrogen) according to the manufacturer’s instructions. These siRNA-transfected cells were used for in vitro assays 48-hour post-transfection. The target sequence for siHOXA11as was as follows: siRNA, 5’-CGGAUAUUGC-5’ (antisense). All experiments were repeated at least three times.

5. Plasmid constructs and generation of stable cell line

Full-length human HOXA11as transcript cDNA was amplified by PCR and inserted into the pLenti6/V5-D-TOPO

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vector according to the ViraPower Lentiviral Expression Systems (Invitrogen) protocol. Briefly, the plasmid was transfected into 293FT cells for packaging, after which the lentivirus was used to infect the desired cell lines. The selection of HOX11as stably transfected cells was performed in medium containing 10 μg/mL blasticidin (Invitrogen).

6. Cell proliferation assay

Cell proliferation was measured by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma). Briefly, cells (1×10^4 cells/well) were seeded into 96-well flat-bottomed plates in 100 μL of complete medium. The cells were then incubated overnight to allow cell attachment and recovery, after which they were transected with siNC or siHOX11as for 24, 48, 72, or 96 hours. MTT solution (10 μL) was then added to each well, after which the cells were incubated for an additional 2 hours. Absorbance was measured at 570 nm using a microplate reader. Three independent experiments were performed in triplicate.

7. Wound healing assay

Cell migration was assessed by monolayer wound healing assay. Briefly, cells were seeded into 6-well culture plates with serum-containing medium and allowed to grow to 90% confluency. The serum-containing medium was then removed, after which cells were starved for 24 hours. When the cell confluence reached nearly 100%, an artificial homogenous wound was created by scratching the monolayer with a sterile 200 μL pipette tip. After the cells were washed with serum-free medium, images of cells migrating into the wound were captured at 0, 24, and 48/60 hours using a microscope. Each experiment was repeated three times.

8. Matrigel invasion assay

Matrigel invasion assay was performed using the BD Bio- coat Matrigel Invasion Chamber (pore size, 8 μm; 24-well; BD Biosciences, Bedford, MA) according to the manufacturer’s instructions. Briefly, overexpression cells or siHOX11as-transfected cells and siNC-transfected cells (5×10^4 cells/mL) were plated in the upper chamber in serum-free medium, and complete medium was added to the bottom chamber. After 24 hours of incubation, cells that had invaded through the membrane were stained using a Differential Quik Stain Kit (Diff Quik, Sysmex, Kobe, Japan), then counted using a light Microscopy Axio Imager M2 (Carl Zeiss, Thornwood, NY; magnification ×200). The assay was performed in triplicate.

9. Western blot analysis

Proteins were extracted with RIPA buffer (Thermo Fisher Scientific, Inc., Waltham, MA) and their concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Next, samples were boiled for 5 minutes, subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred electrophoretically to polyvinylidene difluoride membranes (Millipore, Billerica, MA). The membranes were subsequently blocked with 5% non-fat dried milk in 1× Tris-buffered saline containing 0.1% Tween 20 (TBST; pH 7.6) at room temperature for 1 hour, then incubated with primary antibody at 4°C overnight. Blocked membranes were then incubated with primary antibodies (E-cadherin, N-cadherin, or β-catenin rabbit polyclonal antibodies at final concentrations of 1:1,000, Cell Signaling, Beverly, MA; vimentin, Snail, or β-actin mouse polyclonal antibodies at a dilution of 1:1,000, Sigma). Primary antibodies against each protein were detected by horseradish peroxidase-conjugated secondary antibody (1:2,000). Bands were visualized using a SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific), and band intensities were quantified using the Luminescent Image Analyzer (LAS-4000 mini, Fujifilm, Uppsala, Sweden).

10. Data analysis

Statistical analysis was performed with SPSS ver. 19 for Windows (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used to verify standard normal distributional assumptions. A student’s t test and the Mann-Whitney U test were used for parametric and non-parametric variables, respectively. Differences between proportions were compared using Fisher exact test or a chi-square test. PFS and OS were calculated by the Kaplan-Meier method, while the log-rank test was used to compare survival distribution. Multivariate analysis using the Cox regression model adjusted for known prognostic covariates (age, stage, tumor grade, lymph node metastasis, and residual tumor) was conducted. A p < 0.05 was regarded as statistically significant.

Results

1. Expression of HOX11as is up-regulated in SOC tissues

The expression level of HOX11as was measured in 129 SOC tissues and 38 corresponding normal ovarian tissues by qRT-PCR and normalized to U6. HOX11as expression in cancer tissue was more than 77-fold higher than that of non-
cancerous tissue (p < 0.05) (Fig. 1A). One hundred twenty nine SOC patients were divided into a high (n=27) and low expression group (n=102) based on a HOXA11as/U6 ratio of 60 in cancerous tissue (Fig. 1B).

2. Correlation between HOXA11as expression and clinicopathological characteristics in SOC

Clinicopathological data such as age, stage, histologic grade, extent of residual disease, lymph node metastasis, and preoperative cancer antigen 125 (CA125) level were compared between the high and low HOXA11as expression groups (Table 1). High grade histology and higher level of CA125 were more frequently found in the high HOXA11as expression group (p < 0.05).

3. Downregulation of HOXA11as decreases SOC cell proliferation

To investigate the role of HOXA11as in SOC cells, several cell lines were examined for HOXA11as expression. As shown in Fig. 2A, OVCA429, OVCAR3, and SKOV3 expressed higher levels of HOXA11as than other cell lines and the control (HOSE). The knockdown efficiency of the HOXA11as-specific siRNAs (siHOXA11as) was analyzed by qRT-PCR method, which revealed that siHOXA11as had higher silencing efficiency compared to control (Fig. 2B). The proliferation of siHOXA11as-transfected ovarian cancer cells and siNC was measured by MTT assay. Knockdown of HOXA11as inhibited cell proliferation by 40% and 50% at 96 hours post-transfection in OVCA429 and SKOV3 cell lines, respectively, relative to control cells (Fig. 2C and D).

4. HOXA11as attenuates invasion and migration of SOC cells

The effects of HOXA11as on the invasive and migratory behavior of SOC cells were assessed by Matrigel invasion and wound healing assays. Wound healing assays showed larger width of wound in siHOXA11as-transfected OVCA 429 and SKOV3 cells than siNC-transfected cells, which demonstrated decreased migration of SOC cells via downregulation of HOXA11as (Fig. 3A and B). According to the Matrigel invasion assay, the knockdown of HOXA11as significantly reduced the invasive cell numbers by more than 80% (Fig. 3C). The same results were obtained from migration and invasion assays after enforcing HOXA11as expression in OVCA429 cells (Fig. 3D). The invasive capacity of OVCA429 cells increased after 48 hours upon overexpression of HOXA11as (Fig. 3E and F). Taken together, these results indicate that HOXA11as promotes SOC cell invasion and migration in vitro.
5. Matrix metalloproteinase and vascular endothelial growth factor are involved in HOXA11as-related SOC cell metastasis

The expression of proteins related to tumor progression and metastasis were determined in SOC cells to evaluate the possible mechanism of HOXA11as in cancer cell invasion and migration. As shown in Fig. 4A, knockdown of HOXA11as inhibited the level of matrix metalloproteinase (MMP) 2, MMP-9, and vascular endothelial growth factor (VEGF) expression in OVCA429 cells. The expression of these proteins was further validated by western blot analyses, which showed significantly lower expression in siHOXA11-as transfected cells than siNC-transfected cells (Fig. 4B). Taken together, these findings indicate that HOXA11as regulates SOC cell invasion and migration through the regulation of MMP and VEGF.
**Fig. 3.** *HOXA11 antisense (HOXA11as)* promotes migration and invasion of ovarian cancer cells. (A, B) Wound healing assay was used to determine migration in *HOXA11as*-specific siRNA (siHOXA11as)-transfected OVCA429 and SKOV3 cells (×200). (C) Matrigel invasion assay was used to determine invasion after 24 hours in OVCA429 cells. (D) Overexpression of *HOXA11as* in OVCA429 cells analyzed by quantitative real time polymerase chain reaction. (E, F) Migration and invasion assay after overexpressing *HOXA11as* expression in OVCA429 cells. Overexpression of *HOXA11as* in OVCA429 cells increased the invasive capacity after 48 hours. Each assay was performed in triplicate. Data are mean±standard deviation. *p < 0.05 vs. siNC, vector. (Continued to the next page)
Fig. 3. (Continued from the previous page)

Fig. 4. Knockdown of HOXA11 antisense (HOXA11as) inhibits matrix metalloproteinase (MMP) 2, MMP-9, and vascular endothelial growth factor (VEGF) expression in ovarian cancer cells. Protein lysates were obtained from HOXA11as-specific siRNA (siHOXA11as) and negative control siRNA (siNC)-transfected OVCA429 cells 48-hour post-transfection. MMP-2, MMP-9, and VEGF expression were analyzed by quantitative real time polymerase chain reaction (A) and western blotting (B). Each assay was performed in triplicate. Band intensities were quantitated, and MMP-2, MMP-9, and VEGF protein levels were normalized to that of β-actin. Each assay was performed in triplicate. Data are mean±standard deviation. *p < 0.05 vs. siNC.
6. **HOXA11as modulates EMT marker genes in SOC cells**

To further investigate the molecular mechanisms involved in metastatic features of HOXA11as, the EMT marker gene levels were examined using quantitative PCR and western blot assays. Epithelial marker E-cadherin was significantly upregulated and mesenchymal marker N-cadherin, β-catenin, and vimentin were downregulated in siHOXA11as cells. The impact of HOXA11as knockdown on the expression of Twist and Snail, which are known to modulate EMT, was also assessed. siHOXA11as-transfected cells showed lower expression of Twist and Snail than siNC-transfected cells (Fig. 5).

7. **Higher expression of HOXA11as is correlated with poor patient survival**

The median duration of follow up was 39 months (range, 2 to 116 months). Overall, 45 patients (34.9%) died from the disease, while 91 (70.5%) had disease recurrence. Survival analysis was performed to measure the PFS and OS of patients with SOC with different HOXA11as expression. As shown in Fig. 6A, the 10-year PFS of patients who had high HOXA11as cancer tissue expression was unfavorable relative to those with low HOXA11as expression (median PFS, 12 months vs. 24 months; \( p = 0.013 \)). Similarly, worse OS outcomes were shown in the high HOXA11as expression group than the low expression group (median OS, 53 months vs. 77 months; \( p = 0.045 \)) (Fig. 6B). Furthermore, the receiver operating characteristic curve analysis showed that the HOXA11as level was useful to predict survival of SOC patients (area under the curve, 0.731; 95% confidence interval [CI], 0.646 to 0.817) (Fig. 6C). Multivariate Cox regression analysis for survival revealed that high HOXA11as expression in tumor tissues was an independent predictor of poor PFS (hazard ratio [HR], 1.730; 95% CI, 1.015 to 2.948; \( p = 0.043 \)) and OS (HR, 2.170; 95% CI, 1.062 to 4.431; \( p = 0.033 \)), regardless of age, stage, grade, residual tumor size and lymph node metastasis (Table 2). Upon multivariate analysis for overall survival, age (HR, 1.044; 95% CI, 1.008 to 1.081; \( p = 0.015 \)) and residual tumor size of > 1.0 cm (HR, 2.514; 95% CI, 1.223 to 5.167; \( p = 0.012 \)) were associated with mortality, but not with recurrence.

**Discussion**

In this study, HOXA11as expression levels in SOC were higher than those of noncancerous tissues, and increased HOXA11as expression was correlated with poor patient survival. Moreover, knockdown of HOXA11as expression led to reduced cell proliferation, invasion and migration in SOC cells. The metastatic effects of HOXA11as were related to the regulation of genes involved in cell invasion, migration, and EMT, including VEGF, MMP-9, B-catenin, E-cadherin, Snail, Twist, and vimentin. These findings highlighted the clinical
Fig. 6. Correlation of HOXA11 antisense (HOXA11as) expression with patient survival. Kaplan-Meier curves for progression-free survival (A) and overall survival (B) in serous ovarian cancer patients with different expression levels of HOXA11as. (C) Receiver operating characteristic (ROC) curve for prognosis prediction of patients using HOXA11as level. The area under curve (AUC) is shown in plots.

relevance of HOXA11as to predicting adverse prognosis of SOC and suggested its potential in promoting tumor aggressiveness via the regulation of VEGF and EMT-related mechanisms.

In recent years, accumulating evidence has shown that lncRNAs may play a critical role in cellular biology and human diseases. Several lncRNAs have been identified in gynecological cancer, including HOTAIR, MALAT-1, H19, and LSINCT5 [17]. One of the most popular oncogenic lncRNA studied is HOX transcript antisense intergenic RNA (HOTAIR), which our group recently identified as playing a role in cell proliferation and invasion in cervical cancer cell lines [18]. However, no studies have been published regarding the clinical/prognostic significance of the novel lncRNA HOXA11as in SOC.

The role of HOXA11as, a novel lncRNA in epithelial ovar-
ian cancer has been underexplored. In this study, **HOXA11as** expression was shown to be higher in cancer tissues, and knockdown of **HOXA11as** inhibited cell proliferation in various ovarian cancer cell lines. Furthermore, overexpression of **HOXA11as** in OVCA429 cells enhanced the invasive capacity at 48 hours, suggesting that **HOXA11as** contributes to the invasive and migratory phenotype of ovarian carcinoma cells. LncRNAs that are overexpressed in ovarian tumors similar to **HOXA11as** include AB073614, **HOST2**, **LSINCT5**, **HOTAIR**, and **H19** [19-22]. In *vitro* and *in vivo* observations of these LncRNAs have shown decreased cell growth, migration, and apoptosis when downregulated as in this study. Among these, **HOST2** has been proposed to modulate the availability of tumor suppressor that subsequently suppresses the expression of targets that regulate cell growth and motility at the post-transcriptional level [20]. These observations are relevant to **HOXA11as** because its upregulation is similarly associated with cancer cell growth and migration. To determine if **HOXA11as** promotes SOC metastasis by regulating genes that encode certain metastasis-related proteins, the expression of extracellular matrix degrading protease MMPs and VEGF were analyzed. MMPs degrade basement membrane collagen, which promotes tumor cell invasion and metastasis. These compounds are also known to decrease survival in several types of cancers [23]. VEGF, together with MMPs, is known for its role in tumor angiogenesis and essential for cell motility and metastasis [24]. As expected, our results demonstrated significantly decreased expression of MMP-2, MMP-9, and VEGF with downregulation of **HOXA11as**. These findings indicate that **HOXA11as** plays a role in the aggressive nature of ovarian cancer cells through upregulation of the possible downstream targets, MMPs and VEGF.

Since the molecular mechanisms of LncRNAs related to tumor progression and metastasis are not fully understood, a question was raised as to whether **HOXA11as** promotes SOC metastasis by regulating gene expression that encodes metastasis-related proteins. One of the possible mechanisms is thought to be EMT, which involves epithelial cells with mesenchymal properties such as reduced cell-cell junction and increased motility. These properties of EMT have been reported to contribute to cellular proliferation, invasion and migration in various malignancies [25,26]. The knockdown of LncRNA **HOTAIR** was recently shown to be associated with reversal of the EMT process in gastric cancer cells [27], and LncRNA **MALAT1** was reported to function as an inducer of EMT in breast cancer by activating the phosphoinositide 3-kinase–AKT pathway [28]. Similarly, genes related to EMT (E-cadherin, N-cadherin, B-catenin, Snail, and vimentin) were dysregulated by **HOXA11as** knockdown in serous type OVCA429 cells in the present study. Moreover, E-cadherin was markedly upregulated and the mesenchymal markers N-cadherin, β-catenin and vimentin were downregulated in si**HOXA11as** cells. Furthermore, si**HOXA11as**-transfected cells showed lower expression levels of Twist and Snail than siNC-transfected cells, indicating **HOXA11as**-mediated regulation of EMT modulators. Taken together, dysregulated expression of EMT-related genes appears to take part in **HOXA11as**-related SOC cell invasion and migration.

The results of this study suggest the possible relationship of **HOXA11as** with the EMT and contribute to our understanding of the role of LncRNAs. Additionally, this is the first investigation to demonstrate the correlation between the novel LncRNA **HOXA11as** and SOC cell metastasis in relation to EMT. It should be noted that this study was limited by its retrospective nature; however, a large number of cases were examined along with long term survival data. Many studies have shown that LncRNA acts as an unfavorable prognostic factor in various human cancers [17,18,29]; thus, the clinical and prognostic significance of **HOXA11as** were identified in this study. Moreover, only serous type histology was investigated to overcome the heterogeneity of the study population. Further investigations are required to understand the role and molecular mechanisms of **HOXA11as** in other subtypes of ovarian cancer.

### Table 2. Multivariate Cox regression model analyses of factors associated with survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Progression-free survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR  95% CI  p-value</td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>1.008 0.987-1.031 0.420</td>
<td>1.044 1.008-1.081 0.015</td>
</tr>
<tr>
<td>Stage</td>
<td>1.997 1.80-3.371 0.960</td>
<td>1.120 0.532-2.359 0.765</td>
</tr>
<tr>
<td>Histologic grade (grade 1-2 vs. 3)</td>
<td>0.932 0.587-1.479 0.765</td>
<td>0.722 0.379-1.378 0.324</td>
</tr>
<tr>
<td>Residual disease (&gt; 1.0 cm vs. ≤ 1.0 cm)</td>
<td>1.678 0.944-2.984 0.078</td>
<td>2.514 1.223-5.168 0.012</td>
</tr>
<tr>
<td>LN metastasis (positive vs. negative)</td>
<td>0.795 0.479-1.321 0.376</td>
<td>0.516 0.233-1.141 0.102</td>
</tr>
<tr>
<td><strong>HOXA11as</strong> expression (high vs. low)</td>
<td>1.730 1.015-2.948 0.043</td>
<td>2.170 1.062-4.431 0.033</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; LN, lymph node; **HOXA11as**, **HOXA11 antisense**.
Conclusion

In summary, HOXA11as was overexpressed in patients with SOC, and its overexpression was correlated with poor prognosis. Furthermore, functional studies suggested that HOXA11as plays a critical role in controlling SOC cell proliferation and invasion via regulation of EMT-related genes. These findings contribute to a better understanding of dysregulated IncRNAs in cancer progression and may provide guidance for the development of IncRNA-based biomarkers and precision medicine approaches for treatment of SOC.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References

Dose-Response Relationship between Radiation Dose and Loco-regional Control in Patients with Stage II-III Esophageal Cancer Treated with Definitive Chemoradiotherapy

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Introduction

Esophageal cancer ranks ninth in cancer incidences and sixth in cancer-related deaths globally [1]. More than half of esophageal cancer patients are diagnosed with locally advanced disease and approximately 20% have resectable disease at presentation [2]. Even in patients with resectable disease, prognosis is poor after surgical resection alone, with a 5-year survival rate of < 30% [3,4].

Definitive chemoradiotherapy (CRT) has been recommended as the optimal treatment for patients who are medically inoperable or have an unresectable tumor based on the results of the Radiation Therapy Oncology Group (RTOG) 85-01 trial [5-7], which showed a statistically significant survival benefit with CRT compared to radiotherapy (RT) alone (5-year overall survival [OS], 26% vs. 0%, respectively). The RTOG 94-05 trial further compared OS and loco-regional...
control (LRC) with respect to combined-modality therapy using standard-dose 50.4 Gy versus 64.8 Gy of RT for patients with locally advanced esophageal cancer and found no significant advantage to administering high-dose radiation with respect to LRC and OS [8,9]. Although the recommended dose of RT has remained 50.4 Gy in the definitive CRT setting based on the results of the RTOG 94-05 trial [10], the optimal radiation dose is still controversial. This study was designed to investigate the correlation between radiation dose and LRC in patients with stage II-III esophageal cancer treated with definitive CRT.

Materials and Methods

1. Patient characteristics

We identified all patients treated with CRT for clinical stage II-III esophageal cancer at Yonsei Cancer Center between February 1994 and May 2013. Overall, 418 patients were retrospectively reviewed, among which 182 were excluded because of the following reasons: (1) low-dose RT administered as a palliative measure (n=22), (2) incomplete treatment (n=16), (3) esophagectomy after CRT (n=80), (4) other primary cancer history (n=11), (5) intraluminal brachytherapy (n=46), and (6) follow-up loss after CRT (n=7). Ultimately, 236 patients were included in this analysis, and their electronic medical records were retrospectively reviewed.

Pretreatment evaluation included previous medical history, physical examination, symptoms, and performance status. Laboratory studies included a complete blood cell count and routine chemistry. For staging workup, barium swallow, esophagogastroduodenoscopy (EGD), transesophageal endoscopic ultrasonography, and computed tomography (CT) of the chest and abdomen-pelvis were performed. For evaluation of distant metastases, patients underwent whole-body bone scanning and 18F-fluorodeoxyglucose positron emission tomography (FDG-PET).

2. Treatment

RT was performed with three-dimensional conformal RT (3D-CRT) or intensity-modulated RT (IMRT) with helical tomotherapy (Tomotherapy Inc., Madison, WI) starting on day 1 of chemotherapy. A conventional fractionation schedule (daily 1.8-2.0 Gy per fraction, 5 days per week) and cone-down technique were used in all patients. The gross tumor volume (GTV) was delineated using positron emission tomography and CT fusion on the MIM software (Cleveland, OH) or Pinnacle Radiotherapy Planning System (Phillips Medical System, Andover, MA). The initial clinical target volume (CTV) included the GTV plus a margin of at least 5 cm longitudinally and 2 cm radially. The initial CTV received 30.6-50.4 Gy (median dose, 36 Gy) with anterior-posterior parallel opposite fields to reduce lung dose. At the time of cone-down, final CTV encompassed the GTV with a 2 cm margin longitudinally and radially. The total radiation dose ranged from 45.0 to 66.6 Gy, with a median dose of 63 Gy.

Chemotherapy was administered to all patients using a 5-fluorouracil (5-FU)–based regimen, except for five patients (2.1%) who underwent cisplatin alone because of their medical condition. Overall, 217 patients (91.9% of all patients) were treated with a 5-FU/cisplatin (FP) regimen, while 14 (5.9%) underwent 5-FU monotherapy. During RT, two cycles of FP chemotherapy were administered concurrently. Patients had a 4-week break after completing RT, after which they received additional maintenance chemotherapy if a medical oncologist determined that their performance status and medical condition would allow this. 5-FU was administered at 500-1,250 mg/m² daily as a continuous infusion using a portable electronic pump on days 1-4, while cisplatin was administered at 40-100 mg/m² on day 1 and during RT sessions.

3. Follow-up

All patients were examined weekly during RT to monitor treatment toxicities and their general condition. After completion of CRT, patients were followed at 3-month intervals for the first 3 years, 6-month intervals for the next 2 years, and annually thereafter. Follow-up sessions included physical examination, barium swallow, chest CT, FDG-PET, EGD, and toxicity evaluation. Treatment-related toxicities were recorded according to the Common Toxicity Criteria for Adverse Events ver. 4.0. Tumor response was assessed pathologically based on endoscopic biopsy, as well as clinically based on follow-up imaging studies within three months of completion of CRT according to the Response Evaluation Criteria for Solid Tumors (RECIST) ver. 1.1. Recurrences were confirmed histologically or using conclusive imaging studies if pathological confirmation was not achieved. If loco-regional recurrences were confirmed, they were classified into central, marginal, or outfield based on the location of the recurrent tumor. Marginal recurrences were defined as recurrent tumors located inside the initial RT field, but outside of the cone-down RT field. Disease recurrences outside of the esophagus and regional lymph nodes were considered distant metastases.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard dose (n=120)</th>
<th>High dose (n=116)</th>
<th>p-value</th>
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<td>Age, mean (range, yr)</td>
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<td>67.0 (30-86)</td>
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</tr>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>114 (95.0)</td>
<td>112 (96.6)</td>
<td>0.749</td>
</tr>
<tr>
<td>Female</td>
<td>6 (5.0)</td>
<td>4 (3.4)</td>
<td></td>
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<tr>
<td>Karnofsky performance status</td>
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<td></td>
<td></td>
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<tr>
<td>90-100</td>
<td>97 (80.8)</td>
<td>77 (67.2)</td>
<td>0.017</td>
</tr>
<tr>
<td>60-80</td>
<td>23 (19.2)</td>
<td>38 (32.8)</td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>117 (97.5)</td>
<td>113 (97.4)</td>
<td>0.879</td>
</tr>
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<td>Adenocarcinoma</td>
<td>3 (2.5)</td>
<td>3 (2.6)</td>
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<td>Histologic grade</td>
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<tr>
<td>Well differentiated</td>
<td>15 (12.5)</td>
<td>11 (9.5)</td>
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<tr>
<td>Moderately differentiated</td>
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<td>58 (50.0)</td>
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<td>Poorly differentiated</td>
<td>34 (28.3)</td>
<td>36 (31.0)</td>
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<tr>
<td>Unknown</td>
<td>11 (9.2)</td>
<td>11 (9.5)</td>
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<tr>
<td>Tumor length (cm)</td>
<td></td>
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<tr>
<td>≤ 5</td>
<td>66 (55.0)</td>
<td>58 (50.0)</td>
<td>0.442</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>54 (45.0)</td>
<td>58 (50.0)</td>
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<td>Tumor location</td>
<td></td>
<td></td>
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<tr>
<td>Cervical</td>
<td>5 (4.2)</td>
<td>11 (9.5)</td>
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</tr>
<tr>
<td>Upper thoracic</td>
<td>27 (22.5)</td>
<td>31 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Mid thoracic</td>
<td>55 (45.8)</td>
<td>58 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Lower thoracic</td>
<td>33 (27.5)</td>
<td>16 (13.8)</td>
<td></td>
</tr>
<tr>
<td>Clinical T stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cT1</td>
<td>10 (8.4)</td>
<td>8 (6.9)</td>
<td>0.828</td>
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<td>cT2</td>
<td>27 (22.5)</td>
<td>23 (19.8)</td>
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<tr>
<td>cT3</td>
<td>64 (53.3)</td>
<td>62 (53.5)</td>
<td></td>
</tr>
<tr>
<td>cT4</td>
<td>19 (15.8)</td>
<td>23 (19.8)</td>
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<tr>
<td>Clinical N stage</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>cN0</td>
<td>19 (15.8)</td>
<td>12 (10.3)</td>
<td>0.555</td>
</tr>
<tr>
<td>cN1</td>
<td>86 (71.7)</td>
<td>90 (77.6)</td>
<td></td>
</tr>
<tr>
<td>cN2</td>
<td>14 (11.7)</td>
<td>12 (10.3)</td>
<td></td>
</tr>
<tr>
<td>cN3</td>
<td>1 (0.8)</td>
<td>2 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>II</td>
<td>46 (38.3)</td>
<td>32 (27.6)</td>
<td>0.079</td>
</tr>
<tr>
<td>III</td>
<td>74 (61.7)</td>
<td>84 (72.4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%).

4. Statistical analysis

Patients were grouped by total radiation dose, with the high-dose group receiving ≥ 60 Gy and the standard dose group < 60 Gy. Study endpoints were LRC and survival. Survival duration was calculated from the date of diagnosis to the corresponding event (locoregional recurrence, distant metastasis [DM], or death). Continuous variables between the two groups were compared using independent t tests based on baseline characteristics. The Pearson’s chi-square test or Fisher exact test were used as appropriate to compare categorical variables. The Kaplan-Meier method with log-rank test was used to analyze survival outcomes between groups. Multivariate analysis using the stepwise Cox proportional hazards regression model was performed to identify prognostic factors for LRC and OS (inclusion criteria, p < 0.10). All statistical tests were two-sided with significance defined as p < 0.05. Data were analyzed using the IBM SPSS software ver. 20.0 (IBM Corp., Armonk, NY).
**Results**

Of the 236 patients analyzed in our study, 120 received < 60 Gy of RT (standard-dose group) and 116 patients received ≥ 60 Gy (high-dose group). Patient characteristics are shown in Table 1. No statistically significant differences were observed between groups with respect to age, sex, histologic subtype, tumor length, clinical T stage, N stage, or clinical stage distribution. Most patients were male (96%) and had squamous cell carcinoma (97.5%). More patients with stage III disease were included in the high-dose group, although the difference was not significant (72.4% vs. 61.7%, p=0.079). Karnovsky performance status and tumor location were the only factors that showed statistically significant differences between the two groups, and there were more patients with better performance statuses and lower thoracic esophageal tumors in the standard-dose group. Initial FDG-PET was performed in 71.2% of all patients, with no significant difference between groups (76.7% in the standard-dose group vs. 65.5% in the high-dose group).

1. Details regarding treatment and follow-up

Patient treatment details are summarized in Table 2. Most patients received FP-based chemotherapy, and the proportion of patients treated with FP was similar between groups (p=0.742). The median doses of 5-FU and cisplatin were also similar in both groups. Maintenance chemotherapy following CRT was administered to 147 patients (62.3%), including 66 in the standard-dose group and 81 in the high-dose group (55% vs. 69.8% respectively, p=0.019). With the exception of four patients who underwent IMRT with tomotherapy, RT was performed with 3D-CRT. The median radiation dose was 50.4 Gy (range, 45 to 59.4 Gy) in the standard-dose group and 63 Gy (range, 60 to 66.6 Gy) in the high-dose group. The median follow-up period was 19.4 months (range, 2.2 to 164.7 months) for all patients and 50.8 months (range, 4.9 to 164.7 months) for those who survived.

2. Survival outcomes and tumor response

The median OS and progression-free survival (PFS) times for all patients were 26.2 months and 13.2 months, respectively. Comparisons of LRC, distant metastasis-free survival (DMFS), PFS, and OS between the two dose groups are shown in Fig. 1. All endpoints except DMFS were found to have statistically significant differences favoring the high-dose group. The 2-year and 5-year LRC rates of all patients were 60.0% and 48.4%, respectively. The 5-year LRC rates were significantly different between groups (59.7% in the high-dose group and 37.3% in the standard-dose group, p=0.002) (Fig. 1A). Although DMFS rates were not significantly different according to the RT dose, PFS rates were sig-

| Table 2. Treatment characteristics
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Characteristic</td>
<td>Standard dose (n=120)</td>
<td>High dose (n=116)</td>
<td>p-value</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>RT modality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3D-CRT</td>
<td>119 (99.2)</td>
<td>113 (97.4)</td>
<td>0.363</td>
</tr>
<tr>
<td>IMRT</td>
<td>1 (0.8)</td>
<td>3 (2.6)</td>
<td></td>
</tr>
<tr>
<td><strong>RT dose, median (range, Gy)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50.4 (45.0-59.4)</td>
<td>63.0 (60.0-66.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fractional</td>
<td>1.8 (1.8-2.5)</td>
<td>1.8 (1.8-2.0)</td>
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<tr>
<td><strong>Chemotherapy regimen</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluouracil+cisplatin</td>
<td>111 (92.5)</td>
<td>106 (91.4)</td>
<td>0.742</td>
</tr>
<tr>
<td>5-Fluouracil monotherapy</td>
<td>6 (5.0)</td>
<td>8 (6.9)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3 (2.5)</td>
<td>2 (1.7)</td>
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<tr>
<td><strong>Median dose of chemotherapy</strong></td>
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</tr>
<tr>
<td>5-Fluouracil (mg/m²)</td>
<td>1,000 (500-1,250)</td>
<td>1,000 (500-1,250)</td>
<td>0.942</td>
</tr>
<tr>
<td>Cisplatin (mg/m²)</td>
<td>80 (40-100)</td>
<td>80 (50-100)</td>
<td>0.470</td>
</tr>
<tr>
<td><strong>Maintenance chemotherapy</strong></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>66 (55.0)</td>
<td>81 (69.8)</td>
<td>0.019</td>
</tr>
<tr>
<td>No</td>
<td>54 (45.0)</td>
<td>35 (30.2)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). 3D-CRT, 3 dimensional-conformal radiotherapy; IMRT, intensity-modulated radiotherapy; RT, radiotherapy. *Fisher exact test.
Fig. 1. Kaplan-Meier curves of locoregional control (LRC) (A), distant metastasis-free survival (DMFS) (B), progression-free survival (PFS) (C), and overall survival (OS) (D).

Significantly different between the two dosing groups (median PFS, 11.7 months vs. 16.7 months in the standard-dose and high-dose groups, respectively; p=0.029) (Fig. IB and C). Furthermore, OS rates were significantly different between patients treated with < 60 Gy and ≥ 60 Gy (median, 22.3 months vs. 35.1 months, respectively; p=0.043) (Fig. 1D). Radiation doses were grouped into intervals of 5 Gy and plotted against LRC durations to investigate whether a dose-response relationship exists between RT dose and LRC (Fig. 2). A positive correlation was observed between RT
Fig. 2. Dose response relationship between radiotherapy (RT) dose and loco-regional control (LRC) durations.

dose and LRC rate in the setting of definitive CRT.

The total treatment response rate was 94% for all patients. Complete response (CR) was achieved in 125 patients (53%), including 53 in the standard-dose group and 72 in the high-dose group. CR rates were significantly higher in the high-dose group than in the standard-dose group (62.1% vs. 44.2%, respectively; p=0.006). The partial response rates were 47.5% in the standard-dose group and 34.5% in the high-dose group. The rates of stable disease (SD) and progressive disease (PD) were 5.0% and 3.3% in the standard-dose group, while they were 2.6% and 0.9% in the high-dose group, respectively, with no significant differences (SD, p=0.500; PD, p=0.186). The failure patterns are summarized in Table 3. A total of 141 patients (59.7%) experienced treatment failures, including loco-regional failure (LRF) alone in 77 patients (32.6%), DM alone in 50 (21.2%), and both LRF and DM in 14 (5.9%). The number of patients with LRF alone differed significantly between two groups (39.2% vs. 25.9% in the standard-dose vs. high-dose groups, respectively; p=0.029). The rate of central failures was two-fold higher in the standard-dose group (26.7% vs. 12.1%, p=0.005), and DM occurred more frequently in the high-dose group (25.9% vs. 16.7%, p=0.023).

3. Prognostic factors and treatment-related toxicities

The results of univariate and multivariate analyses are shown in Table 4. Univariate analysis revealed that RT dose and the use of maintenance chemotherapy were significant prognostic factors associated with LRC. Multivariate analysis showed that RT dose ≥ 60 Gy and the use of maintenance chemotherapy remained independent predictors of improved LRC. For OS, Karnofsky performance status, clinical T stage, American Joint Committee on Cancer (AJCC) stage, RT dose, pretreatment stricture, and the use of maintenance chemotherapy were found to be significant risk factors upon univariate analysis. Finally, multivariate analysis identified clinical stage, RT dose ≥ 60 Gy, and use of maintenance chemotherapy as independent prognostic factors correlated with OS.

Treatment-related toxicities of grade ≥ 2 occurred in 38 patients, with 19 in each of the standard-dose and high-dose groups. No significant differences was found between groups (p=0.929). Toxicities of grades ≥ 3 occurred in 21 patients, with six patients in each group having grade 3 esophageal stenosis, one in the high-dose group having grade 3 mediastinitis and two in the standard-dose group having grade 3 radiation-induced pneumonitis. Moreover, one patient in each group had a grade 3 fistula, while one in the high-dose group had a grade 4 fistula. Three patients had treatment-related grade 5 toxicities, with two in the standard-

<table>
<thead>
<tr>
<th>Primary tumor response</th>
<th>Standard-dose group (&lt; 60 Gy)</th>
<th>High-dose group (≥ 60 Gy)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>LRF only</td>
<td>47 (39.2)</td>
<td>30 (25.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>Central failure</td>
<td>32 (26.7)</td>
<td>14 (12.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>Marginal failure</td>
<td>4 (3.3)</td>
<td>6 (5.2)</td>
<td>0.534a</td>
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<td>Out-field failure</td>
<td>11 (9.2)</td>
<td>10 (8.6)</td>
<td>0.883</td>
</tr>
<tr>
<td>DM only</td>
<td>20 (16.7)</td>
<td>30 (25.9)</td>
<td>0.023</td>
</tr>
<tr>
<td>Both LRF and DM</td>
<td>8 (6.7)</td>
<td>6 (5.2)</td>
<td>0.627</td>
</tr>
<tr>
<td>Total</td>
<td>75/120 (62.5)</td>
<td>66/116 (57.0)</td>
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</table>

Values are presented as number (%). LRF, loco-regional failure; DM, distant metastasis. aFisher exact test.
dose group dying from an esophageal fistula and esophageal perforation, respectively, and one in the high-dose dying from massive esophageal hemorrhage. These patients were all receiving maintenance chemotherapy after the end of CRT when they died, having EGD-confirmed residual tumors.

**Discussion**

In this study, we investigated the correlation between radiation dose and LRC in patients with stage II-III esophageal cancer treated with definitive CRT. The results of the current study suggest that patients who received a total dose > 60 Gy of RT had significantly better LRC, PFS, and OS than patients receiving < 60 Gy when treated with concurrent chemotherapy. Furthermore, our data suggest the existence of a positive correlation between radiation dose and LRC rate.

Based on the results of the RTOG 85-01 trial [5], definitive CRT was established as the standard treatment option for localized esophageal cancer selected for nonsurgical treatment. In this trial, the 5-year OS rate was 26% for patients in the combined-modality group and 0% for those in the RT-alone group (p < 0.001). Persistent tumors were also less common in the combined modality group (26% vs. 37%), as were distant metastases as the first site of treatment failure (16% vs. 30%). The radiation dose of 50 Gy used for the combined-modality arm in the RTOG 85-01 trial became a preferred dose of RT in definitive CRT settings.

However, this dose requires further investigation because 50 Gy of radiation with conventional fractionations is generally considered inadequate to control gross tumors [11,12]. In the RTOG 94-05 phase III trial, the optimal radiation dose was further investigated [8,9]. A total of 236 patients with locally advanced esophageal cancer were randomly selected to receive a combined therapy consisting of FP chemotherapy concurrently with high-dose (64.8 Gy) versus standard-dose (50.4 Gy) RT. There were no significant differences in median survival (13.0 months vs. 18.1 months), 2-year survival (31% vs. 40%), or LRF and loco-regional persistence of disease (56% vs. 52%) between the high-dose and standard-dose groups, respectively. Although 11 treatment-related deaths occurred in the high-dose group, while there were only two in the standard-dose arm, seven of the 11 deaths occurred before the radiation dose reached 50.4 Gy. Moreover, the radiation technique used in that study was two-dimensional, and the margins applied to the target volume were larger than those used in current practice, which may have increased the probability of toxicities. Furthermore, a significantly lower dose of 5-FU was administered to patients...
in the high-dose arm, which could have negatively affected the outcomes of the high-dose arm. Because of such drawbacks, the benefit of high-dose RT with modern techniques remains controversial.

Several studies have attempted to verify the benefit of radiation dose escalation in definitive CRT for locally advanced esophageal cancer [13-15]. Zhang et al. [14] investigated 69 patients with stage II-II unresectable esophageal cancer treated with CRT, including 43 who received ≤ 51 Gy and 26 who received > 51 Gy. They found that patients in the higher dose group had better 3-year local control (36% vs. 19%) and disease-free survival (25% vs. 10%) than those in the low-dose group, but that OS was not significantly different (13% vs. 3%, p=0.054). The complete clinical response rate was also significantly greater in the high-dose group (46% vs. 23%, p=0.048). However, their study was limited owing to its small number of patients, retrospective setting, and varying fractionation schedules in the standard-dose arm (30 Gy in 10 fractions). The results of a phase II study also revealed that selective radiation dose escalation in definitive CRT settings yields promising results without surgery or adjuvant chemotherapy [13]. The preliminary results from our previous report [16] suggested a benefit for high-dose RT in stage II-III esophageal cancer patients. The effects of RT ≥ 60 Gy with concurrent chemotherapy were evaluated in 126 patients. The high-dose group showed significantly improved LRC (2-year LRC rate, 69% vs. 32%; p < 0.01) and PFS (2-year PFS, 47% vs. 20%; p=0.01) relative to the standard-dose group. However, there was no significant difference in OS between groups (median, 28 months vs. 18 months respectively; p=0.26).

In this study, we included a relatively large number of patients, all of whom received RT with conventional fractionations (1.8-2.0 Gy per fraction) and modern techniques (3D-CRT or IMRT). For all patients, the median OS and PFS rates were 26.2 and 13.2 months, respectively, which were more favorable than the results of other studies [17]. In the RTOG 94-05 trial [8], the median survival was 18 months in the standard-dose arm and 13.0 months in the high-dose arm. In the FFCD 9102 trial [18,19], which compared CRT alone to CRT followed by surgery in patients with locally advanced tumors, the median OS was 19.3 months in the CRT arm. In a study conducted by Hurmuzlu et al. [20], 46 patients were treated with high-dose RT (66 Gy in 33 fractions) concurrently with FP chemotherapy. The median OS and disease-specific survival were only 10.8 months and 11 months, respectively.

Radiation dose to the heart was recently reported to have adverse effects on survival, with mean heart dose noted in patients with breast cancer and with V5 and V30 noted in lung cancer patients in the RTOG 0617 trial [21,22]. Although a study conducted to determine the independent impact of heart dose on early OS revealed that heart dose was not associated with early survival outcomes when lung dose was taken into account [23], heart dose should not be overlooked during RT planning. Heart dose is also a concern during treatment planning for esophageal cancer because of the close proximity between the two organs. Therefore, we performed survival analysis according to the tumor location by dividing patients into those with lower thoracic tumors and those with tumors in other locations. High-dose radiation ≥ 60 Gy showed no significant OS benefit in patients with lower thoracic lesions (hazard ratio [HR], 0.681; 95% confidence interval [CI], 0.288 to 1.612; p=0.382). Conversely, OS was significantly better in patients with tumors in other locations (HR, 0.678; 95% CI, 0.467 to 0.985; p=0.041). These results indicate a possible detrimental effect of cardiac dose on early survival in esophageal cancer patients. Accordingly, follow-up studies are warranted to assess the effects of cardiac dose on heart disease or mortality.

It should be noted that this study had several limitations. Specifically, this study has limitations stemming from its retrospective nature. Moreover, the chemotherapy regimens used for the patients and the use of maintenance chemotherapy were not uniform, which may have influenced tumor response. Furthermore, patient characteristics were not matched between the two groups, and patients with good performance statuses and lower thoracic lesions were significantly more prevalent in the standard-dose group than in the high-dose group. Better clinical outcomes in the high-dose group despite a lower performance status suggest that the benefit of high-dose RT outweighs this disadvantage. Finally, it is possible that treatment-related toxicities were underestimated due to the study’s retrospective setting.

**Conclusion**

Higher radiation dose (≥ 60 Gy) was found to be associated with increased LRC, PFS, and OS in patients with stage II-II esophageal cancer treated with definitive CRT. These results suggest that radiation dose escalation may improve survival outcomes for such patients. A prospective trial evaluating the optimal dose of radiation is warranted in the future.

**Conflicts of Interest**

Conflict of interest relevant to this article was not reported.
References


ERCC1 Expression-Based Randomized Phase II Study of Gemcitabine/Cisplatin Versus Irinotecan/Cisplatin in Patients with Advanced Non-small Cell Lung Cancer

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Geon Kook Lee, MD, PhD
Kun Young Lim, MD
Young Ju Lee, MD, PhD
Byung Ho Nam, PhD
Jin Soo Lee, MD, PhD

Purpose
We evaluated the clinical utility of excision repair cross-complementation group 1 (ERCC1) expression as a predictive biomarker for platinum-based chemotherapy in advanced non-small cell lung cancer (NSCLC).

Materials and Methods
Eligible patients were randomly assigned to the GP (gemcitabine 1,250 mg/m² on days 1 and 8, and cisplatin 75 mg/m² on day 1 every 3 weeks) or IP (irinotecan 65 mg/m² and cisplatin 30 mg/m² on days 1 and 8 every 3 weeks) arm. The primary goal of this study was to compare the response rate (RR) of the GP and IP arms according to the ERCC1 expression level.

Results
A total of 279 patients were randomly assigned to the GP (n=139) and IP (n=140) arms, among which 63% were ERCC1-positive and 268 patients were assessable for the RR. The GP and IP arms did not differ significantly with respect to the RR (29.8% vs. 27.0%, respectively; p=0.082), median progression-free survival (PFS); 4.5 months vs. 3.9 months, respectively; p=0.117), and overall survival (OS); 16.5 months vs. 16.7 months, respectively; p=0.313. When comparing the efficacy between the ERCC1-positive and ERCC1-negative groups, there was no significant difference in the RR (GP, 28.2% vs. 32.6%, respectively, p=0.509; IP, 30.2% vs. 21.6%, respectively, p=0.536), median PFS (GP, 4.6 months vs. 5.0 months, respectively, p=0.506; IP, 3.9 months vs. 3.7 months, respectively, p=0.748), or median OS (GP, 18.6 months vs. 11.9 months, respectively, p=0.070; IP, 17.5 months vs. 14.0 months, respectively, p=0.821).

Conclusion
Immunohistochemical analysis of the ERCC1 expression level did not differentiate the efficacy of platinum-based chemotherapy in advanced NSCLC.

Key words
ERCC1, Platinum, Non-small-cell lung carcinoma

Introduction
Lung cancer remains the leading cause of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers, and 40% of these patients present at an advanced stage [1]. Recent advances in the understanding of the molecular origins of NSCLC have changed the treatment paradigm according to the target oncogenes [2]. However, the benefit of such target therapies remains limited to less than half of all patients with NSCLC; therefore, platinum-based chemotherapy has remained the mainstay in the treatment of NSCLC. Several large randomized studies have demonstrated the relative equivalence of platinum-based doublets, with response rates (RRs) ranging from 16% to 32% and a median survival of 8-11 months, with 1- and 2-year survival rates of 35% to 45%, and 10% to 20%, respectively. However, most patients gen-
erally experience disease progression after a median of 3-4 months of initiating chemotherapy, and the long-term prognosis remains poor [3,4]. Furthermore, no consensus has been reached for biomarkers predicting a benefit from platinum-based chemotherapy in advanced NSCLC [5].

Excision repair cross-complementation group 1 (ERCC1) plays an essential role in nucleotide excision repair, which removes platinum-DNA adducts [6]; therefore, high ERCC1 levels are usually associated with a lack of clinical benefit with platinum-based chemotherapy [7-10]. The association between ERCC1 expression and cisplatin resistance has been confirmed by ERCC1 immunohistochemistry analysis of postoperative tumor samples from the International Adjuvant Lung Cancer (IALT) trial. These analyses revealed that adjuvant chemotherapy significantly prolonged survival in patients with ERCC1-negative tumors relative to those with ERCC1-positive tumors [11]. Following publication of the ERCC1 protein expression level as a biomarker of survival benefit and treatment efficacy for cisplatin-based adjuvant chemotherapy, numerous studies tested the predictive or prognostic role of ERCC1 expression levels in advanced NSCLC. Some of these studies have suggested that ERCC1 expression levels are associated with a clinical benefit of platinum-based chemotherapy in advanced NSCLC [12,13]. However, these studies investigated patients treated with high ERCC1 levels using a non-platinum regimen, while none have compared the efficacy of platinum chemotherapy according to the ERCC1 level in advanced NSCLC. Thus, we prospectively investigated the role of ERCC1 levels for predicting the efficacy of two different platinum-based regimens.

To date, most platinum-based regimens have demonstrated similar efficacy in unselected patients with advanced NSCLC [3]. Moreover, gemcitabine and cisplatin (GP) and irinotecan and cisplatin (IP) regimens showed similar efficacy in advanced NSCLC. [4] Although some studies have shown an inverse correlation between ERCC1 levels and efficacy toward GP, none have investigated ERCC1 level and the efficacy toward IP regimen in NSCLC. Interestingly, high ERCC1 levels were reportedly associated with irinotecan efficacy in advanced colorectal cancer [14]. Thus, we hypothesized that IP and GP regimens show different efficacy according to ERCC1 levels.

To date, immunohistochemical analysis using the mouse monoclonal antibody 8F1 has been the most commonly used technique for measuring ERCC1 protein expression. Given the feasibility of immunohistochemical analysis of ERCC1 in biopsy samples, we investigated the clinical usefulness of ERCC1 as a predictive biomarker for two different platinum-based regimens in advanced NSCLC.

Materials and Methods

1. Eligibility criteria

The main eligibility criteria included histological confirmation of advanced NSCLC, no prior chemotherapy, age ≥ 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) < 2, and measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST). Additionally, adequate hematologic (white blood cell count ≥ 4,000/mm³, platelet count ≥ 150,000/mm³), hepatic (bilirubin level ≤ 1.5 mg/dL, aspartate aminotransferase/alanine transaminase ≤ 80 IU/L), and renal (creatinine concentration ≤ 1.5 mg/dL) function was required. Patients with brain metastases were enrolled if they were clinically stable without steroid treatment. The exclusion criteria included serious concomitant systemic diseases and second primary malignancies within the preceding 5 years. The protocol was approved by an independent ethics committee/institutional review board, and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Each patient provided written informed consent (NCT-01003964).

2. Study design

This was an open-label, randomized phase II trial that compared the efficacy of GP versus IP chemotherapy in chemo-naïve advanced NSCLC patients according to ERCC1 expression level. To minimize the impact of subsequent therapy on overall survival (OS), second- or third-line therapies were predefined. Patients with non-squamous cell lung cancer randomly received pemetrexed followed by docetaxel or docetaxel followed by pemetrexed as the second- or third-line treatment when the disease progressed. Patients with squamous cell lung cancer received docetaxel as a second-line therapy.

3. Random assignment and treatment plan

After ERCC1 assessment, patients were randomly assigned to either the IP or GP arm using the random block-size permutation method based on a computer-generated block randomization schedule. Patients in the IP arm received 65 mg/m² irinotecan and 30 mg/m² cisplatin on days 1 and 8 every 3 weeks. Patients in the GP arm received 1,250 mg/m² gemcitabine on days 1 and 8 and 75 mg/m² cisplatin on day 1 every 3 weeks. Patients were treated for a maximum of nine cycles or until reaching progressive disease (PD), death, or unacceptable toxicity.

As the disease progressed, patients with non-squamous
cell lung cancer were randomly assigned to receive either pemetrexed (500 mg/m² every 3 weeks) or docetaxel (75 mg/m² every 3 weeks). Patients with squamous cell lung cancer received docetaxel as second-line therapy with disease progression. The randomization process is shown in S1 Fig.

4. Study assessment

The safety assessment included patient history, physical examination, vital signs, ECOG PS, adverse effects and electrocardiography blood chemistry and hematology findings. Safety assessments were performed upon screening, on day 1 of subsequent cycles, and during the final study visit using Common Terminology Criteria for Adverse Events (CTCAE) ver. 3.0 [15].

Objective tumor responses were assessed using RECIST ver. 1.0 [16] after every two cycles of therapy. Progression-free survival (PFS) was calculated from the date of random assignment to PD or death. OS was calculated from the date of random assignment to death or the last follow-up.

5. Immunohistochemistry for ERCC1 expression

This study required the collection of formalin-fixed paraffin-embedded tumor blocks before therapy. The primary antibody for the detection of ERCC1 was clone 8F1 (catalog No. GTX22356 from GeneTex, Irvine, CA). One pathologist (G.K.L.) who was unaware of the clinical data independently evaluated ERCC1 staining under a light microscope at a magnification of ×400. Staining intensity was graded on a scale of 0 to 3. Five images of representative areas were acquired at a magnification of ×400 for each specimen, and a total of 500 to 1,500 positive or negative tumor nuclei per specimen were manually counted. The percentage of positive tumor nuclei was calculated for each specimen, and a proportion score was assigned (1 if 0%, 2 if 0 < to ≤ 10%, 3 if 10 < to ≤ 25%, 4 if 25 < to ≤ 50%, and 5 if > 50%). This proportion score was multiplied by the staining intensity of nuclei to obtain a final semiquantitative H score. Tumors with an H score exceeding 15 (i.e., tumors with a staining intensity score of 3 and 50% or more positive nuclei) were deemed ERCC1 positive.

6. EGFR and KRAS mutations and anaplastic lymphoma kinase–fluorescence in situ hybridization analysis

We analyzed EGFR and KRAS mutations using a polymerase chain reaction–based direct DNA sequencing method [17]. Anaplastic lymphoma kinase (ALK) rearrangements were detected by fluorescent in situ hybridization in formalin-fixed paraffin-embedded specimens using the break-apart probe for the ALK gene (Vysis LSI ALK Dual Color, Abbott Molecular, Abbott Park, IL) [18].

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**Fig. 1.** CONSORT diagram. ERCC1, excision repair cross-complementation group 1; GP, gemcitabine and cisplatin; IP, irinotecan and cisplatin; PFS, progression-free survival; OS, overall survival.
Table 1. Patient demographics and disease characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n=279)</th>
<th>GP arm (n=139)</th>
<th>IP arm (n=140)</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>181 (64.9)</td>
<td>91 (65.5)</td>
<td>90 (64.3)</td>
<td>0.900</td>
</tr>
<tr>
<td>Female</td>
<td>98 (35.1)</td>
<td>48 (34.5)</td>
<td>50 (35.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, median (range, yr)</strong></td>
<td>59 (28-82)</td>
<td>58 (28-79)</td>
<td>60 (32-82)</td>
<td>0.797</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>100 (35.8)</td>
<td>49 (35.3)</td>
<td>51 (36.4)</td>
<td>0.916</td>
</tr>
<tr>
<td>Former</td>
<td>76 (27.2)</td>
<td>37 (26.6)</td>
<td>39 (27.9)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>103 (36.9)</td>
<td>53 (38.1)</td>
<td>50 (35.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>215 (77.1)</td>
<td>109 (78.4)</td>
<td>106 (75.7)</td>
<td>0.664</td>
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<td>Squamous cell</td>
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<td>23 (16.4)</td>
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<td>Large cell</td>
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<td>Sarcomatoid</td>
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<td>1 (0.7)</td>
<td>2 (1.4)</td>
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<tr>
<td>NOS</td>
<td>14 (5.0)</td>
<td>7 (5.0)</td>
<td>7 (5.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>24 (8.6)</td>
<td>12 (8.6)</td>
<td>12 (8.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>IV</td>
<td>255 (91.4)</td>
<td>127 (91.4)</td>
<td>128 (91.4)</td>
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</tr>
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<td><strong>ECOG PS</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>0</td>
<td>21 (7.5)</td>
<td>15 (10.8)</td>
<td>6 (4.3)</td>
<td>0.102</td>
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<tr>
<td>1</td>
<td>171 (61.3)</td>
<td>80 (57.6)</td>
<td>91 (65.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>87 (31.2)</td>
<td>44 (31.7)</td>
<td>43 (30.7)</td>
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<td><strong>ERCC1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
<td>102 (36.6)</td>
<td>49 (35.3)</td>
<td>53 (37.9)</td>
<td>0.710</td>
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<tr>
<td>Positive</td>
<td>177 (63.4)</td>
<td>90 (64.7)</td>
<td>87 (62.1)</td>
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</tr>
<tr>
<td><strong>EGFR mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19DEL or L858R</td>
<td>61 (21.9)</td>
<td>27 (19.4)</td>
<td>34 (24.3)</td>
<td>0.558</td>
</tr>
<tr>
<td>Rare exon 20 mutations</td>
<td>6 (2.2)</td>
<td>3 (2.2)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
<tr>
<td>T790M</td>
<td>5 (1.8)</td>
<td>1 (0.7)</td>
<td>4 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>150 (53.8)</td>
<td>78 (56.1)</td>
<td>72 (51.4)</td>
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</tr>
<tr>
<td>Not determined</td>
<td>57 (20.4)</td>
<td>30 (21.6)</td>
<td>27 (19.3)</td>
<td></td>
</tr>
<tr>
<td><strong>KRAS mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G12X</td>
<td>12 (4.3)</td>
<td>8 (5.8)</td>
<td>4 (2.9)</td>
<td>0.388</td>
</tr>
<tr>
<td>Wild type</td>
<td>128 (45.9)</td>
<td>60 (43.2)</td>
<td>68 (48.6)</td>
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</tr>
<tr>
<td>Not determined</td>
<td>139 (49.8)</td>
<td>71 (51.1)</td>
<td>68 (48.6)</td>
<td></td>
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<tr>
<td><strong>ALK-FISH</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4 (1.4)</td>
<td>2 (1.4)</td>
<td>2 (1.4)</td>
<td>0.819</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (3.6)</td>
<td>4 (2.9)</td>
<td>6 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Not determined</td>
<td>265 (95.0)</td>
<td>133 (95.7)</td>
<td>132 (94.3)</td>
<td></td>
</tr>
</tbody>
</table>

GP, gemcitabine and cisplatin; IP, irinotecan and cisplatin; NOS, not otherwise specified; ECOG PS, Eastern Cooperative Oncology Group performance status; ERCC1, excision repair cross-complementation group 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; FISH, fluorescent in situ hybridization.

7. Statistical analysis

The primary objective was to estimate the efficacy of GP or IP chemotherapy according to ERCC1 expression in patients with advanced NSCLC. The primary end point was the RR, which was defined as the proportion of patients whose best response was complete response or partial response among all per-protocol patients. The study employed a marker by treatment interaction design. We expected that the proportion of ERCC1-positive patients
would be almost the same in both treatment groups. Moreover, it was expected that the ERCC1-negative group would show a better RR than the ERCC1-positive group. The expected RR for the ERCC-negative group was approximately 45%, whereas a 30% RR was expected for the ERCC1-positive group. A total of 142 patients were needed for each group to detect a 15% difference in the RR between the ERCC1-positive and ERCC1-negative groups with 83% power, a 5% level of significance, and a one-sided test. The patients were randomly assigned to either the GP or IP arm at an equivalent ratio.

Additional end points included PFS, OS, and toxicities. All patients who received at least one cycle of chemotherapy were considered assessable for PFS, OS, and safety. All hypotheses were tested at a two-sided α level of 0.05. The log-rank test was used to compare PFS and OS according to the ERCC1 expression levels. The distribution of PFS and OS was estimated using the Kaplan-Meier method. Statistical comparison of the RRs according to mutation status was performed using chi-squared or Fisher exact tests. We also assessed interactions between treatment groups and ERCC1 expression subgroups in relation to RR and survivals, PFS and OS, using logistic regression and Cox regression test, respectively.

Results

1. Patient and treatment characteristics

Between February 2009 and September 2013, 289 patients were enrolled. Among 283 patients who underwent ERCC1 assessment, 177 patients (62.5%) were ERCC1 positive, and 106 patients (37.5) were ERCC1 negative. Finally, 279 patients received treatment per protocol after ERCC1 assessment (Fig. 1). The characteristics of the 279 patients who were randomly assigned to each treatment arm are summarized in Table 1. Most patients were male (64.9%), ever smokers (63.1%), and exhibited good PS (68.8%), stage IV disease (91.4%), and adenocarcinoma histology (78.1%).

EGFR or KRAS mutation testing was not routinely performed at our institution at the time of the study initiation; thus, we retrospectively collected mutation data. The EGFR and KRAS mutation status were available in 222 (79.6%) and 140 (50.2%) patients, respectively, while there was no significant difference in the frequencies between treatment arms. We also did not find any significant association between EGFR or KRAS mutation status and ERCC1 level.

The median number of treatment cycles given to patients in both arms did not differ significantly relative to treatment assignment or ERCC1 expression (Mann-Whitney test; p=0.418 and p=0.503, respectively) (Table 2).

2. Treatment outcome according to ERCC1 level and treatment arm

Of the 279 patients in this study, 268 were assessable for a response. First, we compared the RR according to the treatment arms. The RR was 29.8% (95% confidence interval [CI], 22.0 to 38.4) for the GP arm and 27.0% (95% CI, 19.8 to 35.3) for the IP arm (p=0.082). When the RR was compared according to the ERCC1 level in each treatment arm, there were no significant differences (Table 3). We also compared the efficacy according to the ERCC1 level and found no significant differences. The RR was 29.2% (95% CI, 22.6 to 36.7) for the ERCC1-positive group and 26.8% (95% CI, 18.3 to 36.8) for the ERCC1-negative group (p=0.741). When the RR was compared according to treatment arm in each ERCC1 group, there was no significant difference between the GP and IP arms in the ERCC1-positive group (28.2% for GP vs. 30.2% for IP; p=0.362). Additionally, the GP arm showed a trend toward a higher RR than the IP arm in the ERCC1-negative group (32.6% vs. 21.6%, respectively; p=0.085).

The cutoff for the OS update was June 29, 2015, and the median duration of follow-up was 16.7 months (range, 0.5 to 70.9 months). Of the 279 patients in this study, 266 patients

<table>
<thead>
<tr>
<th>Table 2. Cycles of treatment by arm and assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Standard deviation</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

GP, gemcitabine and cisplatin; IP, irinotecan and cisplatin; ERCC1, excision repair cross-complementation group 1.
Table 3. Outcome according to treatment arms and ERCC1 levels

<table>
<thead>
<tr>
<th>Response</th>
<th>Overall response rate (95% CI, mo)</th>
<th>p-value</th>
<th>Median PFS (95% CI, mo)</th>
<th>p-value</th>
<th>Median OS (95% CI, mo)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>Partial response</td>
<td>Stable disease</td>
<td>Progressive disease</td>
<td>Not determined</td>
<td>GP (n=139)</td>
<td>IP (n=140)</td>
</tr>
<tr>
<td>0</td>
<td>39</td>
<td>68</td>
<td>24</td>
<td>8</td>
<td>29.8 (22.0-38.4)</td>
<td>0.082</td>
</tr>
<tr>
<td>0</td>
<td>37</td>
<td>59</td>
<td>41</td>
<td>3</td>
<td>27.0 (19.8-35.3)</td>
<td>3.9 (3.3-4.5)</td>
</tr>
<tr>
<td>ERCC1 Pos (n=90)</td>
<td>ERCC1 Neg (n=49)</td>
<td>ERCC1 Pos (n=87)</td>
<td>ERCC1 Neg (n=53)</td>
<td>ERCC1 Pos (n=177)</td>
<td>ERCC1 Neg (n=102)</td>
<td>ERCC1 Pos (n=177)</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>43</td>
<td>18</td>
<td>5</td>
<td>28.2 (19.0-39.0)</td>
<td>0.509</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>32.6 (19.5-48.0)</td>
<td>5.0 (4.1-5.9)</td>
</tr>
<tr>
<td>-</td>
<td>26</td>
<td>35</td>
<td>25</td>
<td>1</td>
<td>30.2 (20.8-41.1)</td>
<td>3.9 (2.9-4.9)</td>
</tr>
<tr>
<td>-</td>
<td>11</td>
<td>24</td>
<td>16</td>
<td>2</td>
<td>21.6 (11.3-35.3)</td>
<td>3.7 (2.8-4.6)</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>78</td>
<td>43</td>
<td>6</td>
<td>29.2 (22.6-36.7)</td>
<td>4.4 (3.8-5.0)</td>
</tr>
<tr>
<td>0</td>
<td>26</td>
<td>49</td>
<td>22</td>
<td>5</td>
<td>26.8 (18.3-36.8)</td>
<td>4.1 (3.0-5.2)</td>
</tr>
<tr>
<td>GP (n=90)</td>
<td>IP (n=87)</td>
<td>GP (n=49)</td>
<td>IP (n=53)</td>
<td>GP (n=90)</td>
<td>IP (n=87)</td>
<td>GP (n=49)</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>43</td>
<td>18</td>
<td>5</td>
<td>28.2 (19.0-39.4)</td>
<td>0.362</td>
</tr>
<tr>
<td>0</td>
<td>26</td>
<td>35</td>
<td>25</td>
<td>1</td>
<td>30.2 (20.8-41.1)</td>
<td>3.9 (2.9-4.9)</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>25</td>
<td>6</td>
<td>3</td>
<td>32.6 (19.5-48.0)</td>
<td>5.0 (4.1-5.9)</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
<td>24</td>
<td>16</td>
<td>2</td>
<td>21.6 (11.3-35.3)</td>
<td>3.7 (2.8-4.6)</td>
</tr>
</tbody>
</table>

ERCC1, excision repair cross-complementation group 1; CI, confidential interval; PFS, progression-free survival; OS, overall survival; GP, gemcitabine and cisplatin; IP, irinotecan and cisplatin; Pos, positive; Neg, negative.
Table 4. Subsequent treatment

<table>
<thead>
<tr>
<th>Second-line therapy</th>
<th>GP (n=139)</th>
<th>IP (n=140)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemetrexed</td>
<td>40 (28.8)</td>
<td>39 (27.9)</td>
<td>0.189</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>35 (25.2)</td>
<td>51 (36.4)</td>
<td></td>
</tr>
<tr>
<td>EGFR-TKI</td>
<td>34 (24.5)</td>
<td>33 (23.6)</td>
<td></td>
</tr>
<tr>
<td>Platinum-based doublet</td>
<td>2 (1.4)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Crizotinib</td>
<td>2 (1.4)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>26 (18.7)</td>
<td>13 (9.3)</td>
<td></td>
</tr>
<tr>
<td>Third-line therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>38 (27.3)</td>
<td>46 (32.9)</td>
<td>0.428</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>15 (10.8)</td>
<td>16 (11.4)</td>
<td></td>
</tr>
<tr>
<td>EGFR-TKI</td>
<td>31 (22.3)</td>
<td>26 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Doublet chemotherapy</td>
<td>8 (5.8)</td>
<td>14 (10.0)</td>
<td></td>
</tr>
<tr>
<td>ALK-TKI</td>
<td>2 (1.4)</td>
<td>1 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>2 (1.4)</td>
<td>3 (2.1)</td>
<td></td>
</tr>
<tr>
<td>Anti-PD1 therapy</td>
<td>0</td>
<td>2 (1.4)</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>43 (30.9)</td>
<td>32 (22.9)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). GP, gemcitabine and cisplatin; IP, irinotecan and cisplatin; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; ALK, anaplastic lymphoma kinase.

Table 5. Adverse events ≥ grade 3

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>IP</th>
<th>GP</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Anemia</td>
<td>14</td>
<td>22</td>
<td>0.155</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>14</td>
<td>9</td>
<td>0.385</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>58</td>
<td>53</td>
<td>0.715</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2</td>
<td>12</td>
<td>0.006</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>11</td>
<td>0</td>
<td>0.001</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
<td>6</td>
<td>0.538</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>1</td>
<td>0.371</td>
</tr>
</tbody>
</table>

IP, irinotecan and cisplatin; GP, gemcitabine and cisplatin.

(95.3%) suffered disease progression or died. The median PFS and OS for all patients were 4.3 months (95% CI, 3.7 to 4.9) and 16.7 months (95% CI, 14.0 to 19.4), respectively. When the PFS and OS were compared according to the ERCC1 level or treatment arm, there was no significant difference (Table 3, S2 Fig, A-D). Moreover, subgroup analysis revealed no significant differences between the GP and IP arms in the ERCC-positive (S3 Fig, A and B) or -negative group (S3 Fig, C and D). When the PFS were compared according to the ERCC1 level in the GP arm, no difference was observed (S4 Fig, A). However, the ERCC1-positive group showed a trend toward longer OS than the ERCC1-negative group in the GP arm (18.6 months vs. 11.9 months, respectively; p=0.07) (S4 Fig, B). In the IP arm, there was no significant difference in survival according to ERCC1 level (S4 Fig, C and D).

Interaction tests did not show a significant difference in RR, PFS, and OS between the ERCC1-positive and ERCC1-negative group. The p-values for the interaction treatment with ERCC1 subgroup for RR, PFS, and OS were 0.248, 0.233, and 0.773, respectively.

3. Subsequent treatment

Of the 279 patients in this study, 240 (86%) and 204 (73%) received second-line and third-line therapy, respectively. Of the 240 patients who received second-line therapy, 165 (69%) received predefined second-line therapy. Of the 204 patients
who received third-line therapy, 105 (51%) received predefined third-line therapy. There was no significant difference in the subsequent treatments given to each arm (Table 4).

4. Adverse events

Anemia and neutropenia were the most commonly reported treatment-related adverse events. Overall, the grade 3 or 4 toxicity rates were low in both arms (Table 5). Grade 3 diarrhea was more common in the IP arm (p=0.001), while grade 3 or 4 thrombocytopenia was more common in the GP arm (p=0.006). There were no treatment-related deaths.

Discussion

Treatment of advanced NSCLC has changed significantly since demonstration of the superior efficacy of target therapy against driver oncoproteins such as EGFR mutations and ALK rearrangements. Nevertheless, platinum-based chemotherapy remains the standard therapy for advanced NSCLC without known driver mutations. At the time of this study initiation, platinum-based chemotherapy was the standard for advanced NSCLC in Korea. Routine EGFR mutation testing for selected first-line therapy started in April 2011 in Korea. In this study, we prospectively investigated the predictive value of ERCC1 expression level in advanced NSCLC patients who participated in a randomized phase II study comparing the efficacy of GP versus IP. Furthermore, our study predefined the subsequent treatment course to minimize the impact on OS. This design allowed us to test the prognosis and predictive value of the ERCC1 level in advanced NSCLC. However, we did not find any significant difference in RR, PFS, or OS according to the ERCC1 level in patients with advanced NSCLC.

Correlative biomarker analysis from two randomized phase III trials demonstrated that low ERCC1 protein or mRNA levels are associated with a better RR to platinum-based chemotherapy in advanced NSCLC. Nevertheless, both studies failed to demonstrate any significant association with survival [12,13]. Recently, another randomized phase III study investigated whether ERCC1 protein level-based chemotherapy selection would improve survival in advanced NSCLC; however, this investigation failed to demonstrate any differential benefit in RR or survival [19]. To date, patients with high ERCC1 levels have been assigned to non-platinum chemotherapy in ERCC1 level-based clinical trials for advanced NSCLC. However, no studies have compared the efficacy of the same platinum chemotherapy according to the ERCC1 level. Because the patients in our study received homogeneous platinum chemotherapy, we could prospectively investigate the predictive value of the ERCC1 level on platinum chemotherapy. However, we did not detect any correlation between the ERCC1 level and efficacy of GP or IP in advanced NSCLC. Moreover, the ERCC1 level was not prognostic in these patients.

Recently, a validation study using the ERCC1 8F1 antibody from two independent randomized trials of postoperative adjuvant cisplatin-based chemotherapy failed to validate ERCC1 protein expression as a predictive biomarker in NSCLC [20,21]. The authors suggested that a change in the performance of ERCC1 8F1 antibody since 2006 resulted in discordance in ERCC1 staining, even in sample samples. Furthermore, they found that the currently available ERCC1 antibodies could not distinguish functional from non-functional isoforms, which may result in misclassification of tumors. Moreover, Schneider et al. [22] reported that the results of commercial ERCC1 testing are inconsistent and unreliable. These authors evaluated ERCC1 testing offered by three large commercial laboratories and found a significant difference in the independent laboratory ERCC1 expression rates (70% vs. 60% vs. 44%, p < 0.0001). Furthermore, none of the assays could predict platinum resistance with a specificity greater than 50% [22]. Taken together, these findings suggest that ERCC1 testing is not applicable for routine practice in patients with NSCLC.

Because NSCLC patients are usually treated with combination chemotherapy, it may be necessary to assess multiple biomarkers. In addition to ERCC1, RRMI and BRAC1 levels have been investigated; however, none of these markers have demonstrated an adequate level of evidence for routine clinical use [23,24]. In addition to the ERCC1 expression levels, ERCC1 polymorphisms have been evaluated for cisplatin sensitivity in NSCLC. A meta-analysis of 1,252 NSCLC patients reported that there was no significant link between ERCC1 polymorphism and cisplatin sensitivity [25].

Despite the considerable potential for use of ERCC1 as a biomarker for cisplatin sensitivity, our study showed that immunohistochemical staining of ERCC1 is not adequate for selecting patients for platinum-based chemotherapy in advanced NSCLC. Further efforts are needed to develop clinically useful biomarkers to determine sensitivity to platinum-based chemotherapy in NSCLC.

Conclusion

There was no significant difference in efficacy between GP and IP treatment according to the ERCC1 expression level of the patient. Immunohistochemical analysis of the ERCC1
expression level did not differentiate the efficacy of platinum-based chemotherapy in advanced NSCLC.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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References


Survey of the Patterns of Using Stereotactic Ablative Radiotherapy for Early-Stage Non-small Cell Lung Cancer in Korea

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Purpose
Stereotactic ablative radiotherapy (SABR) is an effective emerging technique for early-stage non-small cell lung cancer (NSCLC). We investigated the current practice of SABR for early-stage NSCLC in Korea.

Materials and Methods
We conducted a nationwide survey of SABR for NSCLC by sending e-mails to all board-certified members of the Korean Society for Radiation Oncology. The survey included 23 questions focusing on the technical aspects of SABR and 18 questions seeking the participants’ opinions on specific clinical scenarios in the use of SABR for early-stage NSCLC. Overall, 79 radiation oncologists at 61/85 specialist hospitals in Korea (71.8%) responded to the survey.

Results
SABR was used at 33 institutions (54%) to treat NSCLC. Regarding technical aspects, the most common planning methods were the rotational intensity-modulated technique (59%) and the static intensity-modulated technique (49%). Respiratory motion was managed by gating (54%) or abdominal compression (51%), and 86% of the planning scans were obtained using 4-dimensional computed tomography. In the clinical scenarios, the most commonly chosen fractionation schedule for peripherally located T1 NSCLC was 60 Gy in four fractions. For centrally located tumors and T2 NSCLC, the oncologists tended to avoid SABR for radiotherapy, and extended the fractionation schedule.

Conclusion
The results of our survey indicated that SABR is increasingly being used to treat NSCLC in Korea. However, there were wide variations in the technical protocols and fractionation schedules of SABR for early-stage NSCLC among institutions. Standardization of SABR is necessary before implementing nationwide, multicenter, randomized studies.

Key words
Non-small cell lung carcinoma, Stereotactic body radiotherapy, Clinical practice pattern, Surveys and questionnaires
Introduction

Surgical resection has long been the mainstay treatment option for early-stage non-small cell lung cancer (NSCLC). In recent decades, the introduction of stereotactic ablative radiotherapy (SABR) has provided an opportunity to cure NSCLC in patients with high surgical risks. SABR showed comparable local control with minimal toxicity compared with surgical resection in a retrospective study [1,2]. However, owing to its short history of development, there are still some controversies regarding the best strategies for SABR, including the optimal indication, dose, fractionation, planning method, and management of respiratory motion. In many countries, several studies have investigated the differences in the patterns of using SABR for NSCLC [1-3]. These differences in practice are potential hurdles to implementing multicenter trials. The purpose of the current study was to understand the current practices of using SABR for early-stage NSCLC in Korea. The lung cancer subcommittee of the Korean Society for Radiation Oncology intends to use these results to develop new protocols and standards for multicenter trials.

Materials and Methods

1. Survey design

This three-part survey was developed by the lung cancer subcommittee of the Korean Society for Radiation Oncology. SABR was defined as hypofractionated radiotherapy (1-8 fractions). The full questionnaire is provided in Supplementary. The first part of the survey recorded the responder and the institution characteristics, including the location; demographic characteristics of radiation oncologists, lung specialists, and medical physicists; how long SABR has been used to treat NSCLC; and the annual number of patients who undergo SABR for NSCLC. The second part of the survey focused on technical aspects of SABR for NSCLC, including the equipment used, planning systems, immobilization and motion-management methods, simulation techniques, image guidance, and quality assurance. The third part of the survey included questions about the clinical decisions and the choice of treatment modality for specific clinical scenarios. Some of the questions allowed multiple responses to reflect diverse opinions.

2. Responses and statistics

The survey was e-mailed to all board-certified members of the Korean Society for Radiation Oncology. The e-mail was sent four times between June 2014 and January 2015 to collect as many responses as possible. The survey results were collected and analyzed on March 2015. A total of 79 radiation oncologists responded, from 61 of the 85 institutions (71.8%) in South Korea that have a radiation oncology department.

Statistical analyses were performed using SPSS software (release 18.0.0, SPSS Inc., Chicago, IL). The $\chi^2$ test and Fisher exact test were used to identify differences among groups. A two-sided p-value of $\leq 0.05$ was considered statistically significant.

Results

1. Characteristics of the institutions and radiation oncologists

Of the 61 institutions that responded to survey, 33 used SABR for NSCLC and were evaluated in this study. There were 28 academic institutions (85%) and five non-academic institutions (15%). Nineteen institutions (58%) were located in Seoul and its metropolitan areas. There were one or two board-certified radiation oncologists at each of 13 institutions (40%), three at each of 12 institutions (36%), and 4 or more at each of 15 institutions (24%). Most institutions had one radiation oncologist who had specialized in lung cancer (64%) together with one medical physicist (61%). Eleven institutions (33%) had used SABR to treat NSCLC for $<2$ years and 10 (30%) had used SABR for $>5$ years. The time that SABR was first used to treat NSCLC is shown in Fig. 1. Overall, 37 radiation oncologists with experience of using SABR for lung cancer completed the survey and provided evaluable responses. Twenty-two of the respondents (59%) worked in Seoul and its metropolitan areas. The characteristics of the radiation oncologists are shown in Table 1.

2. Technical aspects and follow-up policy

Multiple responses were allowed for questions on the technical aspects of SABR. Rotational intensity-modulation radiotherapy (IMRT) (59%) was the most common planning method followed by static IMRT (49%). Immobilization methods included the wing board and vacuum lock system, which were used by 54% and 51% of respondents, respectively. For motion management, 54% of the respondents used abdominal compression to control target movement and 51%
Table 1. Characteristics of the radiation oncologists

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of respondents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Academic affiliation</td>
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</tr>
<tr>
<td>Yes</td>
<td>30 (81)</td>
</tr>
<tr>
<td>No</td>
<td>7 (19)</td>
</tr>
<tr>
<td>Time since board certification (yr)</td>
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</tr>
<tr>
<td>&lt; 2</td>
<td>3 (8)</td>
</tr>
<tr>
<td>2-5</td>
<td>7 (19)</td>
</tr>
<tr>
<td>5-10</td>
<td>9 (24)</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>18 (49)</td>
</tr>
<tr>
<td>How long the respondent has performed SABR (yr)</td>
<td></td>
</tr>
<tr>
<td>&lt; 2</td>
<td>16 (43)</td>
</tr>
<tr>
<td>2-5</td>
<td>11 (30)</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>10 (27)</td>
</tr>
<tr>
<td>No. of cases of SABR per year</td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>14 (38)</td>
</tr>
<tr>
<td>5-10</td>
<td>9 (24)</td>
</tr>
<tr>
<td>10-20</td>
<td>10 (27)</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>4 (11)</td>
</tr>
</tbody>
</table>

SABR, stereotactic ablative radiotherapy.

used respiratory gating. The technical aspects of SABR are summarized in Table 2. With a fractionated schedule, 49% of respondents treat all patients on consecutive days, 38% used a more prolonged schedule, and 13% used both schedules. Most of the respondents (86%) obtained the first follow-up images at 4-7 weeks after SABR, and the most common imaging modalities were computed tomography (CT) (78%), followed by positron emission tomography (PET) (11%), and both CT and PET (11%).

3. Clinical decisions

The surveyed oncologists were asked whether they think it is necessary to obtain pathological confirmation before performing SABR in cases with a high risk of procedure complications or technical difficulties, if clinical T1 or T2 lung cancer is most likely. The oncologists frequently (46%) recommended that patients should undergo pathologic confirmation by biopsy before performing SABR. The other options included that SABR could be performed only after CT or PET studies had been completed (38%), and that SABR could be performed after a close imaging follow-up (14%). The
respondents were also asked how they would handle suspicious mediastinal or hilar lymph node enlargements diagnosed as reactive nodes by CT or PET. Fifty-four percent of the responders recommended pathologic confirmation before SABR, 41% recommended close observation with imaging modalities after SABR, 3% responded that they would not perform SABR in this case, and one clinician would perform SABR in a T1 case and recommend pathologic confirmation in a T2 case. We also asked the respondents to describe how pulmonary function test (PFT) results influence their decision to perform SABR. Most of the respondents (62%) thought that the patient’s clinical condition is more important than the PFT results, while 14% of the respondents stated that they would set limitations on PFT results which would not consider SABR.
4. Case management

Case 1P was a 75-year-old male with stage I, cT1 (1.6 cm), N0 NSCLC located peripherally to the mediastinum and 2.2 cm from the chest wall (Fig. 2A). We assumed that he was diagnosed with adenocarcinoma, had good performance status (Zubrod 1), but his pulmonary function was too poor to perform lobectomy. Nearly all of the respondents (94%) recommended SABR and the most frequently selected fractionation schedule was 60 Gy in four fractions (46%) followed by 48 Gy / four fractions (19%) and 60 Gy / three fractions (16%).

Case 1C was identical to case 1P, except the tumor was located < 2 cm from the mediastinum. For case 1C, SABR was recommended by 43% of the respondents and hypofractionated conventional radiotherapy was recommended by 41%. The most frequently selected fractionation schedule was 60 Gy in 10 fractions, chosen by 14% of respondents; however, there was little consensus with regard to the fractionation schedule. Other schedules used included 60 Gy / 15 fractions (11%) and 50 Gy / five fractions (8%).

Case 2P was a 75-year-old male with stage I, cT2 (4.5 cm), N0 NSCLC with a performance status of Zubrod 1, but his pulmonary function was poor and he was scheduled to undergo lobectomy (Fig. 2B). Most of the respondents recommended radiotherapy alone (76%). Concurrent chemoradiotherapy and sublobar resection were recommended by 14% and 5%, respectively. SABR was selected as the treatment of choice by 59% of respondents and hypofractionated radiotherapy by 19% of respondents. The most frequently selected fractionation schedule was 60 Gy in four fractions (32%).

Case 2C was identical to 2P, except that it was central NSCLC (< 2 cm from the mediastinum). Radiotherapy alone, concurrent chemoradiotherapy, and sublobar resection were recommended by 76%, 16%, and 8% of respondents, respectively. Only 19% of respondents considered SABR as the treatment of choice; most recommended hypofractionated conventional radiotherapy (44%). The treatment options selected for each case are shown in Fig. 3.

Discussion

In this nationwide survey, we assessed the current practices of using SABR to treat early-stage NSCLC in Korea. SABR for NSCLC was rapidly incorporated into clinical practice in Korea (Fig. 1). Most of the institutions started SABR for NSCLC within the last 5 years. The majority of the radiation oncologists (72%) with experience of SABR used this technique in < 10 NSCLC patients per year. This low volume suggests that we should conduct multicenter trials rather than single-center trials in future studies of SABR for NSCLC.

The technical aspects of SABR varied widely among the institutions and could represent potential hurdles to multicenter trials. In particular, there were marked variations in the planning techniques and methods used to manage respiratory motion. Accordingly, it will be necessary to standardize these factors before commencing multicenter trials. Most institutions used modern imaging modalities with radiotherapy, including four-dimensional CT simulation, IMRT, and volumetric image guidance. These rapid adaptations of modern techniques make it feasible to perform high-quality trials.

Although SABR has been rapidly integrated into clinical practice, there are some issues to address. One of the issues is mandatory pathologic examination before treatment in high-risk patients. In our survey, 46% of the respondents sought pathologic confirmation before performing SABR. Another issue is the value of mediastinal staging. More than half of the respondents stated that pathologic confirmation was necessary before SABR in cases with reactive mediastinal nodes. Of course, extensive diagnostic staging procedures will be required in multicenter trials.

Currently, various fractionation schedules are used for SABR, including 20 Gy x 3, 18 Gy x 3, 16 Gy x 3, 15 Gy x 4, 12 Gy x 4, and 12 Gy x 5 fractions [4]. To compare the efficacy or toxicity of two different dose fractionation schemes, we conventionally use the biologically effective dose (BED), which is calculated from a linear quadratic model. It also has been adopted in studies comparing SABR fractionation schemes. It has been widely debated whether we should apply a LQ model to SABR, but multiple studies have revealed that radiotherapy doses exceeding a BED of 100 Gy or 105 Gy increase the local control rate and survival outcome, which suggests that the LQ model is suitable for comparing conventional dose schemes [5-9]. However, there is no consensus regarding the ideal fractionation schedule for SABR of NSCLC. In the present survey, even in cases with peripheral cT1 NSCLC, the most popular fractionation regimen (60 Gy in four fractions) was used by less than half of the respondents. This might be due to the national health insurance program of South Korea, which covers SABR only when SABR is given in four fractions. In T2 or central cases, the fractionation schedule varied considerably. Accordingly, it will be necessary to standardize the dose and fraction schedule for SABR before starting future trials.

We investigated the opinions of radiation oncologists regarding their use of SABR in cases with centrally located and/or large (cT2) cancers. Results of a phase II trial performed at the University of Indiana revealed a trend toward increased grade 3-5 toxicity in central tumors (27.3% vs.
10.4%, p=0.088) [10], raising concern of increased toxicity of SABR for centrally located NSCLC. Accordingly, fewer respondents recommended SABR for central NSCLC than for peripheral NSCLC in the current survey (Fig. 3). In terms of tumor size, T2 NSCLC was associated with a lower local control rate than T1 NSCLC following SABR in prior studies [11,12]. The part of the survey focusing on preferred treatments for T2 NSCLC revealed that concurrent chemotherapy was more common in T1 cases than in T2 cases. Many radiation oncologists hesitated to use SABR for centrally located T2 NSCLC. Only 19% of the respondents stated that they would perform SABR in cases with centrally located T2 NSCLC. Because of the varying influences of location and size of the cancer on clinical practices, clinical trials should include stratified protocols to account for these factors. Our study has several limitations. First, not all of the board-certified radiation oncologists in Korea responded to the survey, which might introduce selection bias. In addition, because multiple responses were allowed for some of the technical questions, we may overestimate the rate of infrequently used techniques. Finally, although we sought to integrate most of the approaches used in actual practice, some practices were not reported because the list of responses was incomplete.

Conclusion

In conclusion, this nationwide survey confirmed that the use of SABR for treating early-stage NSCLC has increased dramatically in Korea in recent years. The technical aspects and clinical decisions for SABR for treating NSCLC varied markedly. The treatment patterns of centrally located T2 NSCLC varied much more than those of peripherally located T1 NSCLC. These results highlight the need to standardize various aspects of the protocol when planning nationwide, multicenter, clinical trials.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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Clinicopathologic Characteristics and Prognosis of Tongue Squamous Cell Carcinoma in Patients with and without a History of Radiation for Nasopharyngeal Carcinoma: A Matched Case-Control Study

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Mian Xi, MD1,2

Purpose
Previous studies reported an association between an increased risk of tongue cancer and radiation treatment for nasopharyngeal carcinoma (NPC). This study compared the clinicopathologic characteristics and outcomes of tongue squamous cell carcinoma (TSCC) in patients with and without a history of radiotherapy for NPC.

Materials and Methods
From 1965 to 2009, a total of 73 patients were diagnosed with TSCC with a history of radiotherapy for NPC. The patients were matched in a 1:3 ratio with patients with sporadic TSCC according to age, sex, and year of the TSCC diagnosis. The primary endpoint was the overall survival.

Results
The median interval from NPC to TSCC was 82 months. The NPC survivors were more likely to be diagnosed with a more advanced T classification, less likely to have lymph node involvement, and more likely to have the tumor located in the dorsum of the tongue than sporadic TSCC. Regarding the histologic characteristics, the NPC survivors were more likely to have a weak lymphocytic host response, low tumor budding, and low risk of a worse pattern of invasion. The sporadic TSCC patients had a better overall survival (hazard ratio, 0.690; p=0.033) than the NPC survivors. In competing risks analysis, the cumulative incidence functions for the competing event (documented non-tongue cancer death) were significantly higher in the NPC survivors (Gray's test, p=0.001).

Conclusion
TSCC patients with a history of radiotherapy for NPC appear to have particular clinicopathologic features, a poorer survival, and are more likely to die from non-tongue cancer causes than those with sporadic TSCC.

Key words
Tongue neoplasms, Second primary cancer, Nasopharyngeal carcinoma, Prognosis, Morbidity

Introduction
In southern China, nasopharyngeal carcinoma (NPC) is endemic with an incidence ranging from 20 to 30 per 100 000 [1]. Advances in technology, particularly intensity-modulated radiotherapy, have brought revolutionary improvements in the management of NPC, with 5-year overall survival (OS) rates exceeding 80% [2,3]. Unfortunately, the ionizing radiation and chemotherapy regimens of the treat-
ment are themselves carcinogenic [4]. Several epidemiological studies have reported an increased incidence of second primary cancer in the NPC survival cohort [5-7]. Chen et al. [5] conducted a population-based study including 23,639 NPC patients and reported the oral cavity and pharynx to be the most common sites of a second primary cancer after radiation for NPC. Previous studies have reported an association between an increased risk of tongue cancer and radiation for NPC [6,8]. Most of this excess risk can be attributed to radiation of the oral cavity [8]. A previous study reported the tongue to be the most common site of second malignancies after NPC [9]. On the other hand, the clinicopathologic characteristics and outcomes of tongue cancer arising in NPC patients have not been explored in detail.

This study analyzed a large series of consecutive patients diagnosed with tongue squamous cell carcinoma (TSCC) at a single institution and identified patients with a prior history of radiotherapy for NPC. The clinicopathologic characteristics and survival were compared with a matched control group of patients with sporadic TSCC.

Materials and Methods

1. Patient and disease characteristics

The inclusion criteria were (1) a prior history of radiotherapy for NPC, (2) histological confirmation of TSCC, and (3) a latency period of at least 3 years between the end of radiotherapy and the diagnosis of TSCC. The criteria were adapted from those described by Cahan et al. [10] and Arlen et al. [11], who applied them to radiation-induced sarcomas. Such cases were excluded from the study sample because of the different treatment principles of tongue-base malignancies owing to the anatomical position.

From January 1965 to December 2009, 56,127 patients with NPC received radiotherapy at the Sun Yat-sen University Cancer Center. Of these, a total of 73 patients fulfilled the inclusion criteria. A total of 2,720 consecutive patients were diagnosed with tongue cancer with no history of concomitant or prior malignancy in the authors’ institution. For each NPC survivor, three matched patients (219 patients) with sporadic tongue cancer were selected. The two groups were matched according to the most relevant variables: sex, age at tongue cancer diagnosis (within 5 years), and year of the tongue cancer diagnosis (within 3 years). During the matching process, the investigators were blinded to the clinical outcomes of the TSCC. All patients underwent a regular follow-up at the authors’ hospital. The date of the last follow-up was September 2015. The institutional ethics committee of Sun Yat-sen University Cancer Center approved this study in accordance with the Declaration of Helsinki and written informed consent was obtained from all surviving patients or family members of the dead patients.

The radiation techniques for NPC are described in detail elsewhere [12,13]. The majority of patients were treated with conventional radiotherapy and the daily fraction was 2 Gy delivered using Cobalt-60 or 6-MV X-rays. Briefly, the two-dimensional radiation technique consisted of two phases. In the first phase, 50 Gy was delivered to the nasopharyngeal region with two lateral facial-cervical fields (Fig. 1A). In the second phase, a total dose of 16-26 Gy was delivered to the primary lesions in a smaller preauricular field (Fig. 1B). The anterior and posterior split fields were used in the neck region. The radiation dose to the cervical region was 50-54 Gy for N0 stage and 60-66 Gy for any positive lymph nodes.

The clinicopathologic characteristics of the TSCC patients and treatment modalities for TSCC were identified, including radiotherapy, surgery, and multimodality therapy. All patients diagnosed with TSCC were restaged according to the seventh edition of the American Joint Committee on the Cancer TNM staging system. Hematoxylin and eosin (H&E)-stained slides have been available at the authors’ institution since 1997. Of the 141 patients, including the NPC survivors and the matched group from 1997 to 2009, 62 underwent biopsies at other hospitals and received treatment without surgery. Therefore, the H&E slides of only 79 patients were available, which included 22 NPC survivors and 57 sporadic TSCC patients. All slides were re-evaluated independently by optical microscopy by experienced pathologists, and then jointly for consensus. The investigators were blinded to the clinical data at the time of the evaluation. The H&E-stained slides were assessed with respect to the presence of the worst pattern of invasion (WPOI), tumor budding, lymphocytic host response (LHR), vascular invasion (VI) or perineural invasion (PNI), and depth of tumor invasion.

The LHR pattern was classified as a three-tiered variable (strong, intermediate, or weak) based on the presence of lymphoid nodules. Tumors with a strong LHR were identified as showing the presence of at least one interface lymphoid nodule per ×4 objective lens on optical microscopy. Intermediate LHR was assigned when tumors with lymphoid responses were below this threshold but with one or more lymphoid nodules. Weak LHR was defined as a limited response that lacked any lymphoid nodules [14]. High-risk WPOI was defined as small tumor islands fewer than 15 cells and satellite tumors, which were located ≥ 1 mm away from the main tumor or the nearest satellite. The presence of PNI and limited or no LHR were also considered to be high-risk WPOI. Low-risk WPOI was assigned when the tumors showed pushing borders, large cohesive invasion, or finger-like growth [15]. Tumor budding was defined as the presence
of a single cancer cell or a cluster of fewer than five cancer cells in the invasive frontal region [16]. The slides were initially scanned at a ×4 magnification to select the areas with the highest density of tumor budding. Tumor budding in the selected areas was then counted at a ×20 magnification; the highest count per slide was identified as the number of tumor buds. Tumor budding ≥ 5 was considered to be high risk. The presence of a large PNI and little or no LHR were also considered to be high risk [17].

2. Statistical analysis

A Pearson chi-square test or Fisher exact test was used to assess the associations between the patient’s characteristics and cohort membership (NPC survivors vs. matched cohort). The OS was the primary endpoint in this study and was calculated from the time of the TSCC diagnosis until death or the last follow-up. The disease-free survival (DFS) was defined as the time from the TSCC diagnosis to the first evidence of any treatment failure or death or the last follow-up. Survival analysis was performed using the Kaplan-Meier method; the log-rank test was used to evaluate the statistical significance of the differences. To assess the unadjusted impact of a history of radiotherapy for NPC, competing risks analysis was applied to compare the different types of deaths between the NPC survivors and matched cohort. The Gray’s test was applied to compare the cumulative incidence functions estimated in competing risks analysis [18]. A multivariate Cox proportional hazards model was fitted to assess the independent prognostic significance of the clinical and pathologic characteristics and calculate the hazard ratios. A p-value of < 0.05 was considered significant.

Results

1. NPC characteristics and treatment in the survivor cohort

The crude incidence of TSCC after receiving radiotherapy for NPC was estimated to be approximately 0.13%. Table 1 lists the patient demographics for the NPC survivor cohort at the time of the NPC diagnosis. The median age at the NPC and TSCC diagnosis was 43 years (range, 23 to 66 years) and 52 years (range, 35 to 72 years), respectively. The majority of NPC cases were diagnosed with stage II and III (95.9%) cancers. The median cumulative radiation dose to the nasopharyngeal and neck region was 68.0 Gy (range, 57.3 to 88.0 Gy) and 52.0 Gy (range, 42.0 to 78.0 Gy), respectively. Fifteen patients (20.5%) received chemotherapy with alkylating agents as the treatment. The median interval from NPC to TSCC diagnosis was 82 months (range, 37 to 308 months). The second tongue cancer occurred within 5 years since the end of radiotherapy for NPC in 37.0% of patients, within 10 years in 68.5% of patients, and within 15 years in 91.8% of patients.
Table 1. Demographic and clinical characteristics of NPC in the survivor cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) (n=73)</th>
</tr>
</thead>
<tbody>
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<td>Before 1990</td>
<td>34 (46.6)</td>
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<tr>
<td>1990-1999</td>
<td>30 (41.1)</td>
</tr>
<tr>
<td>≥ 2000</td>
<td>9 (12.3)</td>
</tr>
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<tr>
<td>Male</td>
<td>54 (74.0)</td>
</tr>
<tr>
<td>Female</td>
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<td>NPC stage at diagnosis</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>II</td>
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<tr>
<td>III</td>
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<td>Orthovoltage X-rays</td>
<td>5 (6.9)</td>
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<td>Cobalt-6</td>
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<td>Conventional radiotherapy</td>
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</tr>
<tr>
<td>3D CRT/IMRT</td>
<td>3 (4.1)</td>
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<td>Radiation course</td>
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<td>Split</td>
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</tr>
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<td>Continuous</td>
<td>38 (52.1)</td>
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<td>Alkylating agents chemotherapy</td>
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</tr>
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<td>Yes</td>
<td>15 (20.5)</td>
</tr>
<tr>
<td>No</td>
<td>58 (79.5)</td>
</tr>
<tr>
<td>Local or regional recurrence</td>
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</tr>
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<td>Yes</td>
<td>9 (12.3)</td>
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<tr>
<td>No</td>
<td>64 (87.7)</td>
</tr>
<tr>
<td>Radiation dose, median (range, Gy)</td>
<td>68.0 (57.3-88.0)</td>
</tr>
<tr>
<td>Age at NPC diagnosis, median (range, yr)</td>
<td>43 (23-66)</td>
</tr>
<tr>
<td>Age at TSCC diagnosis, median (range, yr)</td>
<td>52 (35-72)</td>
</tr>
<tr>
<td>Latency from NPC to TSCC, median</td>
<td>82.0 (37-308)</td>
</tr>
<tr>
<td>(range, mo)</td>
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</table>

NPC, nasopharyngeal carcinoma; 3D CRT, 3-dimensional conformal radiotherapy; IMRT, intensity-modulated radiotherapy; TSCC, tongue squamous cell carcinoma.

2. TSCC characteristics and treatment: NPC survivors in comparison with the matched group

The median age of the sporadic TSCC cohort (2,720 patients: 1,796 male [66.0%] and 924 female [34.0%]) was 52 years (range, 20 to 94 years; mean, 51.7 years). The age of onset of TSCC (p=0.433) and sex distribution (p=0.169) were similar between the NPC survivors and the sporadic TSCC cohort.

As shown in Table 2, the NPC survivors and matched cohort showed a similar TNM stage distribution of TSCC. On the other hand, the NPC survivors were more likely to have a more advanced T classification (T4 classification, 17.8% vs. 2.7%) and less likely to have lymph node involvement (15.1% vs. 24.2%) than the matched cohort. The tumor location in the NPC survivor cohort was more likely to be located in the dorsum of the tongue than that observed in the matched cohort (35.6% vs. 9.1%). In addition, the NPC survivors were more likely to have a family history of cancer (16.4% vs. 8.7%).

The treatment modality used showed significant intergroup differences with 67.1% of the NPC survivors receiving surgery alone or multimodality therapy versus 89.0% in the matched group (p<0.001). A neck dissection was performed in 21.9% of NPC survivors compared to 74.9% in the matched group (p<0.001). The rates of chemotherapy and radiotherapy did not differ significantly between the two groups.

3. Histological comparison

A total of 79 patients were included for a histological comparison (Fig. 2). As shown in Table 2, there was no significant difference between the NPC survivors and matched group with respect to VI and PNI. A weak LHR was evident in the 18 NPC survivors (81.8%), whereas only 25 sporadic TSCC patients (43.9%) had a weak LHR (p=0.003). In addition, a significantly higher number (75.4%) of sporadic TSCC patients had a high intensity of tumor budding (≥5), whereas only 45.5% of NPC survivors had this risk (p=0.016). The high risk of WPOI was significantly higher in the sporadic TSCC patients (68.4%) than in the NPC survivors (40.9%) (p=0.039).

4. Follow-up and survival analysis

The median follow-up time of the NPC survivors and the matched group after the TSCC diagnosis who were alive was 90.0 months and 114.0 months, respectively. The OS was significantly poorer in the NPC survivor group than the matched group (5-year OS rates, 44.0% vs. 63.6%; p=0.006) (Fig. 3A). A significant difference in the DFS was observed between the 2 groups (5-year DFS, 39.0% vs. 53.4%; p=0.026) (Fig. 3B). In competing risks analysis, the cumulative incidence functions for the tongue cancer events were similar in the two groups (Gray’s test, p=0.331), but the rates of the competing event (documented non-tongue cancer death) were significantly higher in the NPC survivors (Gray’s test, p=0.001).

During the follow-up, four patients (5.5%) in the NPC survivor group developed a third primary cancer, namely diffuse large B cell lymphoma, left breast cancer, thyroid cancer, and basal-cell epithelioma in the neck, while five patients
Table 2. Characteristics and treatment in NPC survivors and matched patients with sporadic TSCC.

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<thead>
<tr>
<th>Characteristic</th>
<th>NPC survivors</th>
<th>Sporadic TSCC</th>
<th>p-value</th>
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<td><strong>Stage</strong></td>
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<tr>
<td>II</td>
<td>20 (27.4)</td>
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<tr>
<td>III</td>
<td>14 (19.2)</td>
<td>40 (18.3)</td>
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</tr>
<tr>
<td>IVa</td>
<td>13 (17.8)</td>
<td>22 (10.0)</td>
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</tr>
<tr>
<td>T2</td>
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<td>114 (52.1)</td>
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<td>8 (11.0)</td>
<td>14 (6.4)</td>
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<td>6 (2.7)</td>
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<td>20 (9.1)</td>
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<td>147 (67.1)</td>
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<tr>
<td>II</td>
<td>12 (16.4)</td>
<td>42 (19.2)</td>
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<td>III</td>
<td>2 (2.7)</td>
<td>4 (1.8)</td>
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<td>66 (30.1)</td>
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<td>107 (48.9)</td>
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<td>112 (51.1)</td>
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<td><strong>Treatment</strong></td>
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<td>33 (45.2)</td>
<td>125 (57.0)</td>
<td>&lt; 0.001</td>
</tr>
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<td>14 (19.2)</td>
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<tr>
<td>Radiotherapy alone</td>
<td>10 (13.7)</td>
<td>17 (7.8)</td>
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<tr>
<td>Multimodality therapy</td>
<td>16 (21.9)</td>
<td>70 (32.0)</td>
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</tr>
<tr>
<td><strong>Neck dissection</strong></td>
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<td></td>
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</tr>
<tr>
<td>Surgery with neck dissection</td>
<td>16 (21.9)</td>
<td>164 (74.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Surgery without neck dissection</td>
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<td>23 (10.5)</td>
<td></td>
</tr>
<tr>
<td>No surgery</td>
<td>30 (41.1)</td>
<td>32 (14.6)</td>
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<td><strong>Any chemotherapy</strong></td>
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<tr>
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<td>44 (60.3)</td>
<td>156 (71.2)</td>
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<td><strong>Any radiotherapy</strong></td>
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<td>15 (20.5)</td>
<td>49 (22.4)</td>
<td>0.744</td>
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<td>170 (77.6)</td>
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<td><strong>Vascular invasion</strong></td>
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<td></td>
<td></td>
</tr>
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<td>3 (5.3)</td>
<td>0.614</td>
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<tr>
<td>Absent</td>
<td>20 (90.9)</td>
<td>54 (94.7)</td>
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Table 2. Continued

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<th>Characteristic</th>
<th>NPC survivors</th>
<th>Sporadic TSCC</th>
<th>p-value</th>
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<td>Perineural invasion</td>
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<td>Present</td>
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<td>0.186</td>
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<td>56 (98.2)</td>
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<td>LHR</td>
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<td></td>
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<td>Strong/Intermediate</td>
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<td>32 (56.1)</td>
<td>0.003</td>
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<tr>
<td>Weak</td>
<td>18 (81.8)</td>
<td>25 (43.9)</td>
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<tr>
<td>Tumor budding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (≥ 5)</td>
<td>10 (45.5)</td>
<td>43 (75.4)</td>
<td>0.016</td>
</tr>
<tr>
<td>Low (&lt; 5)</td>
<td>12 (54.5)</td>
<td>14 (24.6)</td>
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</tr>
<tr>
<td>Worst pattern of invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>9 (40.9)</td>
<td>39 (68.4)</td>
<td>0.039</td>
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<tr>
<td>Low</td>
<td>13 (59.1)</td>
<td>18 (31.6)</td>
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</table>

Values are presented as number (%). NPC, nasopharyngeal carcinoma; TSCC, tongue squamous cell carcinoma; LHR, lymphocytic host response.

Fig. 2. Histologic features of tongue squamous cell carcinoma, presence of vascular invasion (arrow) (A), presence of perineural invasion (arrow) (B), strong lymphocytic host response (C), weak lymphocytic host response (D), presence of tumor budding (arrow) (E), and presence of high risk of worst pattern of invasion (F) (H&E staining, ×200).

(2.3%) in the matched group developed a second primary cancer.

Table 3 lists the results of univariate analysis for the OS and DFS. The statistically significant clinical factors in univariate analysis (p < 0.10) were analyzed by multivariate analysis (Table 4). The multivariate Cox proportional hazards regression model yielded a hazard ratio (HR) of 0.690 (95% confidence interval [CI], 0.491 to 0.971; p=0.033) for the matched group compared to the NPC survivor cohort, with the OS as the endpoint. Independent of the NPC history, the risk of death due to any cause was also greater in male patients, patients with a more advanced TNM stage, and those who did not receive surgery or multimodality treatment. On the other hand, the NPC history was not an independent prognostic factor for the DFS (HR, 0.768; 95% CI, 0.554 to 1.063; p=0.112). The TNM stage (p < 0.001) and treatment modality (p=0.025) appeared to have independent effects on the DFS.
Fig. 3. (A) Overall survival for nasopharyngeal carcinoma (NPC) survivors and matched patients with sporadic tongue squamous cell carcinoma (TSCC) (p=0.006). (B) Disease-free survival for NPC survivors and matched patients with sporadic TSCC (p=0.026). TC, tongue cancer.

Discussion

The standardized incidence ratios of tongue cancer were significantly higher in NPC patients, particularly in NPC endemic areas [7]. In this study, 73 TSCC patients, who had a history of radiotherapy for NPC, were compared with a matched cohort of 219 sporadic TSCC cases. To the best of the authors’ knowledge, this is the largest study to compare TSCC patients with a history of radiotherapy for NPC and sporadic TSCC cases. This study showed that the clinicopathologic characteristics and outcomes were significantly different between the two groups.

Up to now, there is no definite conclusion regarding the pathogenesis of radiation-related second primary tumors. Teo et al. [8] suggested that the increasing incidence of tongue cancer in NPC patients was more likely associated with a history of oral cavity radiotherapy than the sharing of common risk factors or mucosal field-cancerization. Welden et al. [19] reported the long-term monitoring results of 694 children and teenagers after treatment for Hodgkin’s disease and reported that 93% of second solid malignancies developed in regions that had received at least 35 Gy. Regarding the radiation field of conventional radiotherapy in NPC, the anterior half of the tongue was shielded routinely. On the other hand, the posterior tongue dorsum and tongue base were inevitably included in the medium- and high-dose region of the radiation field. As reported by Qin et al. [20] the mean radiation dose to the tongue tip, tongue body, tongue base, and entire tongue was 11.2 Gy, 34.1 Gy, 57.0 Gy, and 43.1 Gy, respectively, using a two-dimensional radiation technique. In addition, the distribution of the tongue cancer location between the NPC survivor group and sporadic TSCC cohort is significantly different. TSCC in NPC survivors is more likely to be located in the dorsum of the tongue but less likely in the ventral area. Therefore, the purported correlation between the radiation dose and the location of second primary tumor was confirmed.

Compared to sporadic TSCC, TSCC in NPC survivors was more likely to have a more advanced T classification, but less likely to have lymph node involvement. The relatively higher percentage of T4 classification in NPC survivors may be because the tumor was more likely to be located in the dorsum of the tongue than the sporadic TSCC cohort (35.6% vs. 9.1%, p < 0.001). The lesions located in the dorsum may more easily involve the adjacent structures (e.g., deep muscle of the tongue) and were almost imperceptible in the early stage. On the other hand, TSCC in the NPC survivors was less likely to have lymph node involvement, which may be due to two reasons: a history of radiation to neck region causes the lymphatic atresia and altered the lymphatic drainage; and the significantly higher percentage of neck dissection in
Table 3. Univariate analysis of OS and DFS in relation to clinical characteristics

<table>
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<tr>
<th>Variable</th>
<th>No. (%)</th>
<th>OS 5-Year OS</th>
<th>p-value</th>
<th>DFS 5-Year DFS</th>
<th>p-value</th>
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<td><strong>Group</strong></td>
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<td>NPC survivor</td>
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<td>0.006</td>
<td>39.0</td>
<td>0.026</td>
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<td>Sporadic TSCC</td>
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<td>≤ 52</td>
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<td>0.159</td>
<td>53.5</td>
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<td>&gt; 52</td>
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<td><strong>Sex</strong></td>
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<td>60.6</td>
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<td>61.5</td>
<td>&lt; 0.001</td>
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<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy alone</td>
<td>27 (9.2)</td>
<td>58.3</td>
<td></td>
<td>43.3</td>
<td></td>
</tr>
<tr>
<td>Multimodality therapy</td>
<td>86 (29.5)</td>
<td>49.9</td>
<td></td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td><strong>Neck dissection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery with neck dissection</td>
<td>180 (61.6)</td>
<td>68.3</td>
<td>&lt; 0.001</td>
<td>60.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Surgery without neck dissection</td>
<td>50 (17.1)</td>
<td>55.3</td>
<td></td>
<td>44.8</td>
<td></td>
</tr>
<tr>
<td>No surgery</td>
<td>62 (21.3)</td>
<td>33.0</td>
<td></td>
<td>27.0</td>
<td></td>
</tr>
</tbody>
</table>

OS, overall survival; DFS, disease-free survival; NPC, nasopharyngeal carcinoma; TSCC, tongue squamous cell carcinoma.

The sporadic TSCC cohort (74.9% vs. 21.9%, p < 0.001) leads to a relatively higher percentage of positive lymph node detection.

A detailed histopathologic review showed that TSCC in the NPC survivors was more likely to have weak LHR, low-risk WPOI, and low tumor budding. Previous studies reported that the increased risks of second primary malignancies could be attributed to the compromised immune function in cancer survivors. The strong LHR suggested that an adaptive cytotoxic T-cell immune response was concentrated significantly at the interface of the tumors and the strong LHR has a protective effect in oral cancer patients [21]. Therefore, the weak LHR in NPC survivors indicated that these patients may have an impaired immune surveillance and adaptive immunity. Tumor budding represents two additional aggressive and malignant features (active inva-
Table 4. Multivariate analysis of OS and DFS in relation to clinical characteristics

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Characteristic</th>
<th>HR</th>
<th>95% CI for HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>History of NPC</td>
<td>0.690</td>
<td>0.491-0.971</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.686</td>
<td>0.476-0.988</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>TNM stage</td>
<td>2.146</td>
<td>1.553-2.964</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment modality</td>
<td>1.158</td>
<td>1.029-1.303</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Neck dissection</td>
<td>1.054</td>
<td>0.807-1.376</td>
<td>0.701</td>
</tr>
<tr>
<td>DFS</td>
<td>History of NPC</td>
<td>0.768</td>
<td>0.554-1.063</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>0.786</td>
<td>0.548-1.078</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>TNM stage</td>
<td>1.766</td>
<td>1.285-2.426</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Treatment modality</td>
<td>1.137</td>
<td>1.016-1.271</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>Neck dissection</td>
<td>1.047</td>
<td>0.813-1.349</td>
<td>0.723</td>
</tr>
</tbody>
</table>

OS, overall survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; NPC, nasopharyngeal carcinoma.

sion and cellular dischondition). The presence of high intensity tumor budding was associated with a poorer prognosis, epithelialmesenchymal transition, and cervical lymph node metastasis in TSCC [17,22]. In this study, the sporadic TSCC patients were more likely to have lymph node involvement than TSCC in the NPC survivors, which validated this pathological feature. The high-risk WPOI, which is marked by small tumor islands and satellite tumor, was also confirmed as an independent predictor for a poor prognosis in TSCC [15]. Overall, TSCC in NPC survivors appear to have less aggressive histologic features (low tumor budding and low-risk WPOI) but impaired immune surveillance (weak LHR). The distinct histological features in radiation-associated TSCC patients should be validated in a further study.

There has been increasing concern as to whether a prior history of cancer could impact the prognosis and therapeutic management in patients with head and neck cancer [23]. A major finding of this analysis was the negative prognostic impact of a history of radiotherapy for NPC on the survival of patients subsequently diagnosed with TSCC, which has never been examined previously. On the other hand, death as a result of tongue cancer was similar in the NPC survivors and sporadic TSCC cohort. This is because NPC survivors were more likely to die from non-tongue cancer causes. The accumulation of genetic damage due to a history of radiation and the differences in the immune status may help explain this situation [24,25]. In addition, the prognosis of TSCC in NPC survivors could be negatively influenced by the excess mortality due to NPC and severe late complications. Similar results were obtained in other second cancers, such as breast cancer, among patients with a history of radiation for Hodgkin’s lymphoma [26]. In particular, the cumulative incidence functions for the tongue cancer events were similar in the two groups (Gray’s test, p=0.331). Several possible interpretations can be made. First, the potential survival advantage of less aggressive histologic features in TSCC of NPC survivors (low tumor budding and low-risk WPOI) may be counteracted by the impaired immune surveillance (weak LHR). Second, the TSCC in NPC survivors were more likely to be limited to local disease without lymphatic nodal metastases, which was correlated with better local control and longer survival. Third, previous studies indicated that TSCC in the NPC survivors and sporadic TSCC might have different carcinogenesis [8]. The sporadic TSCC patients were more likely to be accompanied by mucosal field-cancerization, which resulted in a difficulty of loco-regional control.

The main limitations of this study were its retrospective nature and single-institutional data design. Although deaths as a result of tongue cancer could be identified in this study, there was limited information on the specific causes of non-cancer deaths because of the unexplained loss to follow-up. In addition, this study spanned more than 40 years, during which radiotherapy technology for NPC has evolved markedly. The majority of NPC patients in this study received conventional radiotherapy owing to the relatively short follow-up time for the patients who received modern conformal radiotherapy. The relevance of the long-term effects of outdated treatments to patients receiving modern therapy is still questionable. The main strength of this analysis was its very large sample size of radiation-associated TSCC in the NPC survivor cohort. Considering the rarity of radiation-associated TSCC, the generalizability of these results will require further validation in multi-centric studies.
Conclusion

Compared to sporadic TSCC, TSCCs with a history of NPC radiation have specific clinicopathologic features. These results also indicated that the history of radiation for NPC was a poor prognostic factor. TSCC patients with a history of radiotherapy for NPC are more likely to die from non-tongue cancer causes than those with sporadic TSCC. These results may impact cancer-screening strategies in patients with NPC, and those with a high risk should be targeted for more intensive surveillance.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References


A Multicenter Randomized Phase II Study of Docetaxel vs. Docetaxel Plus Cisplatin vs. Docetaxel Plus S-1 as Second-Line Chemotherapy in Metastatic Gastric Cancer Patients Who Had Progressed after Cisplatin Plus Either S-1 or Capecitabine

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Introduction

Despite its decreasing incidence, gastric cancer remains a major global health issue with 951,600 new cases and 723,100 deaths in 2012 [1]. Although palliative chemotherapy has been established as the standard of care in patients with unresectable locally advanced or metastatic gastric cancer (MGC) based on the survival benefits and improved quality of life (QoL) over supportive care alone [2], the median overall survival (OS) of these patients rarely exceeds 12 months. Currently, fluoropyrimidine plus platinum-based combination chemotherapy is the most commonly used first-line reference regimens [3-6]. Recently, with the availability of oral fluoropyrimidines, such as capecitabine and S-1, infusional 5-fluorouracil (5-FU) has been substituted with either capecitabine or S-1, based on the results of phase III studies showing comparable efficacy and more favorable safety profiles with those agents [3-5,7]. The combination of capecitabine or S-1 plus cisplatin is currently one of the most

Purpose

This study evaluated the re-challenge of S-1 or cisplatin in combination with docetaxel in metastatic gastric cancer (MGC) that had progressed on a cisplatin plus either S-1 or capecitabine regimen.

Materials and Methods

Patients with progressive disease after first-line cisplatin plus S-1 or capecitabine were randomized to receive 3-week cycles of docetaxel 75 mg/m² intravenously (IV) on D1 (D), docetaxel 60 mg/m² IV plus cisplatin 60 mg/m² IV on D1 (DC), or docetaxel 60 mg/m² IV D1 plus oral S-1 30 mg/m² twice a day on D1-14 (DS).

Results

Seventy-two patients were randomized to the D (n=23), DC (n=24), or DS (n=25) group. The confirmed response rate was 4.3% (95% confidence interval [CI], 0% to 12.6%), 4.3% (95% CI, 0% to 12.6%), and 8.7% (95% CI, 0% to 20.2%) for the D, DC, and DS groups, respectively. Compared to the D arm, the DS arm had a better progression-free survival (2.7 months vs. 1.3 months, p=0.034) without any deterioration in safety or quality of life, whereas the DC arm had a similar progression-free survival (1.8 months vs. 1.3 months, p=0.804) and poorer overall survival (5.6 months vs. 10.0 months, p=0.035).

Conclusion

A re-challenge with S-1, but not cisplatin, in combination with docetaxel has potential anticancer benefits over docetaxel alone in MGC with progression after prior cisplatin plus S-1 or capecitabine.

Key words
Stomach neoplasms, Antineoplastic agents, Docetaxel, Tegafur-gimeracil-oteracil, Cisplatin
commonly used first-line regimens in both clinical trials and practice. On the other hand, as the disease in most patients inevitably progresses during or after first-line chemotherapy, the role of salvage chemotherapy in gastric cancer has been investigated. Recent randomized phase III studies have revealed a significant survival benefit from second-line chemotherapy with docetaxel or irinotecan [8-10]. Nevertheless, given the modest survival benefits from current second-line chemotherapy, there is still a great need for further treatment improvements after the failure of first-line therapy.

Docetaxel, which inhibits microtubule depolymerization, has been used widely in the treatment of MGC. In particular, based on its different mechanism of action from those of fluoropyrimidine and platinum and the lack of cross-resistance with these agents, docetaxel is used frequently as a second-line regimen after the failure of fluoropyrimidine- and/or platinum-based first-line therapy. Furthermore, docetaxel shows synergistic antitumor activity with fluoropyrimidines, particularly S-1, by modulating the expression of enzymes involved in the 5-FU metabolism, including thymidylate synthase, dihydropyrimidine dehydrogenase, and orotate phosphoribosyltransferase [11,12]. Docetaxel has also shown synergistic activity with cisplatin in gastric cancer, which was attributed partially to the suppression of the cisplatin-induced multidrug resistance-associated protein-1 by docetaxel [13], resulting in the accumulation of intracellular platinum-glutathione complexes. Given that these molecules modulated by docetaxel are involved in the resistance to fluoropyrimidines and cisplatin in gastric cancer [14,15], the hypothesis in this study was that the co-treatment of docetaxel and either S-1 or cisplatin could increase the antitumor activity compared to docetaxel alone, by at least partially overcoming the resistance to fluoropyrimidines or cisplatin in patients whose tumors progressed during or after fluoropyrimidines- or cisplatin-based first-line therapy.

Materials and Methods

1. Patients

Eligible patients were ≥ 18 years old with histologically confirmed metastatic gastric adenocarcinoma. The patients needed to have documented disease progression during or within 6 months of the completion of first-line chemotherapy with either S-1 or capecitabine plus cisplatin. The additional eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, a measurable lesion, adequate major organ function, absence of concurrent uncontrolled medical conditions or other malignancies within the past 3 years, prior taxane treatment, and pre-existing grade ≥ 2 neuropathy. All patients provided written informed consent prior to study entry. The institutional review boards of all participating centers approved the study protocol (ClinicalTrials.gov identifier NCT00980603).

2. Study design and treatment

The patients were assigned randomly 1:1:1 to receive docetaxel (75 mg/m² intravenously on day 1), docetaxel plus cisplatin (each 60 mg/m² intravenously on day 1), or docetaxel plus S-1 (docetaxel 60 mg/m² intravenously on day 1 and oral S-1 30 mg/m² twice daily on days 1-14), administered every 3 weeks. The dose of docetaxel in the docetaxel alone arm was based on a previous phase II study of MGC in a second-line setting [16]. The doses of triweekly docetaxel plus cisplatin in previous studies were 60-75 mg/m² and 60-70 mg/m², respectively, in a second-line setting [17-20]. Based on the safety and efficacy of these trials, the dose in the docetaxel plus cisplatin arm was determined to be 60 mg/m² each for the current trial. In the docetaxel plus S-1 arm, although the recommended doses of triweekly S-1 plus docetaxel were S-1 80 mg/m²/day on days 1 to 14 and docetaxel 40 mg/m² on day 1 in previous studies [21,22], this study used the same dose of docetaxel (60 mg/m²) as in the docetaxel plus cisplatin arm, along with a reduced dose of S-1 (60 mg/m²/day).

Randomization was performed using the random permutation method to stratify the patients according to the study site, ECOG performance status (0-1 vs. 2), and the best response to first-line chemotherapy (complete or partial response vs. stable disease or progressive disease). Treatment was continued until disease progression, unacceptable toxicity, or the withdrawal of consent.

3. Assessments

The medical history, physical examination, and laboratory tests (including a complete blood count with differential, chemistry [including creatinine clearance], and urinalysis) were performed within 1 week before the study treatment. The physical examinations, and the blood hematology and chemistry tests were repeated at the beginning of each cycle. The baseline tumor assessment using the chest X-ray and abdominal/pelvic computed tomography scans were performed within 4 weeks before the study treatment, and these imaging methods were repeated every two treatment cycles until disease progression. The objective tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.0 [23], and all responses were confirmed at least 4 weeks after the initial documentation.
The safety evaluations were based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) ver. 3.0. The QoL was assessed using the European Organization for Research and Treatment of Cancer QoL questionnaires (EORTC QLQ-C30 and gastric module STO22) at the beginning of each cycle for the initial four cycles, and then every other cycle until disease progression.

4. Statistical analysis

The primary endpoint of this study was the objective response rate (ORR). The secondary endpoints included the progression-free survival (PFS; time from the date of start treatment to the date of disease progression or death), OS (time from the date of start treatment to the date of death), safety, and QoL. Based on Simon’s selection design with a probability of 90% for correctly selecting the best treatment with respect to ORR, assuming that the smallest ORR would be 16% and the best treatment would be superior by an absolute difference of 14% (i.e., an ORR of 30%), a total of 144 patients including a 5% dropout rate (i.e., 48 patients per treatment group) were found to be required.

Patients who received at least one dose of the study drug comprised the safety population. Efficacy analysis was performed in a modified intention-to-treat (ITT) population, which excluded patients who were deemed ineligible or never started the study treatment from randomized patients.

The Kaplan-Meier method and the log-rank test were used to estimate and compare the survival distribution, respectively. The discrete data were compared using a Pearson’s chi-square test or Fisher exact test, and the quantitative data were compared using the Kruskal-Wallis test. All tests were two-sided with a 5% significance level.

Results

1. Patient characteristics

Seventy-two patients (50% of the target number) from three institutions were enrolled between November 2008 and September 2012. The restriction of accrual to a previous failed regimen of cisplatin plus either S-1 or capcitabine resulted in slow accrual, which caused early closure of the study. The patients were assigned randomly to the docetaxel alone (D; n=23), docetaxel plus cisplatin (DC; n=24), or docetaxel plus S-1 (DS; n=25) groups. One patient in the DC arm was deemed ineligible because of prior first-line chemotherapy with capcitabine plus oxaliplatin rather than cisplatin. This patient was excluded from the efficacy analysis but included in the safety analysis. Two patients in the DS arm were also excluded from the efficacy and toxicity analyses because of a refusal to receive chemotherapy after randomization in one case and the receipt of a second-line chemotherapy with infusional 5-FU instead of S-1 in the other patient (Fig. 1). The baseline characteristics were well balanced between the treatment arms (Table 1). The majority of patients initially had metastatic disease with multiple metastatic organ sites and
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Modified intention-to-treat population</th>
<th>D (n=23)</th>
<th>DC (n=23)</th>
<th>DS (n=23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (78.3)</td>
<td>20 (87.0)</td>
<td>14 (60.9)</td>
<td>0.147</td>
</tr>
<tr>
<td>Female</td>
<td>5 (21.7)</td>
<td>3 (13.0)</td>
<td>9 (39.1)</td>
<td></td>
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<tr>
<td><strong>Age, median (range, yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 (34-68)</td>
<td>55 (38-74)</td>
<td>55 (39-68)</td>
<td></td>
<td>0.995</td>
</tr>
<tr>
<td><strong>ECOG performance status</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 (8.7)</td>
<td>1 (4.3)</td>
<td>1 (4.3)</td>
<td>0.784</td>
</tr>
<tr>
<td>1</td>
<td>21 (91.3)</td>
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<td>20 (87.0)</td>
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<tr>
<td>2</td>
<td>0</td>
<td>2 (8.7)</td>
<td>2 (8.7)</td>
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</tr>
<tr>
<td><strong>Disease status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initially metastatic</td>
<td>22 (95.7)</td>
<td>18 (78.3)</td>
<td>18 (78.3)</td>
<td>0.201</td>
</tr>
<tr>
<td>Recurrent</td>
<td>1 (4.3)</td>
<td>5 (21.7)</td>
<td>5 (21.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, well differentiated</td>
<td>1 (4.3)</td>
<td>1 (4.3)</td>
<td>3 (13.0)</td>
<td>0.922</td>
</tr>
<tr>
<td>Adenocarcinoma, moderately differentiated</td>
<td>6 (26.1)</td>
<td>6 (26.1)</td>
<td>5 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, poorly differentiated</td>
<td>12 (52.2)</td>
<td>12 (52.2)</td>
<td>10 (43.5)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma, differentiation cannot be assessed</td>
<td>1 (4.3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Signet ring cell carcinoma</td>
<td>3 (13.0)</td>
<td>4 (17.4)</td>
<td>5 (21.7)</td>
<td></td>
</tr>
<tr>
<td><strong>No. of metastatic organ sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5 (21.7)</td>
<td>5 (21.7)</td>
<td>2 (8.7)</td>
<td>0.719</td>
</tr>
<tr>
<td>2</td>
<td>11 (47.8)</td>
<td>10 (43.5)</td>
<td>11 (47.8)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>7 (30.4)</td>
<td>8 (34.8)</td>
<td>10 (43.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Metastatic organ site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>9 (39.1)</td>
<td>9 (39.1)</td>
<td>8 (34.8)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>9 (39.1)</td>
<td>11 (47.8)</td>
<td>8 (34.8)</td>
<td>0.749</td>
</tr>
<tr>
<td>Distant lymph nodes</td>
<td>11 (47.8)</td>
<td>7 (30.4)</td>
<td>9 (39.1)</td>
<td>0.532</td>
</tr>
<tr>
<td>Others</td>
<td>9 (39.1)</td>
<td>15 (65.2)</td>
<td>13 (56.5)</td>
<td>0.241</td>
</tr>
<tr>
<td><strong>First-line chemotherapy regimen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-1 plus cisplatin</td>
<td>15 (65.2)</td>
<td>14 (60.9)</td>
<td>18 (78.3)</td>
<td>0.520</td>
</tr>
<tr>
<td>Capcitabine plus cisplatin</td>
<td>8 (34.8)</td>
<td>9 (39.1)</td>
<td>5 (21.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Best response to first-line chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.815</td>
</tr>
<tr>
<td>Partial response</td>
<td>11 (47.8)</td>
<td>11 (47.8)</td>
<td>8 (34.8)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>7 (30.4)</td>
<td>7 (30.4)</td>
<td>7 (30.4)</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td>5 (21.7)</td>
<td>5 (21.7)</td>
<td>8 (34.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Time to progression on first-line therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6 mo</td>
<td>16 (69.6)</td>
<td>17 (73.9)</td>
<td>15 (65.2)</td>
<td>0.945</td>
</tr>
<tr>
<td>≥6 mo</td>
<td>7 (30.4)</td>
<td>6 (26.1)</td>
<td>8 (34.8)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). D, docetaxel; DC, docetaxel plus cisplatin; DS, docetaxel plus S-1; ECOG, Eastern Cooperative Oncology Group.

had developed disease progression within 6 months during their first-line chemotherapy.

2. Treatment delivery

The median number of treatment cycles was 2 (range, 1 to 10) in the D arm, 2 (range, 1 to 11) in the DC arm, and 3 (range, 1 to 23) in the DS arm. The proportion of patients requiring a dose reduction was higher in the DC and DS arms than in the D arm: 21.7% (5 of 23) with D, 30.4% (7 out of 23) with DC, and 34.8% (8 of 23) with DS. The most common cause of dose reduction was neutropenia (2 of 5) in the D arm, neutropenia (2 of 7) in the DC arm, and mucositis (3 of 8) in the DS arm. The proportion of patients who experienced cycle delay owing to adverse events was similar in the three arms: 26.1% (6 of 23) with D, 21.7% (5 of 23) with
Table 2. Objective tumor response

<table>
<thead>
<tr>
<th>Modified intention-to-treat population</th>
<th>D (n=23)</th>
<th>DC (n=23)</th>
<th>DS (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best overall response, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete response</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Partial response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed partial response</td>
<td>1 (4.3)</td>
<td>1 (4.3)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>Unconfirmed partial response</td>
<td>0</td>
<td>2 (8.7)</td>
<td>1 (4.3)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>6 (26.0)</td>
<td>10 (43.5)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>16 (69.6)</td>
<td>9 (39.1)</td>
<td>11 (47.8)</td>
</tr>
<tr>
<td>Inevaluableb)</td>
<td>0</td>
<td>1 (4.3)</td>
<td>0</td>
</tr>
<tr>
<td>ORR (95% CI, %)</td>
<td>4.3 (0-12.6)</td>
<td>4.3 (0-12.6)</td>
<td>8.7 (0-20.2)</td>
</tr>
<tr>
<td>ORR including unconfirmed partial response</td>
<td>4.3 (0-12.6)</td>
<td>13.0 (0-32.9)</td>
<td>13.0 (0-32.9)</td>
</tr>
</tbody>
</table>

D, docetaxel; DC, docetaxel plus cisplatin; DS, docetaxel plus S-1; ORR, overall response rate; CI, confidence interval. a) The tumor response was not confirmed by a second evaluation at least 4 weeks later following the first documentation of a response. b) Computed tomography measurement was not available due to follow-up loss.

![Graph A](image1.png)
![Graph B](image2.png)

Fig. 2. Kaplan-Meier curves of progression-free survival (A) and overall survival (B). CI, confidence interval; DS, docetaxel plus S-1; DC, docetaxel plus cisplatin; D, docetaxel.

DC, and 26.1% (6 of 23) with DS. The median relative dose intensities were 92.3% for docetaxel in the D arm, 90.6% for docetaxel and 90.6% for cisplatin in the DC arm, and 91.2% for docetaxel and 89.1% for S-1 in the DS arm. The main reasons for the discontinuation of treatment were disease progression (95.7% [n=22] with D, 100% [n=23] with DC, and 91.3% [n=21] with DS) followed by adverse events (4.3% [n=1] with D, 0% with DC, and 4.3% [n=1] with DS).

3. Efficacy

Among the modified ITT population, confirmed ORR was 4.3% (1 of 23; 95% confidence interval [CI], 0% to 12.6%) in the D arm, 4.3% (1 of 23; 95% CI, 0% to 12.6%) in the DC arm, and 8.7% (2 of 23; 95% CI, 0% to 20.2%) in the DS arm (p > 0.990) (Table 2). The ORR, including the unconfirmed partial response, was 4.3% (1 of 23; 95% CI, 0% to 24.4%) in the D arm, 13.0% (3 of 23; 95% CI, 0% to 32.9%) in the DC
arm, and 13.0% (3 of 23; 95% CI, 0% to 32.9%) in the DS arm (p=0.685). The disease control rate (DCR; the percentage of patients who achieved a complete response, partial response and stable disease) was 30.4% (7 of 23; 95% CI, 11.6% to 49.2%) in the D arm, 56.5% (13 of 23; 95% CI, 36.2% to 76.8%) in the DC arm, and 52.2% (12 of 23; 95% CI, 31.8% to 72.6%) in the DS arm (p=0.164). With a median follow-up time of 7.3 months (range, 1.6 to 31.5), the median PFS was 1.3 months with D (95% CI, 1.0 to 1.5), 1.8 months with DC (95% CI, 0.8 to 2.9), and 2.7 months with DS (95% CI, 1.0 to 4.4; p=0.072) (Fig. 2A). The DS arm showed a significantly prolonged PFS compared to the D arm (p=0.034), whereas the DC arm did not show a significant difference in PFS compared to the D arm (p=0.804). In terms of OS, the DC arm was worse than the D arm (median, 5.6 months [95% CI, 4.4 to 6.7] vs. 10.0 months [95% CI, 7.8 to 12.2]; p=0.035), whereas the DS arm (median, 6.9 months; 95% CI, 2.1 to 11.7) was comparable to the D arm (p=0.421) (Fig. 2B).

4. Post-study subsequent therapy

The proportion of patients receiving third-line therapy was higher in the D arm (19 of 23, 82.6%) than in the DC (12 of 23, 52.2%) or DS arms (13 of 23, 56.5%; p=0.089). The ORR in the third-line therapy was 10.5% (2 of 19; 95% CI, 0% to 24.3%) for D, 0.0% (0 of 13) for DC, and 0.0% (0 of 13) for DS (p=0.637).

5. Safety

Table 3 lists the adverse events. The overall incidence of grade ≥ 3 events was 43.5% (10 of 23) in the D arm and 62.5% (15 of 24) in the DC arm, and 32.9% (8 of 23) in the DS arm (DC vs. D, p=0.155; DS vs. D, p=0.382). Although the overall toxicity profiles in the treatment arms were comparable, the incidence of all grade nausea was significantly higher in the DS arm than the D arm (44% vs. 13%, p=0.047). The incidence of all grade aminotransferases elevation (42% vs. 13%, p=0.049) was significantly higher in the DC arm, whereas infection without neutropenia occurred more frequently in the D arm than in the DC arm (26% vs. 4%, p=0.048).

The most common grade 3/4 toxicity was neutropenia (21.7% with D, 25.0% with DC, and 8.7% with DS); febrile neutropenia occurred in 8.7% of cases in the D arm, 8.3% in the DC arm, and 4.3% in the DS arm. There was one possible treatment-related death in each of the DC (infection without

### Table 3. Adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>D (n=23)</th>
<th></th>
<th>DC (n=24)</th>
<th></th>
<th>Total</th>
<th></th>
<th>Total</th>
<th></th>
<th>Total</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade ≥ 3</td>
<td>Total</td>
<td>Grade ≥ 3</td>
<td>Total</td>
<td>(D vs. DC)</td>
<td>p-value</td>
<td>Grade ≥ 3</td>
<td>Total</td>
<td>(D vs. DS)</td>
<td>p-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hematological</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>3 (13)</td>
<td>6 (26)</td>
<td>1 (4)</td>
<td>8 (33)</td>
<td>0.752</td>
<td>0</td>
<td>11 (48)</td>
<td>0.221</td>
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<tr>
<td>Neutropenia</td>
<td>5 (22)</td>
<td>8 (35)</td>
<td>6 (25)</td>
<td>12 (50)</td>
<td>0.380</td>
<td>2 (9)</td>
<td>8 (35)</td>
<td>&gt; 0.990</td>
<td></td>
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</tr>
<tr>
<td>Anemia</td>
<td>0</td>
<td>20 (87)</td>
<td>3 (13)</td>
<td>24 (100)</td>
<td>0.109</td>
<td>1 (4)</td>
<td>23 (100)</td>
<td>0.233</td>
<td></td>
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<td>Thrombocytopenia</td>
<td>0</td>
<td>3 (13)</td>
<td>0</td>
<td>3 (13)</td>
<td>&gt; 0.990</td>
<td>1 (4)</td>
<td>5 (22)</td>
<td>0.699</td>
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<tr>
<td><strong>Non-hematological</strong></td>
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<td>Febrile neutropenia</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td>2 (8)</td>
<td>&gt; 0.990</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>0.665</td>
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<tr>
<td>Infection with neutropenia</td>
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<td>4 (17)</td>
<td>0</td>
<td>0</td>
<td>0.050</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>0.346</td>
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<td>Infection without neutropenia</td>
<td>3 (13)</td>
<td>6 (26)</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>0.048</td>
<td>1 (4)</td>
<td>3 (13)</td>
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<tr>
<td>Stomatitis</td>
<td>1 (4)</td>
<td>8 (35)</td>
<td>1 (4)</td>
<td>8 (33)</td>
<td>&gt; 0.990</td>
<td>3 (13)</td>
<td>13 (57)</td>
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<td>Anorexia</td>
<td>3 (13)</td>
<td>17 (74)</td>
<td>5 (21)</td>
<td>18 (75)</td>
<td>&gt; 0.990</td>
<td>1 (4)</td>
<td>21 (91)</td>
<td>0.243</td>
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<tr>
<td>Nausea</td>
<td>0</td>
<td>3 (13)</td>
<td>1 (4)</td>
<td>9 (38)</td>
<td>0.093</td>
<td>0</td>
<td>10 (44)</td>
<td>0.047</td>
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<tr>
<td>Diarrhea</td>
<td>1 (4)</td>
<td>11 (48)</td>
<td>0</td>
<td>6 (25)</td>
<td>0.135</td>
<td>2 (9)</td>
<td>11 (48)</td>
<td>&gt; 0.990</td>
<td></td>
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<tr>
<td>Fatigue</td>
<td>2 (9)</td>
<td>17 (74)</td>
<td>5 (21)</td>
<td>19 (79)</td>
<td>0.740</td>
<td>2 (9)</td>
<td>20 (87)</td>
<td>0.459</td>
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<td>Peripheral neuropathy</td>
<td>0</td>
<td>10 (44)</td>
<td>1 (4)</td>
<td>10 (42)</td>
<td>&gt; 0.990</td>
<td>0</td>
<td>12 (52)</td>
<td>0.768</td>
<td></td>
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<tr>
<td>AST/ ALT elevation</td>
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<td>3 (13)</td>
<td>0</td>
<td>10 (42)</td>
<td>0.049</td>
<td>1 (4)</td>
<td>6 (26)</td>
<td>0.459</td>
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<td></td>
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<tr>
<td>Hyperbilirubinemia</td>
<td>0</td>
<td>2 (9)</td>
<td>0</td>
<td>3 (13)</td>
<td>&gt; 0.990</td>
<td>2 (9)</td>
<td>5 (22)</td>
<td>0.414</td>
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<tr>
<td>Creatinin elevation</td>
<td>0</td>
<td>2 (9)</td>
<td>0</td>
<td>3 (13)</td>
<td>&gt; 0.990</td>
<td>0</td>
<td>1 (4)</td>
<td>&gt; 0.990</td>
<td></td>
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</tbody>
</table>

Values are presented as number (%). D, docetaxel; DC, docetaxel plus cisplatin; DS, docetaxel plus S-1; AST, aspartate aminotransferase; ALT, alanine aminotransferase. aG5 adverse event, b p < 0.05 when compared with docetaxel; DS vs. D for all grades nausea (p=0.047), DC vs. D for all grades infection without neutropenia (p=0.048) and all grades AST/ ALT elevation (p=0.049).
Fig. 3. Mean quality of life scores by the treatment arms. Global quality of life (A), physical functioning (B), role functioning (C), emotional functioning (D), nausea and vomiting (E), fatigue (F), appetite loss (G), and reflux symptoms (H). For the global quality of life, physical, role, or emotional functioning, higher scores indicate better quality of life or functioning. For nausea and vomiting, fatigue, appetite loss, and reflux symptoms, higher scores indicate a higher level of symptoms. D, docetaxel; DC, docetaxel plus cisplatin; DS, docetaxel plus S-1. *p < 0.05. (Continued to the next page)
neutropenia, peritonitis) and DS arms (infection with neutropenia, pneumonia).

6. Quality of life

More than 60% of the patients in each arm completed the baseline QoL questionnaire and at least one post-treatment questionnaire. QoL compliance was similar in the three arms. Although there were no significant differences in the global QoL scores between the three arms, the combination arms had poorer QoL scores at some time points compared to the D arm. These included lower physical functioning at cycle 2 (p=0.002), role functioning at cycles 2 (p=0.014) and 3 (p=0.018), and emotional functioning at cycle 3 (p=0.014), more fatigue at cycles 2 (p=0.010) and 3 (p=0.049), appetite loss at cycle 2 (p=0.005), insomnia at cycle 3 (p=0.021), and dry mouth at cycle 2 (p=0.028) in the DC arm. Fatigue at cycles 2 (p=0.040) and 3 (p=0.018), constipation at cycle 2 (p=0.047), nausea/vomiting at cycle 3 (p=0.045), reflux symptoms at cycle 2 (p=0.009), and hair loss at cycle 3 (p=0.017) were elevated in the DS arm (Fig. 3).

Discussion

The reported survival benefits of second-line chemotherapy in patients with MGC have prompted efforts to develop more effective second-line chemotherapy regimens. Recent phase III studies demonstrating survival improvement with second- or third-line therapy compared to the best support-

![Appetite loss](image1)

![Refux symptoms](image2)

**Fig. 3. (Continued from the previous page)**
etaxel alone, the addition of cisplatin to docetaxel did not show any improvement in the ORR (4.3% in the DC arm vs. 4.3% in the D arm; p > 0.990), PFS (median, 1.8 months vs. 1.3 months; p=0.804), or OS (median, 5.6 months vs. 10.0 months; p=0.035), whereas this combination led to a deterioration in some QoL scores, including physical functioning, role functioning, emotional functioning, fatigue, appetite loss, insomnia, and dry mouth.

In contrast, the addition of S-1 to docetaxel showed a better PFS (median, 2.7 months vs. 1.3 months; p=0.034) than docetaxel alone without substantial impairment in the QoL or increasing toxicity except for all grades of nausea. Although this PFS benefit was not translated to an OS benefit, this effect might have been due to the higher proportion of patients who received subsequent chemotherapy in the D arm than in the DS arm (82.6% vs. 56.5%). In terms of confirmed ORR, which is similar to the addition of cisplatin to docetaxel, the addition of S-1 to docetaxel showed a very low ORR and did not show any significant difference compared to docetaxel alone (4.3% in the D arm, 4.3% in the DC arm, and 8.7% in the DS arm; p > 0.990). Previous studies of second-line chemotherapy in MGC showed an ORR ranging from 0% to 22% [8,9,24-27], and the ORR of the present study was much lower than the ORRs of previous studies. The small sample size might have affected the result because the baseline characteristics and treatment delivery did not appear to differ from previous studies. On the other hand, after including the unconfirmed response, the ORRs of the combination arms appeared to be comparable to those of previous studies (4.3% in the D arm vs. 13.0% in the DC arm vs. 13.0% in the DS arm; p=0.685).

A recent phase III study (JACCRO GC-05) also evaluated the re-introduction of a previous failed drug (S-1) combined with a new agent (irinotecan) in advanced gastric cancer refractory to first-line S-1 treatment [27]. The combination of irinotecan with S-1 did not show any PFS (3.8 months vs. 3.4 months; HR, 0.85; p=0.16) or OS (8.8 months vs. 9.5 months; HR, 0.99; p=0.92) benefits compared to irinotecan alone, even though grade ≥ 3 leukopenia and febrile neutropenia were significantly higher with the combination regimen. These inconsistent results regarding the re-introduction of a failed drug were also reported in earlier studies of metastatic colorectal cancer. In one report, the combination of oxaliplatin with infusional 5-FU/leucovorin resulted in a significantly better ORR (9.9% vs. 1.3% vs. 0%) and time to progression (median, 4.6 months vs. 1.6 months vs. 2.7 months) than oxaliplatin or infusional 5-FU/leucovorin alone following progression on irinotecan plus bolus 5-FU/leucovorin [28]. In another report, however, the combination of irinotecan with infusional 5-FU/leucovorin did not lead to an improvement in the ORR (16% vs. 11%, p=0.07), PFS (median, 4.4 months vs. 4.3 months; p=0.75), or OS (median, 15.0 months vs. 13.9 months; p=0.16) compared to irinotecan alone after the failure of first-line infusional 5-FU/leucovorin [29]. Currently, in colorectal cancer treatment regimens, 5-FU/leucovorin is normally re-administered after a previous failure in combination with either irinotecan or oxaliplatin [30].

The present study had several limitations. This was a small sized phase II study with an unmet primary endpoint and was terminated early because of the slow accrual. With these limitations, conclusions could not be drawn regarding the role of adding a previous failed agent to a second-line therapy in MGC. Given its importance in clinical practice, this issue needs to be addressed further in future clinical trials.

**Conclusion**

The addition of S-1, but not cisplatin, to docetaxel as a second-line treatment resulted in better efficacy in terms of the PFS compared to docetaxel alone, without clinically significant impairment of safety or QoL aspects, in MGC patients after progression on first-line S-1 or capcitabine plus cisplatin. Given that the re-challenge issue beyond progression might be dependent on a specific agent, molecularly targeted agents as well as cytotoxic chemotherapy will need to be investigated further in this setting to better optimize the second-line regimens in gastric cancer.

**Conflicts of Interest**

S-1 was provided by JEIL Pharm. Co., Ltd.

**Acknowledgments**

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References


Outcomes of Treatment for Malignant Peripheral Nerve Sheath Tumors: Different Clinical Features Associated with Neurofibromatosis Type 1

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Purpose
Malignant peripheral nerve sheath tumors (MPNSTs) are a rare subtype of sarcoma that occur spontaneously or in association with neurofibromatosis type 1 (NF-1). This study aimed to clinically differentiate these types of MPNSTs.

Materials and Methods
The study reviewed 95 patients diagnosed with and treated for MPNST at Yonsei University Health System, Seoul, Korea over a 27-year period. The clinical characteristics, prognostic factors, and treatment outcomes of sporadic MPNST (sMPNST) and NF-1-associated MPNST (NF-MPNST) cases were compared.

Results
Patients with NF-MPNST had a significantly lower median age (32 years vs. 45 years for sMPNST, p=0.012), significantly larger median tumor size (8.2 cm vs. 5.0 cm for sMPNST, p < 0.001), and significantly larger numbers of imaging studies and surgeries (p=0.004 and p < 0.001, respectively). The 10-year overall survival (OS) rate of the patients with MPNST was 52±6%. Among the patients with localized MPNST, patients with NF-MPNST had a significantly lower 10-year OS rate (45±11% vs. 60±8% for sMPNST, p=0.046). Univariate analysis revealed the resection margin, pathology grade, and metastasis to be significant factors affecting the OS (p=0.001, p=0.020, and p < 0.001, respectively). Multivariate analysis of the patients with localized MPNST identified R2 resection and G1 as significant prognostic factors for OS.

Conclusion
NF-MPNST has different clinical features from sMPNST and requires more careful management. Further study will be needed to develop specific management plans for NF-MPNST.

Key words
Sarcoma, Neurilemmoma, Neoplasms, Neurofibromatoses

Introduction
Malignant peripheral nerve sheath tumor (MPNST) is a type of soft tissue sarcoma that originates from the peripheral nerve sheath. These tumors account for 5%-10% of all soft tissue tumors, but their occurrence is rare in the general population with an estimated annual incidence of 1/1,000,000 individuals [1-3]. MPNST is frequently associated with neurofibromatosis, and has an incidence of 2%-5% among patients with neurofibromatosis type 1 (NF-1); this incidence is very high relative to that of the general population [2]. MPNST is considered aggressive and is associated with a low survival rate (34%-52%) [4]. The prognosis of MPNST is
associated with the tumor size and location, resection margin, adjuvant chemotherapy, distant metastasis, stage, and site [5-7].

NF-1 is an autosomal hereditary syndrome characterized by symptoms and signs, such as neurofibromas, café-au-lait spots, osseous lesions, optic pathway glioma, axillary or inguinal freckling, and Lisch nodules, with a global incidence of 1/2,500-1/3,000 individuals [8,9]. Throughout their lives, patients with NF-1 frequently develop benign and malignant tumors consequent to the loss of the tumor suppressive activity of NF1 [8]. The descendants of patients with NF-1 are born with a systemic heterozygous NF1 mutation; however, a mutation of the second normal copy is required for the formation of NF1-related tumors [10]. Patients with NF-1 carry an approximately 2.5- to 4-fold higher risk of malignancy relative to the general population, as well as the risk of optic glioma and soft tissue sarcoma, which have been reported to occur in 15%-20% and 4%-25% of patients, respectively [8].

The prognostic role of a patient’s NF-1 status with regard to the MPNST treatment has been studied because the genetic backgrounds underlying sporadic MPNST (sMPNST) and MPNST arising in patients with NF-1 (NF-1 associated MPNST, NF-MPNST) differ, and the clinical manifestations of NF-1 frequently include multiple benign and malignant tumors [6,11,12]. On the other hand, the differences in survival associated with these subtypea is controversial [6,11-16]. Although many researchers have reported the characteristic features of NF-MPNSTs, there are no standard management guidelines for this subtype. The present study evaluated the clinical differences between the two subtypes of MPNST to further the development of specific diagnostic and treatment strategies for NF-MPNST.

Materials and Methods

1. Patient population

A total of 95 patients diagnosed with and treated for MPNST at the Yonsei Cancer Center, Yonsei University Health System, Seoul, Korea from 1988 to 2015 were enrolled in this study. The clinical data were collected via retrospective chart review. A clinical diagnosis of NF-1 was confirmed by the presence of one of the following criteria: confirmed NF1 mutation and associated symptoms, or ≥2 clinical manifestations that met the National Institutes of Health (NIH) consensus criteria [3,9]. The direct polymerase chain reaction sequencing method of the patient DNA isolated from a blood sample was used for a genetic NF1 test. Among the 33 patients, 32 met the clinical diagnosis criteria fully. Among them, an additional genetic test was performed for six patients. For only one patient who did not meet the clinical diagnostic criteria, the genetic test was used to confirm NF-1. NF-MPNST was defined as MPNST that occurred in a patient diagnosed with NF-1, and sMPNST was defined as MPNST arising in a patient without NF-1. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (No. 4-2016-0401).

2. Treatment

Surgery was generally performed with curative intent. Palliative surgery was performed to relieve the symptoms caused by the tumor. A biopsy alone was performed in two cases involving unresectable lesions. Radiotherapy was generally administered to patients with positive tumor margins after surgery or to reduce symptoms, such as pain or other neurologic signs. Chemotherapy was generally administered for metastatic MPNST or recurrent disease. First-line adjuvant treatments for localized MPNST were evaluated as treatment patterns.

3. Clinical data

The pathologic diagnoses of MPNST were initially made but were reviewed by the institutional pathologist at the time of the study in accordance with the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) pathologic grading system [17]. The FNCLCC system considered the cellularity, nuclear pleomorphism, anaplasia, mitotic rate, necrosis, and microvascular proliferation [17]. For 15 of the 95 patients, the grades could not be defined either because these patients had been referred from other institutions (n=10) or tissues were unavailable because the diagnoses had been made at least 20 years earlier (n=5).

Information on the chemotherapeutic regimens was collected from the medical records. Data regarding the tumor size and depth, lymph node involvement, and distant metastases were collected from imaging studies, which included computed tomography, magnetic resonance imaging, ultrasonography, and positron emission tomography. This study evaluated the total number of imaging studies or operations including biopsies, which are defined as the sum of the number of imaging studies or operations performed between the time of diagnosis and the last follow-up. Disease progression was defined as tumor growth after surgical intervention, radiotherapy, or chemotherapy, based on imaging studies. Staging information according to the seventh American Joint Committee on Cancer (AJCC) staging system was obtained from the surgical records [18]. The margin status was defined as follows: R0, microscopically negative margins (tumor-free
margin > 2 mm; R1, macroscopically negative margins with microscopically positive margins; or R2, macroscopically positive margins.

4. Statistics

The overall survival (OS) was calculated as the interval from the date of diagnosis to the date of death from any cause or the last follow-up. OS was calculated using the number of deaths prior to April 30, 2016. A chi-square test or Fisher exact test was used to analyze the categorical variables, and a Student’s t test or Mann-Whitney test and an analysis of variance (ANOVA) or the Kruskal-Wallis test were used to analyze the continuous variables. The Kaplan-Meier method was used for survival analyses, and the results were compared using a log-rank test. A Cox proportional

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of patients with malignant peripheral nerve sheath tumors</th>
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</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
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<tr>
<td>Age at expire (yr)</td>
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<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Location</td>
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<td>Trunk</td>
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<tr>
<td>Extermity</td>
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<tr>
<td>Head and neck</td>
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<tr>
<td>Tumor size (cm)</td>
</tr>
<tr>
<td>&lt; 5</td>
</tr>
<tr>
<td>≥ 5</td>
</tr>
<tr>
<td>N/A</td>
</tr>
<tr>
<td>Depth</td>
</tr>
<tr>
<td>Superficial tumor</td>
</tr>
<tr>
<td>Deep tumor</td>
</tr>
<tr>
<td>Multiple lesion</td>
</tr>
<tr>
<td>Metachronous metastasis</td>
</tr>
<tr>
<td>Multiple primary</td>
</tr>
<tr>
<td>Histologic grade (n=80)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<td>IB</td>
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<td>IIA</td>
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<tr>
<td>IIB</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>Metastasis</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Margin status (in localized MPNST)</td>
</tr>
<tr>
<td>R0</td>
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<tr>
<td>R1</td>
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<tr>
<td>R2</td>
</tr>
</tbody>
</table>

Values are presented as number (%). NF-1, neurofibromatosis type 1; N/A, not acquired; MPNST, malignant peripheral nerve sheath tumors. aLinear by linear association, bR0/R1/2, resection margin, see details in Materials and Methods section.
Fig. 1. Scheme of first-line treatment modalities. sMPNST, sporadic malignant peripheral nerve sheath tumors; NF-MPNST, malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1; Chemo, chemotherapy; RT, radiotherapy; Both, chemotherapy and radiotherapy; NED, no evidence of disease; R0/R1/2, resection margin, see details in Materials and Methods section; Tx, treatment; F/U, follow-up.
hazard regression model was used for multivariate data analysis and included the factors identified as significant in univariate analysis. Age was also included because this factor was considered clinically meaningful with respect to the treatment tolerability and hereditary cancer syndrome. SPSS ver. 20 for Windows (IBM Corp., Armonk, NY) was used for the statistical analysis of each outcome measure.

Results

1. Clinical characteristics

The overall median age was 40.4 years (interquartile range, 28.3 to 54.0 years; range, 8.3 to 80.4 years) (Table 1). In particular, the 33 patients (35%) with NF-MPNST had a significantly lower median age (31.9 years vs. 45.3 years for sMPNST, p=0.012). Forty-five patients (47%) in the overall group were female, and no significant sex-related difference was observed between the patients with sMPNST and NF-MPNST.

Overall, the tumors presented most frequently in the extremities (n=45, 47%), followed by the trunk (n=34, 35%) and head and neck (n=16, 17%). Among patients with NF-MPNST, the trunk was the most common tumor site (n=15, 46%), whereas the extremities were the most common sites among patients with sMPNST (n=31, 50%). On the other hand, these inter-group differences in MPNST sites were not significant (p=0.325). The median size of the primary NF-MPNSTs was greater than that of the sMPNSTs (8.2 cm vs. 5.0 cm, p < 0.001), and the proportion of large primary tumors (size > 5 cm) was significantly higher among the patients with NF-MPNST than those with sMPNST (28 [85%] vs. 26 [42%], p < 0.001).

Generally, most tumors were located deep beneath the fascia (88% overall; 89% for sMPNST vs. 88% for NF-MPNST, p=0.904). The histologic grade was similar in the two groups (p=0.302, linear by linear association). In contrast, the NF-MPNST patients were significantly more likely to have a higher tumor stage (p=0.002, linear by linear association) (Table 1). Among the cases of localized MPNST, the frequency of patients with NF-MPNST who had positive tumor margins comprised the majority of such patients and was significantly higher than the frequency among patients with sMPNST (66.7% vs. 47.6%, p=0.035). Similar frequencies of metastases were observed in both groups (6 [10%] vs. 3 [9%], p=0.926).

2. Management patterns

Overall, 93 patients (98%) underwent primary surgery. Only two patients (2%) underwent a biopsy alone. In addition, 46 patients (48%) received radiotherapy, and 27 (28.4%, 27/95) received chemotherapy; of these, 16 had sMPNST. Seven patients (28%, 7/25) received neoadjuvant chemotherapy. Anthracyclines (15%), ifosfamide (27%), cisplatin (17%), and etoposide (5%) were the most commonly used chemotherapeutic agents, and the most common regimen was anthracycline-ifosfamide (41%, 11/27), followed by ifosfamide-cisplatin-etoposide (26%, 7/27). Only two patients were treated with anthracycline-ifosfamide-cisplatin (7%, 2/27), and one patient used anthracycline-cisplatin and ifosfamide-cisplatin (S1 Table).

The first-line treatment schemes administered to patients with localized sMPNST and NF-MPNST were compared (Fig. 1). In the R0 group, 60% (18/30) of patients with sMPNST received no further therapy, whereas 56% (5/9) with NF-MPNST received adjuvant treatments (p=0.202). In the R1 group, 50% (4/8) of patients with sMPNST received no treatment, whereas 75% (6.8) of those with NF-MPNST received further adjuvant treatments (p=0.315). In the R2 group, 9% (1/11) of patients with sMPNST and 30% (3/10) with NF-MPNST received chemotherapy and radiotherapy both as the adjuvant treatment (p=0.181). When the analysis was limited to localized MPNST in total, 76 patients (49 sMPNSTs and 27 NF-MPNSTs) underwent a primary resection. Among them, 38% (n=29) received no adjuvant treatment, 54% (n=41) received chemotheraphy or radiotherapy, and 8% (n=6) received both treatments. A total of 45% (22/49) of patients with sMPNST received no further treatment, whereas 74% (20/27) of those with NF-MPNST received adjuvant treatment, including chemotherapy, radiotherapy, or both (p=0.04999). In addition, the NF-1 patients underwent a higher median annual number of imaging studies (7.54 vs. 3.67 for sMPNST, p=0.004) and operations, including biopsy (2.45 vs. 0.70, p < 0.001) during the follow-up period (S2 Table).

3. Survival outcomes

The 10-year OS rate of the entire study cohort was 52±6% (Fig. 2). In particular, although the 10-year OS rate was lower among patients with NF-MPNST than among those with sMPNST, this difference was not significant (44±11% vs. 56±7%, p=0.084) (S3 Fig.). On the other hand, among patients with localized MPNST, those with NF-MPNST had a significantly lower 10-year survival rate (45±11% for NF-MPNST vs. 60±8% for sMPNST, p=0.046) (Fig. 3A). In contrast, patients with metastasis had similar survival outcomes, regardless of the subtype (5-year OS, 33±27% for NF-MPNST
vs. 17±15% for sMPNST, p=0.875) (Fig. 3B).

Patients with R0 margins had a significantly higher 10-year OS rate than the patients with other margin statuses (65±9% for R0; 41±7% for R1 or R2; p=0.001) (Fig. 4A). The patients with pathologic G1 disease had a better 10-year OS rate than those in the other staging groups (G1 vs. G2 vs. G3 vs. N/A; 82±9% vs. 31±12% vs. 44±12% vs. 51±13%, respectively, p=0.020) (Fig. 4B). Metastasis at the time of diagnosis was associated with a poorer prognosis (5-year OS, 55±6% for localized MPNST vs. 22±14% for MPNST with metastasis, p < 0.001) (Fig. 4C). No survival difference was observed with respect to the use of a doublet vs. triplet regimen (50±14% vs. 53±15%, p=0.738) or to the tumor location and size ≥ 5 cm (p=0.264 and p=0.113, respectively; data not shown).

4. Multivariate analysis of clinical risk factors affecting OS in localized MPNSTs

Because NF-1 was identified as a significant prognostic factor for OS among patients with localized MPNSTs in the univariate analysis, multivariate analysis was performed to confirm the risk factors for OS. The R2 status was confirmed to be a significant risk factor for OS (hazard ratio, 2.61; 95% confidence interval [CI], 1.03 to 6.61), and histologic G1 disease was identified to be more favorable for OS compared to G3 disease (odds ratio [OR], 0.18; 95% CI, 0.05 to 0.71). On the other hand, the NF-1 status was found to be an insignificant prognostic factor for OS (OR, 1.18; 95% CI, 0.48 to 2.90). Age > 45 years and a positive resection margin were not identified as significant prognostic factors (Table 2).

**Discussion**

The identified frequency of NF-MPNSTs in this study was 35%; previous studies have reported a frequencies of 20% to 70% [7,16,19-21]. NF-MPNST was found to be related to a
younger age of onset, greater tumor size, and higher disease stage; these findings are in line with those of many previously reports [5,6,12,16,20,21]. Consistent with previous reports, a lower frequency of R0 resection was observed among patients with NF-MPNST compared to those with sMPNST [19,21,22]. Overall, these findings might affect the survival of patients with NF-MPNST.

In previous reports, the 10-year survival rates of MPNST ranged from 30% to 60% [6,11,12,21,23]. The observed 10-year OS rate of 52±6% was consistent with previous reports. The survival differences of patients with NF-MPNST and those with sMPNST are controversial. In the present study, the survival of patients with NF-MPNST was inferior to that of patients with sMPNST when cases of localized disease were analyzed, even though this was not confirmed in multivariate analysis. Many studies reported poorer survival among patients with NF-MPNST [5,11,20]; in contrast, others have reported no significant difference in survival between the two MPNST groups [7,10,12,16]. To the best of the authors’ knowledge, no previous report has demonstrated a better survival among patients with NF-MPNST. In other words, the survival of patients with NF-MPNST is equivalent to or poorer than that of patients with sMPNST.

Given the rarity of MPNST, few studies have been able to analyze more than 100 MPNSTs from a single institution, and existing large studies have tended to report lower survival among patients with NF-MPNST [5,6,11,23-25]. This article is similar to previous reports that also demonstrated a survival difference in univariate analysis but failed to confirm the significance of this difference in multivariate analysis [6,15]. This pattern might be due to confounding factors, such as poor prognostic factors associated specifically with NF-MPNST [5,6,15]. In particular, articles published before 2000 tended to report poorer survival outcomes among patients with NF-MPNST. Since 2000, however, the differences in survival between the two subtypes have narrowed [5,12,24,25]. The treatment protocols used prior to 2000 were different from the current treatment trends, which tended toward more intensive treatment. Over time, previous articles may represent the natural history of NF-MPNST, as well as the essential differences related to the genetic background and clinical characteristics. Despite the reduction or absence of survival differences in recent articles, the considerable heterogeneity among the study cohorts prevents a direct comparison [12,21].

As in many previous articles, metastasis was identified as a poor prognostic factor for both NF-MPNST and sMPNST [5,6,11]. Porter et al. [11] reported significant survival differences between the subtypes in an analyses of both localized and total MPNST’s, whereas Anghileri et al. [15] reported a survival difference among localized MPNSTs. Carl et al. [5] reported a survival difference between the subtypes, even in

**Fig. 4.** Overall survival according to the risk factors. (A) Overall survival associated with the margin status (positive vs. negative). (B) Overall survival associated with the pathologic grade (not acquired [N/A] vs. G1 vs. G2 vs. G3). (C) Overall survival associated with the metastatic status (localized vs. metastasis).
Table 2. Multivariate analysis of the overall survival of patients with localized malignant peripheral nerve sheath tumors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 45 yr vs. &lt; 45 yr</td>
<td>0.81</td>
<td>0.38-1.70</td>
<td>0.571</td>
</tr>
<tr>
<td>NF-1 vs. sporadic</td>
<td>1.18</td>
<td>0.48-2.90</td>
<td>0.716</td>
</tr>
<tr>
<td>Margin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0</td>
<td>1.00 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>1.47</td>
<td>0.54-3.98</td>
<td>0.449</td>
</tr>
<tr>
<td>R2</td>
<td>2.61</td>
<td>1.03-6.61</td>
<td>0.043</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5 cm vs. &lt; 5 cm</td>
<td>1.040</td>
<td>0.40-2.68</td>
<td>0.935</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>0.18</td>
<td>0.05-0.71</td>
<td>0.014</td>
</tr>
<tr>
<td>G2</td>
<td>1.19</td>
<td>0.50-2.82</td>
<td>0.699</td>
</tr>
<tr>
<td>G3</td>
<td>1.00 (reference)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; NF-1, neurofibromatosis type 1.

a total MPNST group (including localized and metastatic disease) and attributed this difference to the more frequent incidence of metastatic disease among patients with NF-1. In the present cohort, a significant survival difference was observed between NF-MPNST and sMPNST among localized MPNSTs, whereas only a non-specific tendency was observed in the total group. In contrast to Carli et al. [5], the frequency of metastasis was similar in patients with NF-MPNST and sMPNST. Hence, the survival difference between these subgroups in the total cohort might not have been evident. In addition, as metastasis might be a stronger prognostic factor than NF-1, the survival difference between the subtypes might be more evident when metastatic disease is excluded.

Patients with NF-1 tend to develop numerous benign tumors, including preexisting neurofibromas. Therefore, it is challenging for clinicians to differentiate newly developed malignancies from underlying benign tumors associated with NF-1 [8,10,12,20]. The life time risk of MPNST in NF-1 patients is high (8%-13%) [20]. This unique finding and the clinical characteristics associated with NF-MPNST affect the disease management patterns, including treatments and cancer surveillance. Regarding the treatment patterns, patients with NF-MPNST also tended to receive adjuvant treatments more frequently than patients with sMPNST. NF-MPNST is associated with a low likelihood of a R0 resection and a higher probability of a large tumor size, and clinicians tend to choose additional treatments to avoid the risk of recurrence even if a curative resections is possible [5,19,21,23]. Patients with NF-MPNST undergo both imaging studies and operations more frequently, relative to those with sMPNSTs, in an attempt by clinicians and patients to achieve an early diagnosis and resection, and overcome the challenges in distinguishing MPNSTs from benign neurofibromas [6,12,26]. All of these differences could burden both the patients with NF-1 and clinicians during the process of MPNST management [6,12,19-21]. These changes in modern management patterns might have reduced the previously mentioned survival difference between sMPNST and NF-MPNST [5,12]. Therefore, the role of these management strategies should be investigated prospectively [20].

The increased identification of genetic cancers in recent years has led to the development of agents that target genetic alterations [27,28]. NF1 acts as a tumor suppressor; hence, the genetic alteration of NF1 is among the etiologies of MPNST in patients with NF-1 [8]. On the other hand, other genetic abnormalities contribute to the malignant transformation of benign neurofibromas [29,30]. Given the previously mentioned clinical characteristics, the management of NF-MPNST requires the development of specific clinical prevention methods and molecular targeting agents [22,30].

The present study had some unique points, such as a relative large single-institution cohort and a focus on Asian ethnicity. On the other hand, this study was retrospective, and the gradual changes in treatment modalities and intensities over the long study period need to be acknowledged.
Conclusion

MPNSTs associated with NF-1 tend to arise at a younger age, are generally larger, and are diagnosed at a higher stage compared to sMPNST. In addition, NF-MPNST and sMPNST have different diagnostic and treatment patterns. These results highlight the need for further investigation of specific strategies that account for the differences in clinical and genetic backgrounds.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

References

Why Do Some People Choose Opportunistic Rather Than Organized Cancer Screening? The Korean National Health and Nutrition Examination Survey (KNHANES) 2010-2012

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Purpose
Although the Korean government has implemented a universal screening program for common cancers, some individuals choose to participate in opportunistic screening programs. Therefore, this study was conducted to identify factors contributing to the selection of organized versus opportunistic screening by the Korean general population.

Materials and Methods
Data from 11,189 participants aged ≥40 years who participated in the fifth Korean National Health and Nutrition Examination Survey (2010-2012) were analyzed in this study.

Results
A total of 6,843 of the participants (58.6%) underwent cancer screening, of which 6,019 (51.1%) participated in organized and 824 (7.5%) participated in opportunistic screening programs. Being female, older, highly educated, in the upper quartile of income, an ex-smoker, and a light drinker as well as having supplementary private health insurance and more comorbid conditions and engaging in moderate physical activity 1-4 days per week were related to participation in both types of screening programs. Being at least a high school graduate, in the upper quartile for income, and a light drinker, as well as having more comorbid conditions and engaging in moderate physical activities 1-4 days per week had a stronger effect on those undergoing opportunistic than organized screening.

Conclusion
The results of this study suggest that socioeconomic factors such as education and income, as well as health status factors such as health-related quality of life and number of comorbid conditions and health behaviors such as drinking and engaging in moderate physical activity 1-4 days per week had a stronger influence on participation in an opportunistic than in an organized screening program for cancer.

Key words
Early detection of cancer, Socioeconomic factors, Quality of life, Health behavior

Introduction

Cancer is as a leading cause of death in Korea [1]; therefore, the Korean government launched a National Cancer Screening Program (NCSP) in 1999 in an attempt to save lives [2]. The NCSP is a well-organized program that has offered screening for five types of cancer (stomach, breast, colorectal, cervical, and liver) to all Korean individuals since 2005. The program is organized according to population group. Recipients of the Medical Aid Plan (MAP) and National Health Insurance (NHI) who are in the lower 50% of the premium scale can access its services free of charge, whereas NHI beneficiaries in the upper 50% of the premium scale are required to pay 10% of the cost of screening [3]. In contrast, individuals who use opportunistic cancer screening programs must pay for all procedure-related costs [4].

Even though the Korean government has ensured that the
entire population can use the organized cancer screening program free of charge, opportunistic screening remains popular despite its out-of-pocket costs. Most medical institutions, tertiary hospitals, and clinics provide both organized and opportunistic health examination programs, including cancer screening [5]. Since the Korean government controls the medical fee schedule for most medical services provided by NHI, the fees for these services are insufficient [6]. Therefore, most institutions attempt to earn money through opportunistic health examination services, which have large profit margins because they are not constrained by the government’s fee schedule. Overall expenditures on health examinations were estimated at 1.5 billion US dollars in 2009 [7]; however, given the rapid growth in the health examination market in Korea, total expenditures may increase substantially.

Participation in cancer screening programs according to the recommended schedule is the best way to reduce the burden of cancer, especially by increasing the survival rate and improving the prognosis of cancer patients [8]. Increasing the rate of participation in cancer screening programs is an important approach to reducing the burden of this disease because screening is an efficient method of ameliorating the morbidity and mortality associated with cancer [9].

Previous studies have identified several factors associated with participation in cancer screening, many of which have focused on socioeconomic differences in such participation [4,8,10]. Kang et al. [10] identified educational and income disparities among those in attendance for opportunistic screening, while there were no significant differences in the organized screening. Lee et al. [4] reported trends in socioeconomic disparities in organized and opportunistic gastric cancer screening. Further, they found that socioeconomic disparities were still present for the opportunistic screening because of widening socioeconomic differences in Korea [4].

Some studies have reported a correlation between health-related quality of life (HRQOL) and participation in cancer screening programs [9,11-14]. These studies have suggested that higher cancer screening rates are associated with a better HRQOL. Health behaviors such as smoking, drinking, and physical activity have also been found to be associated with participation in cancer screening programs [7,12]. Most studies that have attempted to identify or examine the effects of relevant variables such as socioeconomic status, quality of life, health behaviors, and health status on participation in cancer screening [12,14] have focused exclusively on socioeconomic status or health behaviors.

It is important to determine why people participate in opportunistic cancer screening, despite their eligibility to use organized screening programs. Focusing on Korea, this study examined the factors that contribute to individual decisions to participate in organized versus opportunistic screening using data from the fifth Korean National Health and Nutrition Examination Survey (KNHANES V). Moreover, we attempted to identify factors that were more strongly associated with participation in opportunistic than organized cancer screening.

Materials and Methods

1. Study population

This study was based on data derived from the 2010-2012 KNHANES, a nationwide survey examining the general health and nutrition status of the Korean general population conducted by the Korea Centers for Disease Control and Prevention (KCDC) [15]. The KNHANES relies on four methods of data collection, a health interview survey, a health behavior survey, a health examination, and a health nutrition survey [15]. The sample was selected using a stratified, multistage probability sampling design. Overall, 31,641 subjects from 11,400 households (3,800 households annually) and 573 districts (192 districts annually) were selected based on location and type of residence to achieve representativeness of the entire Korean population [15]. The overall response rate was more than 80% for the 3-year study period (81.7% in 2010, 80.4% in 2011, and 80.0% in 2012). In total, 25,534 individuals participated, and data from 13,661 subjects older than 40 years were analyzed in this study (Fig. 1).

We excluded 624 people who had already been diagnosed with cancer and 1,516 people who did not answer the questions about participation in cancer screening or about the type of cancer screening in which they participated. We also excluded 332 subjects who did not participate in one or more of the screening programs for stomach, breast, or colon cancer because these are the most common cancers in Korea [16].

The final sample consisted of 11,189 participants, and written informed consent was obtained from each participant prior to the survey. The study protocol was approved by the Institutional Review Board of the KCDC (Nos. 2010-02CON-21-C, 2011-02-CON-06-C, and 2012-01-EXP-01-2C).

2. Measurements and variables

Data regarding the variables analyzed in this study were obtained through the health interview and health behavior surveys of the KNHANES. We categorized those who participated in cancer screening programs according to whether they were screened as part of an organized or opportunistic program, as determined by responses to the following question: “In last 2 years, have you undergone a cancer screen-
Respondents were considered to have been screened if they underwent screening for one of three cancers (stomach, colon, and breast cancer), as determined by the following two yes or no questions: (1) “In the last 2 years, have you undergone cancer screening provided by the government or National Health Insurance (NHI) service free of charge or with a coinsurance payment of 5%?” and (2) “In last 2 years, have you undergone cancer screening provided by medical institutions such as hospitals or clinics in exchange for out-of-pocket costs?” We identified those who participated in organized cancer screening with the former question and those who participated in opportunistic cancer screening with the latter question. Subjects who had used both types of screening programs in the last 2 years were considered to have undergone organized screening because this study focused on factors correlated with opportunistic screening even though the government guaranteed organized screening to all Koreans. The Korean government recommends that people aged 40 years or older undergo screening for stomach and breast cancer, and that those aged 50 years or older undergo screening for colorectal cancer.

To identify factors associated with participation in cancer screening, we classified the variables correlated with such screening into four groups: demographic characteristics, socioeconomic characteristics, variables related to health status, and health behaviors. Socioeconomic status was based on education level (four categories), monthly household income (four categories), job level (four categories), and marital status (two categories). We measured health insurance status to identify the effects of type of health insurance and private health insurance on participation in cancer screening programs. In Korea, public universal health insurance is divided into NHI and the MAP.

Because HRQOL has a major influence on the use of healthcare services, including cancer screening [11,14], we added a measure of quality-adjusted life-years (QALY) to the study model and divided the sample into two groups based on whether respondents’ scores on the EQ-5D were or were not in the lowest quintile, 0.854. The influence of comorbid conditions on participation in cancer screening was also examined with an index of comorbidity calculated by totaling each participant’s reported history of the following diseases: hypertension, stroke, cardiovascular disease, arthritis, tuberculosis, asthma, diabetes mellitus, thyroid disease, depression, atopic disease, renal failure, hepatitis B and C, cirrhosis, hyperlipidemia, and chronic obstructive pulmonary disease. The total number of comorbid diseases was categorized into four groups (0, 1, 2, and 3 or more). Health
Table 1. Participation in cancer screening in Korea by demographic characteristics, socioeconomic status, health status, and health behaviors (2010-2012)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Overall screening (A+B)</th>
<th>Not screened</th>
<th>Screened</th>
<th>p-value$^{ab}$</th>
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<td>Organized screening (A)</td>
<td>Opportunistic screening (B)</td>
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<td>824 (7.5)</td>
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<td>2,766 (53.9)</td>
<td>2,089 (46.1)</td>
<td>2,362 (45.8)</td>
<td>404 (8.1)</td>
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<td>2,257 (36.9)</td>
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<td>971 (49.5)</td>
<td>906 (49.2)</td>
<td>879 (45.2)</td>
<td>92 (5.6)</td>
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<td>1,094 (42.3)</td>
<td>1,534 (51.2)</td>
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<tr>
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<td>1,866 (64.7)</td>
<td>891 (35.3)</td>
<td>1,538 (53.3)</td>
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<td>Unemployed</td>
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<tr>
<td>Medical Aid</td>
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<td>178 (50.6)</td>
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<tr>
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<td>1,973 (48.3)</td>
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<tr>
<td>Upper four quintiles (&gt; 0.854)</td>
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<td>5,318 (59.7)</td>
<td>3,137 (40.3)</td>
<td>4,621 (51.4)</td>
<td>697 (8.3)</td>
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<tr>
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<tr>
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<td>3,310 (56.7)</td>
<td>2,217 (33.3)</td>
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<td>3,012 (24.9)</td>
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<td>1,129 (39.0)</td>
<td>1,643 (53.1)</td>
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<tr>
<td>2</td>
<td>1,695 (12.4)</td>
<td>1,056 (62.1)</td>
<td>639 (37.9)</td>
<td>940 (54.6)</td>
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<tr>
<td>≥ 3</td>
<td>955 (7.6)</td>
<td>594 (59.7)</td>
<td>361 (40.3)</td>
<td>527 (52.8)</td>
<td>67 (6.9)</td>
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<td></td>
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</tr>
<tr>
<td>Non-smoker</td>
<td>6,536 (53.5)</td>
<td>4,219 (62.9)</td>
<td>2,317 (37.1)</td>
<td>3,768 (55.9)</td>
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<tr>
<td>Ex-smoker</td>
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<td>972 (39.6)</td>
<td>1,352 (51.4)</td>
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<tr>
<td>Current smoker</td>
<td>2,055 (23.5)</td>
<td>1,015 (49.2)</td>
<td>1,040 (50.8)</td>
<td>881 (40.2)</td>
<td>134 (7.0)</td>
</tr>
</tbody>
</table>
behaviors such as smoking, alcohol consumption, and physical activity were also measured. Respondents were categorized as non-smokers, ex-smokers, and current smokers (three groups), as well as into those who had never consumed alcohol, ex-drinkers or current non-drinkers, light drinkers, and heavy drinkers (four groups). Respondents were classified according to whether they engaged in physical activity less than 1 day per week, 1-4 days per week, or more than 4 days per week (three groups).

3. Statistical analyses

Participation rates in cancer screening programs were compared using the chi-square test according to socioeconomic status, type of health insurance, quality of life, health behaviors, and comorbidity. Survey sample weights were used to produce non-biased estimates for the chi-squared test and logistic regression model [15].

The polychotomous (multinomial) logistic regression model was used to identify correlates of participation in cancer screening programs, including organized and opportunistic screening programs. This model estimated the simultaneous odds ratio (OR) for organized and opportunistic cancer screening and independent variables with respect to unscreened subjects. All statistical analyses were performed with the SAS software ver. 9.3 (SAS Institute, Cary, NC).

Results

1. Study population and participation in cancer screening

Of the 11,189 people aged older than 40 years who participated in the survey, 6,843 (58.6%) had been screened for cancer. The mean age of the study population was 56.1 years, and 82.2% of participants lived with their spouse. More than 52% of the subjects had at least graduated from high school, 96.6% were covered by NHI, and 69.0% had one or more supplementary private health insurance plans.

Of the 6,843 respondents who participated in cancer screening programs, 6,019 (51.1%) participated in the organized screening program and 824 (7.5%) had used an opportunistic screening program and paid out-of-pocket for the full price of the screening. Additionally, 681 respondents used both programs and we categorized those subjects into organized screening. Table 1 presents the overall descriptive statistics for the participants and shows differences in the participation rates in the two screening program types by demographic characteristics, socioeconomic status, quality of life, and health behaviors.

The results revealed significantly different participation rates in the two program types according to demographic and socioeconomic characteristics such as sex, age, education, household income, job, possession of health insurance, quality of life, number of comorbid conditions, and health behaviors (e.g., smoking, drinking, and physical activity). Females were more likely to be screened, but males were more likely to use opportunistic screening programs (8.4%).
Table 2. Results of univariate logistic regression analyses identifying risk factors for organized and opportunistic screening

<table>
<thead>
<tr>
<th>Variable</th>
<th>Organized screening</th>
<th>Opportunistic screening</th>
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<td></td>
<td>OR</td>
<td>95% CI</td>
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<tr>
<td><strong>Total</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
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<td></td>
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</tr>
<tr>
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<td>1.25-1.65</td>
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<td>Employee</td>
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<tr>
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<td>1.00</td>
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<td>Lowest quintile (≤ 0.854)</td>
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</tr>
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<td>1.09-1.46</td>
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<td>1.15</td>
<td>0.96-1.38</td>
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<td>Ex-drinker</td>
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<tr>
<td>Heavy drinker</td>
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Table 2. Continued

<table>
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<tr>
<th>Variable</th>
<th>Organized screening</th>
<th>Opportunistic screening</th>
</tr>
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<tbody>
<tr>
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</tr>
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</tr>
<tr>
<td>1-4 days per week</td>
<td>1.21</td>
<td>1.06-1.37</td>
</tr>
<tr>
<td>More than 4 days per week</td>
<td>1.26</td>
<td>1.04-1.53</td>
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</tbody>
</table>

OR, odds ratio; CI, confidence interval. aUnmarried, divorced, or widowed.

2. Factors associated with participation in organized and opportunistic cancer screening programs

The results of the univariate logistic regression analysis are presented in Table 2. All risk factors were significantly associated with participation in the organized screening program for cancer. However, sex, age, and number of comorbid conditions were not significantly associated with participation in opportunistic programs.

Table 3 presents the results of the multivariate logistic regression model. Females were more likely than males to participate in both screening programs and the odd ratios for organized screening was slightly higher than those for opportunistic screening program (OR, 1.59; 95% confidence interval [CI], 1.53 to 1.89 for the organized screening and OR, 1.49; 95% CI, 1.08 to 2.05). Subjects in their 60s were more likely than those in their 30s to be screened for cancer, and the OR for organized screening was slightly higher than those for the opportunistic screening program (OR, 2.28; 95% CI, 1.88 to 2.76 for organized screening and OR, 1.87; 95% CI, 1.34 to 2.62 for opportunistic screening). Currently married respondents were more likely than all others to be screened in organized screening programs (OR, 1.38; 95% CI, 1.18 to 1.61). Having supplementary private health insurance was an important contributor to participation in a screening program (OR, 1.84; 95% CI, 1.59 to 2.13 for organized cancer screening and OR, 1.76; 95% CI, 1.31 to 2.35 for opportunistic cancer screening).

People with a lower quality of life were significantly less likely to participate in opportunistic screening programs (OR, 0.69; 95% CI, 0.52 to 0.89). Suffering from more comorbid conditions was correlated with being screened, especially via opportunistic screening programs (OR, 2.15; 95% CI, 1.39 to 3.32 for opportunistic screening).

Health behaviors such as smoking, drinking, and physical activity were important risk factors correlated with being screened for cancer. Ex-smokers were more likely than current smokers to be screened by both program types (OR, 1.60; 95% CI, 1.37 to 1.89 for organized cancer screening and OR, 1.62; 95% CI, 1.17 to 2.24 for opportunistic cancer screening). The OR for ex-smokers was higher than that for non-smokers. Light drinkers were more likely to be screened than non-drinkers (OR, 1.26; 95% CI, 1.08 to 1.47 for organized cancer screening and OR, 1.42; 95% CI, 1.07 to 1.87 for opportunistic cancer screening). People who engaged in a moderate level of physical activity (1-4 times per week) were more likely to be screened by both types of cancer screening programs than those who did not exercise, and the OR for screening was elevated for opportunistic screening programs (OR, 1.20; 95% CI, 1.05 to 1.38 for organized cancer screening and OR, 1.41; 95% CI, 1.11 to 1.80 for opportunistic cancer screening).

Discussion

Why do people choose to undergo opportunistic screening despite the availability of free or almost free-of-charge organized screening? We used national-level data from the KNHANES V to answer this question. Screening for cancer is a cost-effective approach to reducing the burden of cancer and saving the lives of potential cancer patients [17]. Therefore, the Korean government implemented a universal organized screening program for five types of cancer (stomach, colon, breast, cervical, and liver) in 2005 [3,17]. However, the participation rate in this cancer screening program remains relatively low, and many people use opportunistic screening services. In this study, we sought to identify factors associated with using opportunistic cancer screening programs.

The overall participation rate in cancer screening programs was 58.6% (6,843 subjects; 51.2% for organized cancer screening and 7.5% for opportunistic screening). Specifically, 54.1%, 34.1%, and 32.4% of the subjects were screened for stomach cancer, colorectal cancer, and breast cancer, respectively. The Korean National Cancer Screening Survey (KNHCS) shows participation rate in cancer screening of 33.6% to 73.6% by
Table 3. Results of multivariate logistic regression analyses identifying risk factors for organized and opportunistic screening

<table>
<thead>
<tr>
<th>Variable</th>
<th>Organized screening</th>
<th>Opportunistic screening</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>Lowest quintile (≤ 0.854)</td>
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<td>1</td>
<td>1.23</td>
<td>1.07-1.41</td>
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<td>2</td>
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<td>1.11-1.55</td>
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<td>≥ 3</td>
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Table 3. Continued

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<th>Opportunistic screening</th>
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<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
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<td>Smoking status</td>
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<td>Alcohol consumption</td>
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<td>Heavy drinker</td>
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<tr>
<td>p-value for trend&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<tr>
<td>1-4 days per week</td>
<td>1.20</td>
<td>1.05-1.38</td>
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<td>More than 4 days per week</td>
<td>1.19</td>
<td>0.97-1.46</td>
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<tr>
<td>p-value for trend&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0068</td>
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OR, odds ratio; CI, confidence interval. <sup>a</sup>Calculated using continuous values. <sup>b</sup>Unmarried, divorced, or widowed.

types of cancer in 2013 [18].

There were several significant differences between those who used organized and opportunistic screening programs. Females and those in their 50s and 60s were more likely to use the organized cancer screening program than males and those in their 40s. Highly educated people were significantly more likely than those who were in the least educated group to participate in organized screening. Interestingly, the probability of participating in opportunistic screening increased as education level increased (p < 0.001).

Those with higher incomes were more likely to be screened using an opportunistic rather than an organized program (OR, 2.17; 95% CI, 1.53 to 3.09 for opportunistic screening vs. OR, 1.23; 95% CI, 1.03 to 1.47 for organized screening). We found little difference between those with and without supplementary private health insurance in the probability of being screened by the two programs (OR, 1.76; 95% CI, 1.30 to 2.37 for opportunistic screening vs. OR, 1.82; 95% CI, 1.57 to 2.12 for organized screening).

We obtained several significant results with respect to health status. The likelihood of participating in opportunistic screening was decreased among those with a poor quality of life, whereas more comorbid conditions increased the likelihood of using both organized and opportunistic screening programs. People with a better HRQOL might be more likely to engage in health behaviors [14]. Indeed, previous studies evaluating the impact of health status indicators on cancer screening have reported that poor subjective health status decreased the likelihood of getting screened, and that the rate of getting screened was higher among those with more chronic diseases [12]. Gandhi et al. [14] reported that poor general and physical health were associated with decreased screening rates. Conversely, another study reported no association between quality of life and participation in colorectal cancer screening [9].

We used the EQ-5D, a simple and widely used tool, to measure health status, including HRQOL. The EQ-5D, which is also commonly used to calculate QALY [11,19], addresses the mobility, self-care, usual activities, pain/discomfort, and anxiety/depression [20]. Therefore, EQ-5D provides an index of not only physical but also mental health. Our results suggest that people with poor quality of life are less likely to receive opportunistic screening, but that individuals who self-reported comorbid conditions were more likely to participate in not only organized screening, but also opportunistic screening. Suh et al. [21] reported that poor subjective health might be associated with a high screening rate for colorectal cancer. Subjective health may be influenced by an awareness of one’s objective health status, such as having a chronic disease. Additionally, the probability of participating in opportunistic screening was much higher than that of participating in organized screening among people with poor subjective health.

Health behaviors were associated with participation in cancer screening programs. The participation rate among people with moderately good health habits was higher than
it was among those with extremely good health habits. Ex-smokers were more likely than current smokers to be screened by both types of screening programs. Non-smokers were more likely than current smokers to participate in the organized screening program, which is in accordance with previous results regarding breast and cervical cancer screening among women in the United States [14]. We also found that light drinkers were more likely to be screened by both opportunistic and organized screening programs than non-drinkers, and the OR for opportunistic screening was higher than that for organized screening (OR, 1.36; 95% CI, 1.02 to 1.82 for opportunistic screening vs. OR, 1.26; 95% CI, 1.08 to 1.64 for organized screening). Several previous studies did not reveal significant associations between alcohol consumption and cancer screening behavior [22,23], whereas other studies suggested that drinking was associated with an increased rate of cancer screening [10,23]. However, working with data from the third KNHANES, Kwon et al. [24] found that frequent binge drinkers were less likely to be screened for gastric cancer than non-binge drinkers.

People who engaged in moderate physical activity 1-4 days per week were more likely to be screened by both types of screening programs than were people who did not, and the OR was much higher for opportunistic than for organized screening (OR, 1.41; 95% CI, 1.11 to 1.80 for opportunistic screening vs. OR, 1.20; 95% CI, 1.05 to 1.38 for organized screening). Previous studies have noted that individuals who engaged in physical activity or exercise were more likely to be screened for cancer [14]. Interestingly, we found no relationship between moderate physical activity more than 4 days per week and participating in screening.

Individuals who were screened for one of three cancers (stomach, colon, and breast cancer) were defined as having undergone cancer screening. In Korea, it is recommended that individuals aged 40 years or older undergo screening for stomach and breast cancer once every two years, and that all individuals undergo screening for colon cancer using a fecal occult blood test once per year [3]. In Korea, most people who visit medical institutions to be screened for cancer are screened for one or more types of cancer [3]. In this study, two-thirds (68.9%) of those who participated in a cancer screening program were screened for more than two or more types of cancer.

It is important to understand why people visit medical institutions for opportunistic rather than organized screening for cancer. In this regard, convenience should be considered. For example, a colonoscopy is not accepted as a primary screening method for colorectal cancer in Korea and in other Westernized countries [3,25]. However, a colonoscopy is the preferred test for this purpose because it allows visualization of the entire large bowel and immediate removal of clinically significant precancerous lesions [25]. Therefore, people seek-

ing to be screened for this condition might choose to undergo opportunistic screening because they can receive a colonoscopy. Second, people tend to believe that those who perform opportunistic screenings are more qualified than those who perform organized screening.

It is important to note that this study has several limitations. First, we did not consider the effect of family history of cancer on cancer screening behavior because the KNHANES did not include relevant questions. However, the health belief model indicates that the perception that one is at risk for developing a certain disease may influence preventive health behaviors [26,27], and previous studies have found a positive association between family history and cancer screening [21,28,29]. Conversely, screened people were less likely to perceive that they were at risk of developing cancer [30]; therefore, additional research is required to determine the effects of family history on cancer screening.

Second, KNHANES data describing cancer screening, socioeconomic status, health status, and health behaviors are self-reported based on a cross-sectional design. Therefore, recall bias and interviewer bias may have affected our results despite the interviewers’ efforts to control these phenomena [21]. Third, we could not identify factors associated with each type of cancer screening program because this study assumed that individuals visiting medical institutions for screening might participate in one or more types of cancer screening. However, it is necessary to consider that factors related to cancer screening behavior would differ based on cancer type. Most previous studies focused on one type of cancer screening program. However, the present study defined screened individual as those who had received screening for three types of cancer (stomach, colon, and breast cancer).

Finally, we categorized people screened for cancer into two groups; those who participated in organized and opportunistic screening. If individuals used both programs, we placed them into the organized screening group because this study focused why some people participated in opportunistic screening although the entire population can use organized screening. Overall, 681 people used both programs; therefore, it is necessary to identify the characteristics of people who used both screening programs in a future study.

Despite these limitations, this study relied on data from a nationwide sample with a high response rate. Therefore, our results may be representative of the general population of Korea.
Conclusion

In conclusion, we identified factors that are more strongly associated with participation in opportunistic than organized cancer screening. Those who were married, highly educated, high earners, in better quality of life, light drinkers, and who engaged in moderate physical activity 1-4 days per week were more likely than others to undergo opportunistic screening.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

This work was supported by the Soonchunhyang University Research Fund (No. 20130589).

References

Incorporating Risk Factors to Identify the Indication of Post-mastectomy Radiotherapy in N1 Breast Cancer Treated with Optimal Systemic Therapy: A Multicenter Analysis in Korea (KROG 14-23)

Hae Jin Park, MD1
Kyung Hwan Shin, MD, PhD2
Jin Ho Kim, MD, PhD3
Seung Do Ahn, MD, PhD4
Ja Young Kim, MD5
Won Park, MD, PhD6
Yong Bae Kim, MD, PhD7
Yeon-Joo Kim, MD8
Jin Hee Kim, MD, PhD9
Kyub Kim, MD, PhD10
Kyung Ran Park, MD, PhD11
Hyun Soo Shin, MD, PhD12
Bae Kwon Jeong, MD, PhD13
Sun Young Lee, MD, PhD14
Suzy Kim, MD, PhD15

*Purpose*
In a recent meta-analysis, post-mastectomy radiotherapy (PMRT) reduced any first recurrence (AFR) and improved survival in N1 and N2 patients. We investigated risk factors for AFR in N1 after optimal systemic therapy without PMRT, to define a subgroup of patients who may benefit from PMRT.

*Materials and Methods*
One thousand three hundred eighty-two pT1-2N1M0 breast cancer patients treated with mastectomy without PMRT between 2005 and 2010 were retrospectively analyzed. Only 0.6% had no systemic therapy.

*Results*
After a median follow-up of 5.9 years, there were 173 AFR (53 loco-regional recurrence [LRR] without distant metastases [DM], 38 LRR with DM, and 82 DM without LRR). The 5-year LRR and AFR rates were 6.1% and 12.0%, respectively. Multivariate analysis revealed that close resection margin (p=0.001) was the only independent risk factor for LRR. Multivariate analysis for AFR revealed that age < 35 years (p=0.025), T2 stage (p=0.004), high tumor grade (p=0.032), close resection margin (p=0.035), and triple-negative biological subtype (p=0.031) were independent risk factors. Two or three positive lymph nodes (p=0.078) were considered a marginally significant factor. When stratified by these six factors, the 5-year LRR rates were 3.6% with 0-1 (n=606), 7.5% with 2-3 (n=655), and 12.7% with 4-6 (n=93) risk factors. The 5-year AFR rates were 7.1% with 0-1, 15.0% with 2-3, and 24.5% with 4-6 risk factors.

*Conclusion*
Patients with pT1-2N1M0 breast cancer who underwent mastectomy and optimal systemic therapy showed excellent loco-regional control and disease control. The patients with four or more risk factors may benefit from PMRT, and those with two or three risk factors merit consideration of PMRT.

Key words: Breast neoplasms, Post-mastectomy radiotherapy, Risk factors

**Introduction**
Radiotherapy (RT) has played an important role in the management of breast cancer by eradicating microscopic tumor cells not only for all of the patients treated with breast-conserving surgery but also for selected patients treated with mastectomy [1-3]. Current guidelines generally recommend postmastectomy RT (PMRT) for locally advanced cancer (T3-T4) or four or more positive axillary lymph nodes (LN) (N2 or higher) [1,2,4]. However, postoperative PMRT for early breast cancer (T1-T2) and limited nodal metastasis (N1) are controversial.

Studies have reported the effects of PMRT on tumor recurrence and mortality in patients with 1-3 axillary LNs, including the Danish Breast Cancer Cooperative Group trial and a...
recent meta-analysis by the Early Breast Cancer Trialists’ Collaborative Group (EBCTCG) [5,6]. The Danish trial showed the substantial benefit of PMRT on loco-regional recurrence (LRR) and overall survival in patients with N1 and N2 disease. EBCTCG analysis also showed a similar result such that PMRT to the chest wall and regional lymphatics reduced both recurrence and breast cancer mortality in patients with N1 and N2 disease, even when axillary dissection at least at level II was performed, and systemic therapy was given. Nonetheless, the use of PMRT has been controversial for all T1-T2/N1 patients. One reason is that the absolute risk of any type of recurrence has decreased with modern systemic therapy in recent decades. Another reason is related to breast cancer being not a single entity but biologically distinct diseases [7-9]. The biological subtype can also predict the risk of recurrence and response of treatment as well, although the current guidelines suggest the indication of PMRT based on only tumor and nodal stage. Large randomized trials or meta-analyses including the Danish trial and EBCTCG analysis did not address this issue.

The EBCTCG meta-analysis in 2005 showed that the avoidance of LRR led to a reduction of breast cancer mortality in node-positive patients treated with mastectomy and PMRT [10]. On the other hand, the updated analysis in 2014 showed that the avoidance of any first recurrence (AFR) led to a decrease in breast cancer mortality [5]. In this study, we investigated the risk factors for LRR as well as AFR in N1 patients after optimal systemic therapy but not PMRT, thus defining a subgroup of patients who may or may not benefit from PMRT.

Materials and Methods

This study was approved by the Korean Radiation Oncology Group (KROG 14-23) and Institutional Review Board of each participating institution. After the approval, we retrospectively reviewed the medical records of breast cancer patients treated with mastectomy without PMRT between 2005 and 2010 at 11 institutions in Korea. Patients with a tumor size ≤ 5 cm (pT1 and pT2) and 1-3 axillary LN metastases (pN1) were exclusively included in this study. We excluded patients who received neoadjuvant systemic treatment, had distant metastasis at diagnosis, had a history of malignancies other than thyroid cancer, or were diagnosed with bilateral breast cancer. A total of 1,382 breast cancer patients met the eligibility criteria.

Clinico-pathological information of eligible patients was collected; it included the age at diagnosis, menopausal status, tumor histology, tumor size, tumor grade, number of involved and examined LNs, and estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 status. The positivity of ER, PR, HER2, and Ki-67 was determined by immunohistochemical staining. HER2-positivity was defined as a 3+ immunohistochemical result or a 2+ immunohistochemical result confirmed by fluorescence in situ hybridization. The breast cancer subtypes were approximated based on hormone receptor status, HER2 status, and histologic grade. Because Ki-67 status data were incomplete (available only in 757 patients [54.8%]), we used the histologic grade as a surrogate for Ki-67 based on St. Gallen Expert Consensus [11]. The five surrogate biological subtypes were defined accordingly: luminal A (ER+ or PR+/HER2−/low-intermediate grade), luminal B (ER+ or PR+/HER2−/high grade), HER2+ (ER−/PR−/HER2+), luminal HER2 (ER+ or PR+/HER2+), and triple negative (TN) (ER−/PR−/HER2−).

The primary outcome of interest was AFR, irrespective of LRR or distant metastasis (DM). We defined local recurrence (LR) as tumor recurrence in the ipsilateral chest wall, and regional recurrence (RR) as recurrence in ipsilateral draining LNs (axillary, supraclavicular, or internal mammary LNs). LRR was defined as LR or RR or both. Following the EBCTCG meta-analysis, we used LRR as a first event for statistical analysis. Hereafter, LRR refers to LRR with or without synchronous DM. DM was defined as tumor recurrence outside regions identified as LRR sites. The information on date of death was taken from Korea’s national database, in which death by breast cancer was not distinguished from death by other causes. Time to any recurrence or death was measured from the date of mastectomy.

Cumulative incidence function curves for AFR, LRR, and overall mortality were constructed using the Kaplan-Meier method, and comparisons between groups were performed using log-rank tests. Statistical analysis was performed using SPSS ver. 18.0 (SPSS Inc., Chicago, IL). p-values lower than 0.05 were deemed to indicate statistical significance.

Results

1. Patient and tumor characteristics

Table 1 summarizes the demographic and clinico-pathologic parameters, as well as treatment details, of the study cohort. The median age at diagnosis was 48 years (range, 24 to 85 years). All 1,382 patients underwent mastectomy with clear resection margins, and 189 of these patients (13.7%) had resection margins less than 2 mm. The most common histology was invasive ductal carcinoma (97.5%). There were 820
Table 1. Patient, tumor, and treatment characteristics

<table>
<thead>
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<th>Variable</th>
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<td>562 (40.7)</td>
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<td>T2</td>
<td>820 (59.3)</td>
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<td>547 (39.6)</td>
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<td>28 (2.0)</td>
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<td>Close (&lt; 2 mm)</td>
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<td>&lt; 10</td>
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<tr>
<td>Luminal A</td>
<td>554 (40.1)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>187 (13.5)</td>
</tr>
<tr>
<td>Luminal HER2</td>
<td>209 (15.1)</td>
</tr>
<tr>
<td>HER2+</td>
<td>198 (14.3)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>157 (11.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>77 (5.6)</td>
</tr>
</tbody>
</table>

HER2, human epidermal growth factor receptor 2.

Table 2. Patterns of failure

<table>
<thead>
<tr>
<th>Site of recurrence</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td>39 (2.8)</td>
</tr>
<tr>
<td>Regional</td>
<td>70 (5.1)</td>
</tr>
<tr>
<td>Axilla</td>
<td>54 (3.9)</td>
</tr>
<tr>
<td>Internal mammary</td>
<td>36 (2.6)</td>
</tr>
<tr>
<td>Supraclavicular</td>
<td>47 (3.4)</td>
</tr>
<tr>
<td>Loco-regional</td>
<td>91 (6.6)</td>
</tr>
<tr>
<td>Distant</td>
<td>138 (10.0)</td>
</tr>
</tbody>
</table>

Fig. 1. Cumulative incidence of loco-regional recurrence, any first recurrence, and overall mortality.

T2 tumors (59.3%), and 547 high-grade tumors (39.6%). Complete axillary LN dissection was performed in 1,276 patients (92.3%), and sentinel LN biopsy alone was performed in 103 patients (7.5%). The median number of examined LNs was 15 (range, 1 to 64), and more than 10 LNs were examined in 1,107 patients (80.7%). In all, 813 patients (58.8%) had one involved LN, 371 (26.8%) had two involved LNs, and 198 (14.3%) had three involved LNs. There were 1,022 patients (74.0%) with positive hormone receptor status, and 14 of them did not receive hormone treatment. On the other hand, 38 of 360 patients with negative hormone receptor status did receive hormone treatment. There were 407 patients (29.5%) with positive HER2 expression; 62.1% of them (n=253) were treated with trastuzumab. In addition, 1,333 (96.5%) and 1,046 (75.7%) patients received adjuvant chemotherapy (83% received a taxane-containing regimen) and hormonal therapy, respectively. Eight patients (0.6%) did not receive sys-
Table 3. Univariate and multivariate analysis for loco-regional recurrence and any first recurrence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n=1,382)</th>
<th>Loco-regional recurrence</th>
<th>Any first recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Event (n=91)</td>
<td>Univariate analysis, p-value</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 55</td>
<td>101</td>
<td>13</td>
<td>0.009</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>1,281</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
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<tr>
<td>T1</td>
<td>562</td>
<td>29</td>
<td>0.073</td>
</tr>
<tr>
<td>T2</td>
<td>820</td>
<td>62</td>
<td></td>
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<td>Resection margin</td>
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<tr>
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<td>Close</td>
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</tr>
<tr>
<td>No. of positive nodes</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>813</td>
<td>49</td>
<td>0.320</td>
</tr>
<tr>
<td>2-3</td>
<td>569</td>
<td>42</td>
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</tr>
<tr>
<td>Tumor histologic grade</td>
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<tr>
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<td>Unknown&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28</td>
<td>1</td>
<td></td>
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<tr>
<td>HER2 status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>917</td>
<td>63</td>
<td>0.714</td>
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<tr>
<td>Positive and trastuzumab−</td>
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<tr>
<td>Positive and trastuzumab+</td>
<td>253</td>
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<td></td>
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<td>Unknown&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
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<td></td>
</tr>
<tr>
<td>Biological subtype</td>
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</tr>
<tr>
<td>Luminal A</td>
<td>554</td>
<td>26</td>
<td>0.016</td>
</tr>
<tr>
<td>Luminal B</td>
<td>187</td>
<td>19</td>
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<td>Luminal HER2</td>
<td>209</td>
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<tr>
<td>Unknown&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77</td>
<td>4</td>
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<tr>
<td>Biological subtype</td>
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</tr>
<tr>
<td>Triple negative</td>
<td>157</td>
<td>17</td>
<td>0.026</td>
</tr>
<tr>
<td>Others</td>
<td>1,225</td>
<td>74</td>
<td></td>
</tr>
</tbody>
</table>

HER2, human epidermal growth factor receptor 2. <sup>a</sup>Patients with unknown tumor grade, HER2 status, or biological subtype were not included in the statistical analysis.
temic treatment. Adjuvant systemic treatment was delivered at the physician’s discretion.

2. Tumor recurrence and death

The median follow-up period was 5.9 years (range, 0.6 to 10.4 years). In total, 94 patients (6.8%) died, and 79 of them had experienced tumor recurrence before death. Table 2 shows the sites of total recurrence. There were more distant recurrences (n=138) than LRRs (n=91). Of 91 LRRs, 17 were synchronous LR and RR. Fifty-seven patients experienced LRR as well as DM. Finally, 81 had isolated DM.

There were 173 AFRs, among which 53 (30.6%) were LRRs without DM, 38 (22.0%) were LRRs with DM, and 82 (47.4%) were DMs without LRR. The cumulative incidence curves of LRR, AFR, and overall mortality are illustrated in Fig. 1. The cumulative incidence of AFR and LRR increased steeply within 5 years from the date of mastectomy, and the mortality curve was not particularly steep during the specific time period. The cumulative LRR rates at 5, 7, and 10 years were 61.1%, 77.7%, and 105.5%, respectively. Those for AFR at 5, 7, and 10 years were 120.0%, 138.8%, and 179.9%, respectively; and those for overall mortality at 5, 7, and 10 years were 45.4%, 6.9%, and 11.6%, respectively.

3. Effect of biological subtype on LRR and AFR

When classified into five biological subtypes, luminal A was the most common subtype (40.1%), and the remaining four subtypes had similar proportions (Table 1). The 5-year AFR rates of luminal A, luminal B, HER2, luminal HER2, and TN were 8.2%, 11.7%, 10.5%, 11.2%, and 18.8%, respectively. And the 10-year AFR rates were 16.7%, 24.7%, 13.6%, 12.6%, and 25.5%, respectively (Fig. 1A). The 5-year locoregional recurrence-free survival rates of luminal A, luminal B, HER2, luminal HER2, and TN were 4.3%, 9.5%, 5.9%, 5.3%, and 10.4%, respectively. And the 10-year AFR rates were 9.4%, 12.7%, 9.2%, 6.8%, and 14.6%, respectively (Fig. 1B). In univariate analyses, the TN and luminal B subtypes predicted more LRR and AFR than the luminal A subtype (all, p < 0.001) (Table 3).

Because the use of trastuzumab for HER2 patients was not covered with Korean national health insurance in the early period of enrollment, 253 of 407 HER2 patients (62.2%) were treated with trastuzumab. For this reason, we subdivided HER2-positive patients (among the HER2 and luminal HER2 subtypes) into those treated with and without trastuzumab. Thus, we reclassified those patients into five biological subtypes: luminal A, luminal B, HER2- trastuzumab+, HER2- trastuzumab-, and TN. The results were similar to those of the former analysis in which the TN and luminal B subtypes had significantly more LRR and AFR than the luminal A subtype (all, p < 0.001). There was slightly more LRR and AFR in the HER2 group without trastuzumab treatment than in the HER2 group with the treatment, but this difference was not statistically significant (p=0.674 and p=0.415, respectively).

4. Risk factors

The results of univariate and multivariate analyses of LRR and AFR are presented in Table 3. To facilitate the comparison between groups in multivariate analyses using the Cox proportional hazards model, the biological subtypes were redefined as binary variables: TN tumors and others (p=0.001) (Table 3). In multivariate analyses, age ≤ 35 years, a close resection margin, and a high tumor grade were significantly associated with a high LRR (p=0.009, p < 0.001, and p=0.005, respectively). T2 stage was marginally significant with a high LRR (p=0.073). In multivariate analyses of LRR, a close resection margin was the only independent risk factor (hazard ratio [HR], 1.504; p=0.001), and age (HR, 1.721; p=0.076), high tumor grade (HR, 1.500; p=0.068), and TN biological subtype (HR, 1.596; p=0.095) were marginally significant factors for LRR.

In univariate analyses of AFR revealed that age ≤ 35 years, T2 stage, a close resection margin, two or three positive LNs, positive HR, and high tumor grade were independent risk factors for AFRs (p=0.001, p < 0.001, p=0.023, p=0.010, p=0.483, and p < 0.001, respectively). In multivariate analyses of AFRs, age ≤ 35 years (HR, 1.671; p=0.025), T2 stage (HR, 1.183; p=0.04), a close resection margin (HR, 1.228; p=0.035), a high tumor grade (HR, 1.409; p=0.032), and the TN biological subtype (HR, 1.550; p=0.031) were independent risk factors. Two or three positive LNs (HR, 1.312; p=0.078) were a marginally significant factor for AFR.

5. Risk stratification to identify PMRT indication

Because the purpose of our study was to identify patients who may benefit from PMRT mainly based on overall recurrence, we utilized risk factors proven to be independent in multivariate analyses for AFRs to define risk groups. The six risk factors were patients’ age (< 35 years vs. > 35 years), tumor size (T1 vs. T2), the resection margin status (negative vs. close), the number of metastatic LNs (1 vs. 2-3), tumor grade (low-intermediate vs. high), and biological subtype (TN vs. others). Two patients had no risk factors, and 411, 414, 241, 76, and 17 patients had one, two, three, four, and five risk factors, respectively. No patients had all six risk factors.

The risk of both LRR and AFR increased with increasing number of risk factors. The results stratified by risk group are illustrated in Fig. 2. The 5-year cumulative LRR rates

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were 3.6% with 0-1 risk factor (n=606), 7.5% with 2-3 risk factors (n=655), and 12.7% with 4-6 risk factors (n=93). The 10-year cumulative LRR rates were 9.1% with 0-1 risk factor, 11.6% with 2-3 risk factors, and 17.5% with 4-6 risk factors (Fig. 2A). The 5-year cumulative AFR rates were 7.1% with 0-1 risk factor, 15.0% with 2-3 risk factors, and 24.5% with 4-6 risk factors. The 10-year cumulative AFR rates were 12.8% with 0-1 risk factor, 22.3% with 2-3 risk factors, and 30.5% with 4-6 risk factors (Fig. 2B).

Discussion

PMRT following mastectomy in pT1-2N1 breast cancer patients has not been generally recommended because of the low risk of recurrence. Although the updated EBCTCG meta-analysis proved the benefit of PMRT in N1 and N2 patients who had axillary dissection and systemic therapy [5], the recent consensus from the St. Gallen breast cancer meeting in 2015 did not adopt the routine use of PMRT for all N1 patients, just those with adverse pathology [1]. The present study was designed to identify a subset of N1 patients who may benefit from PMRT.

The entire cohort did not receive PMRT after mastectomy. Our analysis showed that the 10-year AFR and LRR rates were 17.9% and 10.5%, respectively. These rates are comparable to those of the recent contemporary study by Lai et al. [12], which include almost the same study population with ours between 2004 and 2008 and utilized modern systemic treated for most of the patient. They reported the 10-year progression-free survival and LRR rates were 75.1% (24.9%, free from recurrence) and 10%, respectively. However, these results are far superior to the outcomes of the no-RT subset in the EBCTCG meta-analysis (the 10-year AFR and LRR rates were 45.7% and 20.3%, respectively). One possible explanation may be the difference in the number of positive LNs. The rates of only one positive LN were 58.8% in our cohort and 31.4% in the EBCTCG no-RT subset (34.9% were unknown). Our analysis did show a statistically significant difference in AFR between one and two or three positive LNs (p=0.010) but not in LRR (p=0.320) (Table 3). Similarly, in the EBCTCG no-RT and systemic therapy subset, the 10-year AFR rate was lower in the one positive LN group than in the two or three positive LN group (36.3% vs. 47.8%). The LRR rate was similar (20.2% vs. 19.3%). Another explanation could be the difference in the systemic treatment used in the two studies. In our study, 99.4% (1,374 of 1,382 patients) were treated with any type of systemic treatment (24% chemotherapy alone, 3% endocrine therapy alone, 73% both, and 0% none) (Table 1). On the other hand, 86% were treated with systemic treatment in the EBCTCG N1 subset (62% chemotherapy alone, 21% endocrine therapy alone, 3% both, and 14% none), and 87% were treated with systemic treatment in the EBCTCG N1 and no-RT subset (detailed information not shown). Importantly, the most common chemotherapy was cyclophosphamide, methotrexate, and fluorouracil, and the most common endocrine therapy was tamoxifen in the EBCTCG meta-analysis. On the other hand,
80% (1,104 of 1,382 patients) received a taxane-containing chemotherapy, and one-third of the patients received an aromatase inhibitor as endocrine therapy; more than half of the HER2-positive patients received trastuzumab in our cohort. Therefore, it is reasonable that these superior results of our analysis are attributable to modern systemic treatment [13,14]; however, further discussion is beyond the scope of this study. The final possible explanation is that our data were immature to exhibit entire recurrence considering the long natural history of breast cancer. The studies included in the EBCTCG meta-analysis were carried out between 1964 and 1986, and the patients were followed up for more than 10 years (median follow-up period, 9.4 years). The patients in our study were treated between 2005 and 2010, and the median follow-up period was 5.9 years.

Although the difference in the LRR and AFR rates between our study and the EBCTCG meta-analysis seems large, the relationship between them is similar. The closest subset to our cohort in the EBCTCG meta-analysis was the N1 patients treated with systemic treatment and without PMRT. In this subset, the 5-year LRR rate was 17.4% and the 10-year AFR rate was 45.5%, which was 2.6 times higher. In our study, the 5-year LRR rate was 6.1% and the 10-year AFR rate was 17.9%, which was 2.9 times higher. Considering that PMRT reduced any recurrence by one-third in the EBCTCG meta-analysis, if PMRT were administered, the 10-year overall recurrence would be decreased to 12%. The EBCTCG data also showed that the 10-year AFR rate and 20-year breast cancer mortality rate were similar, and PMRT reduced the 20-year breast cancer mortality by more than one-fifth. We predict that the 20-year breast cancer mortality would be 18%; if PMRT were administered, this rate would decrease to 14% in our cohort.

Classification of breast cancer according to biological subtype has proven to be a strong predictor of LRR, DM, and survival [9,15-19]. As mentioned above, the patients in the EBCTCG meta-analysis were diagnosed and treated more than 30 years ago. Thus, it did not address the effects of biological subtypes on the risk of recurrence, which is being utilized for treatment decision making and predicting the prognosis. This is one reason why PMRT could not be recommended for all N1 patients, despite evidence that PMRT does reduce LRR and increase survival. Our study’s finding that patients with the luminal A subtype had lower rates of LRR and those with the TN subtype had higher rates of LRR relative to other subtypes is similar to other studies in the setting of mastectomy [12,16,18,20]. In the systemic review by Lowery et al. [16], the luminal, HER2-overexpressing, and TN tumor subtypes were defined according to the expression of ER, PR, and HER receptor as determined by immunohistochemical staining. Luminal tumors were less likely to develop LRR than HER2-overexpressing or TN tumors, but there were no differences in LRR between HER2-overexpressing and TN tumors following mastectomy. Tseng et al. [18] defined five biological subtypes, which were the same as ours, and evaluated LRR after mastectomy and the impact of PMRT by breast cancer subtype. Compared to luminal A patients, TN patients had the highest risk of LRR and the least benefit from PMRT. Patients with HER2 tumors treated with trastuzumab had a low risk of LRR. However, in our study, the use of trastuzumab in HER2 tumors did not affect LRR or AFR.

We defined AFR as the primary endpoint, similar to the EBCTCG meta-analysis. Therefore, to suggest a PMRT indication in N1, we utilized the risk factors by multivariate analyses of AFR to identify a high-risk subgroup. The 10-year AFR rates were as follows: 30.5% with four or more risk factors present simultaneously (young age, T2 tumors, a close resection margin, high-grade tumors, and the TN subtype); 22.3% with 2-3 risk factors, and 12.8% with 0-1 risk factor. If the proportional risk reductions were applied to each risk group after adding PMRT, the 10-year AFR rates would decline to the following: 22.8% with 4 or more risk factors, 16.7% with 2-3 risk factors, and 9.6% with 0-1 risk factor, respectively. However, in the latter group, the absolute gain of the overall recurrence would be only 3.2%.

This retrospective study had several limitations. First, pathologic findings such as HR/HER2 positivity or tumor grade were not centrally reviewed or reassessed according to common criteria. Criteria discrepancies among participating institutions might hamper an accurate analysis. Second, the follow-up period was relatively short, and our data on patient deaths were all-cause mortality. Therefore, our study could not address survival benefits related to overall recurrence reduction obtained by PMRT in T1-2N1 patients. Despite these limitations, our study had important strengths. This was a large cohort study in which 1,382 T1-2N1 breast cancer patients treated with mastectomy without PMRT were included. About half (n=682) patients were included in N1 and no-RT subset of EBCTCG meta-analysis compared to our study cohort. More importantly, because our study adopted current diagnostic and therapeutic strategies, the conclusions drawn by our analyses are more relevant to real practice than those of the EBCTCG meta-analysis.

The United Kingdom Medical Research Council SUPREMO (Selective Use of Postoperative Radiotherapy After Mastectomy) trial, which randomly allocated approximately 1,600 patients with high-risk N0 as well as N1, completed patients accrual [21]. We expect that the result of this trial help clarify the indication of PMRT.
Conclusion

In summary, patients with pT1-2N1 breast cancer who underwent mastectomy and optimal systemic therapy showed favorable LRR and overall recurrence without PMRT. However, the concomitant presence of multiple risk factors contributes to higher tumor recurrence. The patients with four or more risk factors may benefit from PMRT, and those with two or three risk factors merit consideration of PMRT. In addition, PMRT may be omitted for patients without risk factors and with only one risk factor because the absolute benefits are small.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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Effects of Postoperative Radiotherapy on Leptomeningeal Carcinomatosis or Dural Metastasis after Resection of Brain Metastases in Breast Cancer Patients

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Seok-Gu Kang, MD\textsuperscript{1}  
Chang-Ok Suh, MD\textsuperscript{2}

Purpose
In this retrospective study, we compared the incidence of leptomeningeal carcinomatosis or dural metastasis (LMCDM) in patients who received whole brain radiotherapy (WBRT), partial radiotherapy (PRT), or no radiotherapy (RT) following resection of brain metastases from breast cancer.

Materials and Methods
Fifty-one patients with breast cancer underwent surgical resection for newly diagnosed brain metastases in two institutions between March 2001 and March 2015. Among these, 34 received postoperative WBRT (n=24) or PRT (n=10) and 17 did not.

Results
With a median follow-up of 12.4 months (range, 2.3 to 83.6 months), 22/51 patients developed LMCDM at a median of 8.6 months (range, 4.8 to 51.2 months) after surgery. The 18-months LMCDM-free survival (LMCDM-FS) rates were 77.5\%, 30.0\%, and 13.6\%, in the WBRT, PRT, and no RT groups, respectively (p=0.013). The presence of a tumor adjacent to cerebrospinal fluid flow and no systemic treatment after treatment for brain metastases were also associated with poor LMCDM-FS rate. Multivariate analysis showed that WBRT compared to PRT (p=0.009) and systemic treatment (p < 0.001) were independently associated with reduced incidence of LMCDM.

Conclusion
WBRT improved LMCDM-FS rate after resection of brain metastases compared to PRT in breast cancer patients.

Key words
Breast neoplasms, Meningeal carcinomatosis, Whole brain radiotherapy, Partial radiotherapy

* A list author's affiliations appears at the end of the paper.
Introduction

There has been steady improvement in the survival of breast cancer patients in recent decades; however, late development of brain metastases is an increasing problem in long-term survivors. In fact, breast cancer is the second most common origin of brain metastasis in cancer patients [1]. Intensive treatments, including surgery, radiosurgery, and conventional radiotherapy (RT), can substantially increase survival in some subgroups of patients with a limited number of brain metastases, controlled extracranial disease, and good performance status [2,3].

Whole-brain RT (WBRT) following surgical resection of brain metastases has a well-established role in preventing intracranial failure [4,5]. Because of concern about possible long-term neurocognitive sequelae, partial radiotherapy (PRT) to the tumor bed has been proposed as a substitute for WBRT [6,7]. However, PRT after surgery is associated with increased risk of leptomeningeal carcinomatosis (LMC) or distant brain failure relative to WBRT [8,9].

The risk of LMC should be carefully considered when applying PRT after surgery because of the theoretical possibility of cerebrospinal fluid (CSF) contamination with tumor cells during surgical resection of brain metastases. A few retrospective studies have compared postoperative WBRT and PRT [8,9]. However, these studies included patients with any type of primary cancer, and none of these studies included breast cancer patients alone. Therefore, the present study was conducted to determine the incidence of LMC according to the extent of brain RT after surgical resection of brain metastases in breast cancer patients.

Materials and Methods

1. Patients

We retrospectively reviewed the medical records of breast cancer patients with brain metastases who underwent surgical resection between March 2001 and March 2015 at the National Cancer Center of Korea (Goyang) or between November 2003 and March 2015 at the Severance Hospital of Korea (Seoul). After excluding patients who received RT before surgical resection of brain metastases, 51 patients were included in this study. Overall, 34 patients received postoperative RT, being WBRT in 24 patients and PRT to the tumor bed in 10 patients.

2. Radiotherapy

The postoperative treatment strategy; namely, whether or not to give RT and to what extent, was selected according to the physician’s preference. A WBRT of 25 or 30 Gy in 10 fractions (fx) or 30 Gy in 12 fx (median 30 Gy/10 fx) was administered using a linear accelerator with conventional two opposing lateral fields. There was a difference in treatment strategy in terms of the boost to the tumor bed between the centers. A boost (dose range, 7.5 to 25 Gy; median, 15 Gy in 3-10 fx) after WBRT was routinely prescribed in one center, but not the other. PRT was applied to the resection cavity with a suitable margin using stereotactic or three-dimensional conformal techniques at various dose fractions: 36 Gy/6 fx (n=4), 45 Gy/15 fx (n=2), 45 Gy/10 fx (n=1), and 36 Gy/12 fx (n=1). In two patients who were treated with gamma knife radiosurgery, 16 Gy and 7.5 Gy were prescribed to the 50% isodose line in a single fraction, respectively.

3. Clinical factors

In addition to the extent of brain RT, the following clinical variables were reviewed as potential prognostic factors for the recurrence of leptomeningeal disease: age at diagnosis of brain metastasis, systemic disease status (none/stable vs. progressive), biological subtype of primary tumor, Eastern Cooperative Oncology Group (ECOG) performance status, breast specific graded prognostic assessment score (Breast-GPA) [10], size and number of metastases, extent of resection (gross total resection vs. subtotal resection), hormone therapy, and systemic treatment. Systemic treatment included chemotherapy, targeted therapy, or both and was given after brain surgery and before the development of LMC or dural metastasis (DM) (LMCDM). Systemic treatment at any time after brain surgery was separately analyzed as a prognostic factor for overall survival (OS). Biological subtypes of primary tumors were classified as follows: estrogen receptor-positive and/or progesterone receptor-positive/human epidermal growth factor receptor (HER2)–negative, HER2-positive, and triple-negative according to the previous studies [11]. Continuous variables were dichotomized by their median values. We also analyzed the location of the tumor relative to CSF flow. The tumor was classified as adjacent to CSF flow if there was contact of the tumor’s surface with the pia mater or ventricle wall [12].

4. Outcome evaluation

The patients were followed-up with magnetic resonance imaging (MRI) at intervals of 3 months after surgery. The primary outcome was the development of LMCDM after treat-
ment for brain metastases. LMC was diagnosed based on MRI and/or CSF cytology confirmation. The conservative definition of LMC (i.e., presence of malignant cells in CSF or typical leptomeningeal enhancement on MRI in the brain, spinal cord, or cauda equina) was used. DM was defined as a presence of multiple enhancing nodules on the dura mater on MRI (Fig. 1). Both incidence of LMC and DM were analyzed together as a LMCDM.

Local recurrence (LR) was defined as the appearance of new enhancing lesions within the resection cavity. Distant brain recurrence (DBR) was defined as the presence of new metastatic nodules in distinct brain parenchyma outside the resection cavity.

Considering the emerging evidence of the importance of biological subtype and targeted therapy in breast cancer prognosis and treatment [11], subgroup analysis according to the HER2 status was conducted to evaluate the impact of targeted therapy on the development of LMCDM and OS.

5. Statistical analysis

Patient characteristics were compared among the three treatment groups using Fisher exact test for categorical variables and the Mann-Whitney U test or one-way analysis of variance for continuous variables, as appropriate. The Kaplan-Meier method was used to assess event-time distributions. The time to recurrence was calculated from the date of surgical resection to the date of MRI showing LR, DBR, or LMCDM. Otherwise, patients were censored at the time of their last MRI or the clinical visit when their neurologic signs were last evaluated. OS was calculated from the date of surgery to the date of death, or living patients were censored at the date of their last clinical visit. Log-rank tests were used to compare the event-time distributions among treatment groups. The Cox proportional hazard model was used in multivariate analysis to identify factors associated with LMCDM by calculating hazard ratio (HR) with 95% confi-
Table 1. Patient, tumor, and treatment characteristics

<table>
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<tr>
<th>Characteristic</th>
<th>All (n=51)</th>
<th>WBRT (n=24)</th>
<th>PRT (n=10)</th>
<th>No RT (n=17)</th>
<th>p-value</th>
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<td>46 (34-68)</td>
<td>45 (34-68)</td>
<td>60 (34-75)</td>
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<td>29.9 (7.8-148.0)</td>
<td>32.4 (9.8-67.5)</td>
<td>41 (5.0-115.9)</td>
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<td>15 (63)</td>
<td>10 (100)</td>
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<td>3 (13)</td>
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<td>7 (70)</td>
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<td>9 (90)</td>
<td>16 (94)</td>
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<td>1 (10)</td>
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<td>9 (90)</td>
<td>12 (71)</td>
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<td>Extent of resection</td>
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<td>HRT after brain surgery</td>
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<tr>
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<td>2 (20)</td>
<td>1 (6)</td>
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</tr>
<tr>
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<td>8 (80)</td>
<td>16 (94)</td>
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<td>4 (40)</td>
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<td>Targeted Tx±CTx</td>
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<td>CTx</td>
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<td>11 (46)</td>
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<td>4 (24)</td>
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</tr>
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<td>Systemic treatment after brain surgery (at any time)</td>
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<tr>
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<td>16 (31)</td>
<td>7 (29)</td>
<td>1 (10)</td>
<td>8 (47)</td>
<td>0.291⁰</td>
</tr>
<tr>
<td>CTx with targeted Tx</td>
<td>15 (29)</td>
<td>6 (25)</td>
<td>5 (50)</td>
<td>4 (24)</td>
<td></td>
</tr>
<tr>
<td>CTx without targeted Tx</td>
<td>20 (39)</td>
<td>11 (46)</td>
<td>4 (40)</td>
<td>5 (29)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). WBRT, whole brain radiotherapy; PRT, partial radiotherapy; RT, radiotherapy; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; ECOG, Eastern Cooperative Oncology Group; Breast-GPA, breast specific graded prognostic assessment score; CSF, cerebrospinal fluid; GTR, gross total resection; STR, subtotal resection; HRT, hormone therapy; Tx, therapy; CTx, chemotherapy. ¹One-way ANOVA, ²Fisher exact test, ³n=50 (WBRT, 23; PRT, 10; no RT, 17).
dence intervals (CI). A stepwise backward linear regression was employed, and variables with p-values greater than 0.10 were removed from the model. The study was sufficiently powered (88%) with a one-sided type I error probability of 5% to detect a HR of 3.3 for 27 patients who did not received postoperative WBRT relative to 24 patients who did. The HR of 3.3 was based on previous studies in which the HRs for LMC were 2.44 and 5.67 in patients who underwent postoperative PRT compared with those who underwent WBRT [8,9]. The review and analysis of patient data in this study were approved by the Institutional Review Boards of both institutions.

Results

1. Patient, tumor, and treatment characteristics

The patient, tumor, and treatment characteristics are summarized in Table 1. The median age was 48 years (range, 34 to 75 years) in all patients, and was higher in the no RT group (60 years) than the WBRT (46 years) and PRT (45 years) groups, although this difference was not statistically significant (p=0.805). A boost RT was applied to the tumor bed in 16/24 patients who received WBRT. Overall, 69% of patients had no or stable extracranial disease and 71% had one brain lesion. All patients in the PRT group had no or stable extracranial disease, but the prevalence of extracranial disease was not significantly different among the three groups. The median size of the metastatic brain lesion was 3.5 cm (range, 1.0 to 8.0 cm). Additionally, 80% of all patients had tumors adjacent to the CSF flow. Hormone therapy was applied in 6/19 patients (32%) with hormone receptor-positive primary tumors.

Systemic treatment was administered after surgical resection and before the development of LMCDM in 71% of patients in the WBRT group, compared with 60% and 41% of patients in the PRT and no RT group (p=0.265). In a subgroup of 31 patients with HER2-positive primary tumors, 12 patients were given targeted therapy. The regimens of systemic treatment after brain surgery and before the development of LMCDM are listed in S1 Table. The three most commonly used regimens were capecitabine/lapatinib (37%), gemcitabine/cisplatin (33%), and capecitabine (30%).

2. Intracranial recurrence rate and pattern of failure

During the follow-up period, no intracranial recurrence was noted in 12 of the 24 patients in the WBRT group, two out of 10 in the PRT group, and seven out of 17 in the no RT group (Fig. 2). LR and LMCDM were the major patterns of failure rather than DBR. Eight out of 17 patients (47%) in the no RT group developed LR, as did two out of 10 patients (20%) in the PRT group and five out of 24 patients (21%) in

![Fig. 2. Intracranial recurrence rate and pattern of failures in three treatment groups: no radiotherapy (RT) group (n=17), partial RT (PRT) group (n=10), and whole brain radiotherapy (WBRT) group (n=24). LR, local recurrence; DBR, distant brain recurrence; LMCDM, leptomeningeal carcinomatosis or dural metastasis.](image-url)
the WBRT group. LMCDM was less common in the WBRT group (25%, 6/24 patients) than in the no RT (47%, 8/17 patients) or PRT groups (80%, 8/10 patients). Patterns of failures according to the treatment groups and dose-fractionation of RT are listed in S2 Table.

3. Leptomeningeal carcinomatosis or dural metastasis

At a median follow-up of 12.4 months (range, 2.3 to 83.6 months), LMCDM was detected in 22 out of 51 patients (43%) after surgery. The median time to the diagnosis of LMCDM after surgery was 8.6 months (range, 4.8 to 51.2 months). The 18-month LMCDM-free survival (LMCDM-FS) rate was higher in the WBRT group (77.5%) than in the PRT group (30.0%, p=0.021) and no RT group (13.6%, p=0.007) (Fig. 3A). There was no difference in 18-month LMCDM-FS between the PRT and no RT groups (p=0.449). When patients were divided into two groups according to whether they received WBRT or not, the LMCDM-FS was significantly better in the WBRT group than in the no WBRT group (77.5% vs. 22.6%, p=0.004) (Fig. 3B).

Table 2 shows the results of univariate analyses aimed at identifying which factors were potentially associated with the incidence of LMCDM. Tumors adjacent to the CSF flow were associated with increased risk of LMCDM. Postoperative WBRT and systemic treatment decreased the incidence of LMCDM. Tumor size, extent of resection, and biological subtype of the primary tumor were not associated with LMCDM upon univariate analyses. In the WBRT group, there were no differences in the incidence of LMCDM according to the dose-fractionation scheme of the WBRT. Multivariate analysis showed that WBRT and systemic treatment were significantly associated with reduced incidence of LMCDM. The HR relative to WBRT was 5.2 (95% CI, 1.9 to 14.8; p=0.009) for PRT and 2.7 (95% CI, 0.9 to 7.9; p=0.122) for no RT.

4. LR and OS

The 18-month LR-free survival was 83.6%, 75.0%, and 14.1% in the WBRT, PRT, and no RT groups, respectively (p=0.002). The LR-free survival was significantly lower in the no RT group than in the other groups (Fig. 4).

The median OS in all patients was 28.7 months. The median OS was 24.0 months in the WBRT group and 37.7 months in the PRT group, while it was 11.6 months in the no RT group. However, the OS did not differ significantly among the three groups (p=0.255) (Fig. 5). The OS values did not differ among biological subtypes of the primary tumor upon univariate analysis (p=0.562). The median OS was longer in patients with no or stable extracranial disease than those with progressive disease (median, 32.6 months vs. 11.6 months; p=0.067), although the difference was not statistically significant. Systemic treatment was also associated with a longer median survival time (yes, 32.6 months vs. no, 24.0 months; p=0.019).
Table 2. Univariate and multivariate analysis for LMCDM-FS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<tr>
<td></td>
<td></td>
<td>18-Month LMCDM-FS (%)</td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Extracranial disease status</strong></td>
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<td></td>
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<tr>
<td>None or stable</td>
<td>35</td>
<td>42.1</td>
<td>0.113</td>
</tr>
<tr>
<td>Progressive</td>
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<td>64.8</td>
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<td><strong>Biological subtype of primary tumor</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ER&lt;sup&gt;+&lt;/sup&gt; and/or PR&lt;sup&gt;+&lt;/sup&gt;, HER2&lt;sup&gt;−&lt;/sup&gt;</td>
<td>7</td>
<td>53.6</td>
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<td>&lt; 3.5</td>
<td>23</td>
<td>51.3</td>
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<tr>
<td>≥ 3.5</td>
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<tr>
<td>30 Gy/10 fx</td>
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<td>71.4</td>
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LMCDM-FS, leptomeningeal carcinomatosis or dural metastasis–free survival; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; CSF, cerebrospinal fluid; GTR, gross total resection; STR, subtotal resection; Tx, therapy; CTx, chemotherapy; RT, radiotherapy; WBRT, whole brain radiotherapy; PRT, partial radiotherapy; fx, fractions. <sup>a</sup>Log-rank test, <sup>b</sup>Hazard ratio refers to the risk of leptomeningeal carcinomatosis or dural metastasis per unit time, <sup>c</sup>Cox proportional hazard model (backward likelihood ratio), <sup>d</sup>n=50 (WBRT, 23; PRT, 10; no RT, 17).

5. Subgroup analysis according to HER2 status

In the 31 patients with HER2-positive primary tumors, systemic treatment including targeted therapy was associated with improved LMDCM-FS when compared with no systemic treatment (p=0.002). In the 20 patients with HER2-negative primary tumor, systemic treatment decreased the incidence of LMCDM (p < 0.001) (S3 Fig.). Systemic treatment including targeted therapy was also associated with better OS than no systemic treatment in the
HER2-positive subgroup of patients (p=0.047). In the HER2-negative subgroup of patients, systemic treatment did not improve median OS (p=0.159) (S4 Fig.).

**Discussion**

This study was conducted to assess the effects of postoperative WBRT on LMCDM and to identify the risk factors associated with LMCDM in patients who underwent surgical resection of brain metastases from breast cancer. LMCDM occurred in 22/51 patients (43%) and was the most common pattern of disease recurrence after surgical resection in this study. Patients who received WBRT had less LMCDM than those who received PRT. These results are consistent with those of previous retrospective studies that compared the outcomes of WBRT and PRT in postoperative settings. Patel et al. [8] reported 18-month LMC rates of 13% with WBRT and 31% with stereotactic radiosurgery (SRS) (p=0.045). Hsieh et al. [9] also reported a lower rate of LMC with WBRT (p=0.02).

Our study also demonstrated an association between the presence of a tumor adjacent to CSF flow and increased risk of LMCDM. Several other studies found an association between tumor location and LMC. Some studies have reported that the rate of LMC after surgical resection was higher for posterior fossa tumors than for supratentorial tumor [13,14]. Theoretically, there is a greater chance of CSF exposure during resection of posterior fossa tumors. Ahn et al. [12] classified tumor location relative to CSF flow. Their study showed an increased risk of LMCDM in patients with a tumor adjacent to the CSF flow (HR, 9.00; p < 0.01), consistent with our results. Although this association was not significant in our multivariate analysis, we assume that the patients with tumors adjacent to CSF flow could be at a high risk of LMCDM and that postoperative WBRT needs to be considered.

Concern about the risk of neurotoxicity from WBRT is the main reason postoperative local RT is often applied instead of WBRT in many clinical situations [6,7,15,16]. However, intracranial disease progression is another important cause of neurocognitive deterioration [17]. Occurrence of LMCDM is associated with significant morbidity, and intensive treatment of LMCDM can markedly compromise patient quality of life. Thus, it is particularly important to identify patients at higher risk of LMCDM and prevent the development of LMCDM, especially among those in whom long-term survival is expected. In the WBRT group, various dose-fractionation schemes (30 Gy/10 fx, 30 Gy/12 fx, or 25 Gy/10 fx) were used, all of which resulted in favorable outcomes. In patients with small cell lung cancer, prophylactic cranial irradiation of 25 Gy in 10 fx has been a standard regimen because increased risk of chronic neurotoxicity at higher doses had been reported [18]. Taken together, these results
indicate that postoperative WBRT 25 Gy in 10 fx followed by local boost can be considered after surgical resection of brain metastases to minimize neurotoxicity while achieving an acceptable tumor control.

The incidence of LMCDM was higher in our study than the incidence of LMC in previous reports, which included patients with brain metastases from any primary tumor [19,20]. The broader criterion for the diagnosis of LMCDM used in our study may explain the higher incidence. Twenty two patients developed LMCDM, eight of whom were diagnosed with dural metastasis. Like the patients with LMC, those with dural metastasis eventually require salvage therapy, including WBRT and intrathecal chemotherapy, and are at greater risk of toxicity because of these aggressive treatments. Thus, multiple enhancing dural nodules were also analyzed as LMCDM rather than DBR. Because the incidence of both LMC and dural metastasis can be affected by the extent of postoperative RT, we expected this broad criterion of LMCDM would be useful to identify patients in whom WBRT needs to be considered.

The lower incidence of LMCDM in the WBRT group was not associated with prolonged survival. The median survival was longer in PRT groups (37.7 months) than those in the no RT (11.6 months) or WBRT group (24.0 months), but this difference was not statistically significant. We assume that these results were caused by differences in baseline characteristics among groups. In the PRT group, there were more patients with a Breast-GPA score of 3.5-4.0, who were expected to have better prognosis than others, although this difference was not statistically significant. There was also a possibility that physicians had selected patients with good prognostic factors including Breast-GPA and were expected to live longer to receive PRT rather than WBRT to avoid neurotoxicity. The longer survival time in the PRT group suggests that patients might have had a greater chance to develop LMCDM because they lived longer than others. This possibility cannot be thoroughly investigated based on the present study. However, considering that the median time to develop LMCDM was only 8.6 months, the median survival time of the no RT or WBRT group was not too short to underestimate the LMCDM.

In addition to WBRT and tumor location, several other factors were reportedly associated with the development of LMC in previous studies. For example, primary tumor histology, size of resected lesion, piecemeal resection, and previous intracranial recurrence were associated with the incidence of LMC following surgical resection of brain metastases [12,20-24]. The number of brain metastasis and location of systemic disease progression were prognostic factors for LMC in patients who underwent upfront SRS for brain metastases [19,25,26]. In the current study, we found that systemic treatment after resection of brain metastases was significantly associated with reduced risk of LMCDM, with a HR of 0.1 compared to the patients who did not receive systemic treatment (p < 0.001). These findings are contrary to the widely accepted concept that systemic treatment is less effective in patients with brain metastasis because of the blood-brain barrier (BBB).

However, there is evidence that some targeted agents can cross the BBB [27]. Lapatinib is known to cross the BBB because of its very low molecular weight (581 Da), and several studies evaluating the effects of lapatinib in patients with metastatic brain tumor from the breast are ongoing (NCT-01622868 and NCT01218529). This can explain the improved LMCDM-FS in patients with HER2-positive primary tumor who underwent targeted therapies in this study. Among 12 patients who received targeted therapy, 11 were treated with lapatinib.

Patients with HER2-negative primary tumor also had an improved LMCDM-FS with chemotherapeutic agents upon subgroup analysis. Because all patients in this study underwent surgical resection of brain metastasis, the systemic agents might be able to cross the disrupted BBB, and hence eliminate the microscopically seeded tumor cells in the CSF. However, the heterogeneity of the regimens and diverse clinical situations make it difficult to determine the impact of systemic treatment on the development of LMCDM in the current study.

The strength of our study lies in the homogeneous study population. All of the patients underwent surgical resection of brain metastases from breast cancer. It should be noted that most of the previous studies included mixed populations of patients with metastases from a variety of primary solid tumor types, and more than half of the study populations in prior studies had primary lung cancer. To date, very few studies have determined the incidence of LMC after resection of brain metastases in breast cancer patients. Brain metastases of breast cancer should be separated from those of other primary tumors because of the much longer survival time. De Ieso et al. [28] reported that intensive treatment, including surgery and/or stereotactic RT, of brain metastases can result in long-term survival of more than 2 years in breast cancer patients with a limited number of brain metastases. Thus, both the effective disease control and preservation of quality of life should be pursued in these patients, and determining the proper extent of RT after surgical resection of brain metastasis is of particularly great importance.

There are limitations inherent to retrospective studies. The small number of patients and the baseline imbalance in patient characteristics among treatment groups might have biased the apparent effects of the WBRT. The effects of several factors known to increase the risk of LMC in previous studies, including the location and size of the tumor or the number of lesions, were not fully demonstrated in this study.
Given the inherent bias in retrospective analyses, a large prospective study of breast cancer patients with brain metastases is needed to precisely define high-risk patients who need to receive WBRT after surgery.

**Conclusion**

WBRT improved the LMCDM-FS rate after resection of brain metastases compared to PRT in breast cancer patients. Patients with tumors adjacent to the CSF flow are at higher risk of LMCDM, and these patients might need to be treated with postoperative WBRT. The effects of systemic treatment on LMCDM needs to be evaluated in further studies.

**Electronic Supplementary Material**

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

**References**


**Conflicts of Interest**

Conflict of interest relevant to this article was not reported.

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Efficacy of Chemotherapy in Patients with Unresectable or Metastatic Pancreatic Acinar Cell Carcinoma: Potentially Improved Efficacy with Oxaliplatin-Containing Regimen

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Tae Won Kim, MD, PhD
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Heung-Moon Chang, MD, PhD

Purpose
Pancreatic acinar cell carcinoma (ACC) is a rare cancer of the exocrine pancreas. Because of its rare incidence, the efficacy of chemotherapy in this patient population has been largely unknown. Therefore, we retrospectively analyzed the outcomes of patients with advanced pancreatic ACC who received chemotherapy.

Materials and Methods
Between January 1997 and March 2015, 15 patients with unresectable or metastatic pancreatic ACC who received systemic chemotherapy were identified in Asan Medical Center, Korea.

Results
The median age was 58 years. Eleven and four patients had recurrent/metastatic and locally advanced unresectable disease. The median overall survival in all patients was 20.9 months (95% confidence interval [CI], 15.7 to 26.1). As first-line therapy, intravenous 5-fluorouracil were administered in four patients (27%), gemcitabine in five (33%), gemcitabine plus capecitabine in two (13%), oxaliplatin plus 5-fluorouracil/leucovorin (FOLFOX) in two (13%), and concurrent chemoradiotherapy followed by capecitabine maintenance therapy in two (13%). The objective response rate (ORR) to chemotherapy alone was 23% and the median progression-free survival (PFS) was 5.6 months (95% CI, 2.8 to 8.4). After progression, second-line chemotherapy was administered in eight patients, while four patients received FOLFOX and the other four patients received gemcitabine. The ORR was 38%, and patients administered FOLFOX had significantly better PFS than those administered gemcitabine (median, 6.5 months vs. 1.4 months; p=0.007). The ratio of time to tumor progression (TTP) during first-line chemotherapy to TTP at second-line chemotherapy was significantly higher in patients administered FOLFOX (4.07; range, 0.87 to 8.30) than in those administered gemcitabine (0.12; range, 0.08 to 0.25; p=0.029).

Conclusion
Our results suggest that oxaliplatin-containing regimens may have improved activity against pancreatic ACC.

Key words
Acinar cell carcinoma, Pancreatic neoplasms, Antineoplastic agents, Oxaliplatin

Introduction
Acinar cell carcinoma (ACC) is a rare pancreatic exocrine malignancy that accounts for < 1% of all pancreatic neoplasms [1-3]. Because of its rare incidence, with the exception of analyses based on large national registries, current evidence of pancreatic ACC is primarily dependent on a few case series. These studies have shown that pancreatic ACC has distinct clinicopathological characteristics and treatment outcomes when compared with common pancreatic ductal adenocarcinoma (PDAC). In addition, there is lack of com-
mon molecular alterations shared with PDAC. Most previous studies have suggested that pancreatic ACC has a better prognosis than PDAC [4,5].

Surgical resection is the only curative treatment modality for localized pancreatic ACC, and patients who received surgery were associated with better survival outcomes [6]. However, approximately half of the patients have metastatic disease at presentation [5,7], and a considerable proportion of patients (57%-100%) develop recurrence even after curative surgery [3,5,8]. These findings indicate that development of effective systemic chemotherapy is essential for improving survival outcomes in patients with pancreatic ACC.

Despite recent advances in chemotherapy for PDAC after long stagnation, data regarding the chemotherapy for pancreatic ACC remains insufficient, and the most appropriate regimen for first-line chemotherapy is unclear. Moreover, no prospective studies focusing on pancreatic ACC patients alone have been conducted to date, and most clinical studies investigating novel agents in pancreatic malignancy usually exclude pancreatic ACC. Therefore, more retrospective analyses of chemotherapy in patients with pancreatic ACC may help improve our understanding of this rare disease. Here, we present the clinical outcomes of patients with unresectable or metastatic pancreatic ACC who received chemotherapy in a tertiary referral cancer center.

**Materials and Methods**

We searched the clinical data warehouse of the Asan Medical Center (ABLE; Asan Biomedical Research) and found 24 patients who had histologically documented pancreatic ACC with locally advanced unresectable, recurrent, or initially metastatic disease between January 1997 and March 2015. Among them, five patients were lost to follow-up after recurrence or refused chemotherapy and four patients were histologically diagnosed with mixed acinar-neuroendocrine carcinoma. Therefore, a total of 15 patients were included in the current analysis. We obtained clinical and pathological data from the review of patients’ medical records. All radiological images were reviewed by the investigators.

Tumor responses were graded according to the Response Evaluation Criteria in Solid Tumor (RECIST) ver. 1.1 [9]. Progression-free survival (PFS) was calculated from the administration date for the first dose of chemotherapy to the date of disease progression or any cause of death, whichever occurred first. Overall survival (OS) was calculated from the first dose of chemotherapy to the date of death due to any cause. If patients were alive, they were censored at the time of last follow-up. Time to tumor progression (TTP) was estimated as the time between the start of chemotherapy and documented tumor progression. PFS and OS were estimated using the Kaplan-Meier method and compared by the log-rank test.

To compare the activity of second-line regimens used, we analyzed the Growth Modulation Index (GMI) using the ratio of TTP from first-line chemotherapy (TTP1) to the TTP at second-line chemotherapy (TTP2) in patients who received second-line chemotherapy, similar to a previous study of another rare cancer [10]. The regimen with the higher GMI is considered to have better clinical efficacy. Considering only a small numbers of patients with pancreatic ACC were available to assess the efficacy of chemotherapy, this approach may be advantageous for measuring the relative activity among chemotherapeutic agents because patients serve as their own control. This generally increases statistical sensitivity because it eliminates the between-patient variability.

**Results**

1. Clinical characteristics

Baseline characteristics of the study population are summarized in Table 1. Overall, 13 patients received systemic chemotherapy, while two with locally advanced disease initially received concurrent chemoradiotherapy (CCRT) followed by systemic chemotherapy. The median age was 58 years (range, 29 to 72 years), and 13 patients (87%) were male. Pancreatic head was the most common site of disease (n=10, 67%). Approximately half of the patients (n=6, 40%) had recurrent disease after curative resection and four (27%) had locally advanced unresectable disease. The most common metastatic site was the liver (n=7, 47%), followed by intra-abdominal lymph nodes (n=5, 33%) and peritoneum (n=3, 20%). Individual patient characteristics and their responses to treatment are summarized in SI Table.

2. Treatment and efficacy

As first-line therapy, intravenous 5-fluorouracil (5-FU) was administered to four patients (27%), gemcitabine to five (33%), gemcitabine plus capecitabine (GEM-CAP) to two (13%), oxaliplatin plus 5-FU/leucovorin (FOLFOX) to two (13%), and CCRT followed by capecitabine maintenance therapy to two (13%) (Table 2). In patients who received chemotherapy alone, partial response (PR) was achieved in three patients, indicating an overall response rate (ORR) of 23%. Additionally, three patients with PR received infusional...
Table 1. Patient characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range, yr)</td>
<td>58 (29-72)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>13 (87)/2 (13)</td>
</tr>
<tr>
<td>Primary tumor location</td>
<td></td>
</tr>
<tr>
<td>Pancreatic head</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Pancreatic body/Tail</td>
<td>5 (33)</td>
</tr>
<tr>
<td>CA 19-9 (elevated)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Disease setting</td>
<td></td>
</tr>
<tr>
<td>Recurrent</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Locally advanced</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Initially metastatic</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Metastatic site</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>7 (47)</td>
</tr>
<tr>
<td>Distant lymph nodes</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Others</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Previous surgery in curative intent</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Previous adjuvant chemotherapy (n=6)</td>
<td>2 (33)</td>
</tr>
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</table>

Table 2. First-line treatment and response

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment regimen</td>
<td></td>
</tr>
<tr>
<td>Infusional 5-FU/Leucovorin</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Gemcitabine monotherapy</td>
<td>5 (33)</td>
</tr>
<tr>
<td>GEM-CAP</td>
<td>2 (13)</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>2 (13)</td>
</tr>
<tr>
<td>CCRT followed by capecitabine maintenance</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Response to the first-line treatment</td>
<td></td>
</tr>
<tr>
<td>CR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (7)</td>
</tr>
<tr>
<td>PR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 (27)</td>
</tr>
<tr>
<td>SD</td>
<td>5 (33)</td>
</tr>
<tr>
<td>PD</td>
<td>2 (13)</td>
</tr>
<tr>
<td>NA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 (20)</td>
</tr>
</tbody>
</table>

5-FU, 5-fluorouracil; GEM-CAP, gemcitabine plus capecitabine; FOLFOX, oxaliplatin plus 5-FU/leucovorin; CCRT, concurrent chemoradiotherapy; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NA, not applicable; sLV5FU2, simplified leucovorin and 5-FU regimen. <sup>a</sup>One CR and one PR patients received CCRT with capecitabine followed by capecitabine for their locally advanced disease. The other three PR patients received sLV5FU2, GEM-CAP, and FOLFOX. <sup>b</sup>Among three patients with NA for response evaluation, two patients were lost from early follow-up and one had no measurable lesion.

Fig. 1. Progression-free survival with first-line chemotherapy of patients with chemotherapy alone (A) and overall survival of all patients (B). CI, confidence interval.
Table 3. Second-line chemotherapy and response

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) (n=8)</th>
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</thead>
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<tr>
<td><strong>Chemotherapy regimen</strong></td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Gemcitabine monotherapy</td>
<td>4 (50)</td>
</tr>
<tr>
<td><strong>Response to the second-line chemotherapy</strong></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
</tr>
<tr>
<td>PR&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3 (37)</td>
</tr>
<tr>
<td>SD</td>
<td>1 (13)</td>
</tr>
<tr>
<td>PD</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

FOLFOX, oxaliplatin plus 5-fluorouracil/leucovorin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. <sup>1</sup>All PR patients received FOLFOX.

5-FU/leucovorin (n=1), GEM-CAP (n=1), and FOLFOX (n=1). Among two patients who received CCRT followed by capcitabine maintenance therapy for locally advanced disease, one patient achieved complete response and another one achieved PR. No patient treated with gemcitabine monotherapy achieved objective response (Table 2). The median PFS of patients with chemotherapy alone was 5.6 months (95% confidence interval [CI], 2.8 to 8.4) (Fig. 1A). The median PFS was 11.2 months (95% CI, 0.0 to 27.1) with intravenous 5-FU, 7.3 months with GEM-CAP, 5.6 months with FOLFOX, and 3.2 months (95% CI, 3.0 to 3.4) with gemcitabine monotherapy. The median PFS of patients who received CCRT followed by capcitabine maintenance therapy was 14.5 months. Median OS for all patients was 20.9 months (95% CI, 15.7 to 26.1) (Fig. 1B).

After disease progression while on first-line chemotherapy, second-line chemotherapy was administered to eight patients, with four receiving FOLFOX and four gemcitabine (Table 3). Objective response was achieved in three of the eight patients, indicating an ORR of 38%. All three patients with PR received FOLFOX, and no patients who received gemcitabine achieved objective response (Table 3). Among the patients treated with second-line FOLFOX, gemcitabine monotherapy (n=2), GEM-CAP (n=1), and infusional 5-FU/leucovorin (n=1) had previously been administered. Patients treated with FOLFOX had significantly better PFS than those treated with gemcitabine monotherapy (median, 6.5 months; 95% CI, 2.8 to 10.2 vs. 1.4 months; 95% CI, 0.5 to 2.3; p=0.007) (Fig. 2). The GMI was significantly higher in patients with FOLFOX (4.07; range, 0.87 to 8.30) than in those with gemcitabine (0.12; range, 0.08 to 0.25; p=0.029) (Table 4).

Discussion

In the current study, we retrospectively analyzed the clinical outcomes of patients with unresectable or metastatic pancreatic ACC. Our results suggest that oxaliplatin-contain-

![Image](https://via.placeholder.com/150)

Fig. 2. Progression-free survival with second-line chemotherapy. CI, confidence interval; FOLFOX, oxaliplatin plus 5-fluorouracil/leucovorin; GEM, gemcitabine.

Table 4. Comparison of the ratio of TTP1 to TTP2 in patients who received second-line chemotherapy

<table>
<thead>
<tr>
<th>Second-line chemotherapy</th>
<th>Gemcitabine alone</th>
<th>FOLFOX</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTP1, median (mo)</td>
<td>5.8</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>TTP2, median (mo)</td>
<td>1.4</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>GMI (TTP2/TTP1)</td>
<td>0.12 (0.08-0.25)</td>
<td>4.07 (0.87-8.30)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

TTP1, time to progression at first-line chemotherapy; TTP2, time to progression at second-line chemotherapy; GMI, Growth Modulation Index.
ing regimens may have better efficacy than gemcitabine monotherapy.

The baseline characteristics of our study population were similar to the results of previously published epidemiological studies in terms of age, sex, and tumor location [6,11-13]. Most patients were male (87%) and the pancreatic head was the most common site of primary tumor (67%). Consistent with the results of a previous retrospective study [14], the median OS in our patients was 20.9 months (95% CI, 15.7 to 26.1). These results suggest that the overall prognosis of patients with unresectable or metastatic pancreatic ACC seems to be better than that of PDAC.

With first-line chemotherapy, the ORR was 23% and the median PFS was 5.6 months (95% CI, 2.8 to 8.4). Monotherapy with intravenous 5-FU showed numerically longer PFS (median, 11.2 months) than other regimens, such as monotherapies with GEM-CAP (7.3 months), FOLFOX (5.6 months), and gemcitabine (3.2 months). The two patients with locally advanced disease who received upfront CCRT followed by capcitabine maintenance therapy showed the longest PFS (20.1 and 14.5 months).

In the second-line setting, FOLFOX showed better efficacy than gemcitabine monotherapy in terms of PFS and GMI (i.e., the ratio of TTP1 to TTP2). GMI was suggested as a potential end point of drug efficacy [15] and showed a strong relationship with survival outcome in pre-treated patients with sarcoma [16]. Patients administered FOLFOX had significantly better PFS than those administered gemcitabine monotherapy (median, 6.5 months; 95% CI, 2.8 to 10.2 vs. 1.4 months; 95% CI, 0.5 to 2.3; p=0.007). GMI was also significantly higher in patients administered FOLFOX (4.07; range, 0.87 to 8.30) than in those administered gemcitabine monotherapy (0.12; range, 0.08 to 0.25; p=0.03). Despite the large difference in terms of PFS between FOLFOX and gemcitabine, the number of patients in the second-line setting was too small to conclude whether FOLFOX was superior to gemcitabine, because of probable imbalance in baseline characteristics, including prognostic factors. Nevertheless, the significantly higher GMI with FOLFOX (4.07) than gemcitabine (0.12) suggests that oxaliplatin-containing regimens have better efficacy than gemcitabine, which has been the most popular regimen in pancreatic cancer to date. Indeed, a previous study conducted by the French Sarcoma Group found that a GMI > 1.33 was highly associated with improved OS in the setting of second-line chemotherapy for patients with soft-tissue sarcoma [16]. In very rare types of cancer such as pancreatic ACC, GMI may be a good indicator to estimate the activity of agent through intra-patient comparison, which may decrease the issues related with confounding factors.

The promising efficacy of oxaliplatin-containing regimens in this study might be explained by the distinctive molecular characteristics of pancreatic ACC. A recent study showed that the molecular signature of ACC is different from that of PDAC. KRAS, TP53, CDKN2A (p16), and SMAD4 gene mutations were not typically found in pancreatic ACC, whereas the frequency of mutations in the adenomatous polyposis coli–β catenin pathway, which is rarely detected in PDAC, was similar to those found in colorectal cancer (7%–24%) [17-19]. These findings suggest that the chemotherapeutic approaches for ACC patients include agents known to have activity in colorectal cancer [14,20-22].

Improved efficacy with oxaliplatin in pancreatic ACC may be because of the frequent genomic alterations associated with inactivation of DNA repair genes. In preclinical studies, pancreatic tumors from BRCA2 mutation carriers that showed evidence of loss of heterozygosity at the mutation site were associated with the development of ACC [23]. A recent Japanese study using whole-exome sequencing revealed that the loss of BRCA2 expression was observed in 45% [24] of patients (5/11) with liver metastasis, one of whom achieved complete remission after cisplatin-based chemotherapy. Comprehensive genomic profiling of 44 pancreatic ACC also showed that approximately half of the pancreatic ACC patients (45%) had inactivating genomic alterations in DNA repair genes (BRCA1/2, ATM, MSH1/2, RAD50, BRIPI1, RANCA, and PALB2), and that BRCA2 mutations were detected in 20% of pancreatic ACC [19]. Loss of function in DNA repair genes predisposes susceptibility to the platinum-based chemotherapy or poly(ADP-ribose) polymerase inhibitor; hence, the findings regarding DNA repair deficiencies in pancreatic ACC support our results with regard to the promising efficacy of oxaliplatin-containing regimens.

Multivariate analysis to exclude the impact of confounding factors could not be performed in this study because of the small number of patient included. Moreover, this study has inherent selection bias caused by its retrospective nature. Despite these limitations, this study has advantages in terms of a relatively large number of patients in the setting of unresectable or metastatic disease and that detailed information about the chemotherapeutic agents used was available.

**Conclusion**

In conclusion, our results suggest that the oxaliplatin-containing chemotherapy may have improved activity against pancreatic ACC compared with gemcitabine. This is supported by the results of recent studies demonstrating the distinctive genetic background of pancreatic ACC, including the high frequency of BRCA mutations. We applied GMI during statistical analysis to overcome the limitations associated
with the small populations and retrospective nature of our study. Nevertheless, it is still difficult to apply our results in general. Moreover, a large prospective multicenter trial is needed to address the rare incidence of pancreatic ACC.

Overall, recent findings, including those of the present study, indicate that chemotherapy strategies for unresectable or metastatic pancreatic ACC should be different from those for PDAC.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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References

FGFR4 Arg388 Is Correlated with Poor Survival in Resected Colon Cancer Promoting Epithelial to Mesenchymal Transition

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Purpose
Fibroblast growth factor receptor 4 (FGFR4) plays an important role in cancer progression during tumor proliferation, invasion, and metastasis. This study evaluated the prognostic role of FGFR4 polymorphism in patients with resected colon cancer, including the underlying mechanism.

Materials and Methods
FGFR4 polymorphism was characterized in patients who received curative resection for stage III colon cancer. FGFR4-dependent signal pathways involving cell proliferation, invasion, and migration according to genotypes were also evaluated in transfected colon cancer cell lines.

Results
Among a total of 273 patients, the GG of FGFR4 showed significantly better overall survival than the AG or AA, regardless of adjuvant treatment. In the group of AG or AA, combination of folic acid, fluorouracil, and oxaliplatin (FOLFOX) resulted in better survival than fluorouracil/leucovorin or no adjuvant chemotherapy. However, in GG, there was no difference among treatment regimens. Using multivariate analyses, the Arg388 carriers, together with age, N stage, poor differentiation, absence of a lymphocyte response, and no adjuvant chemotherapy, had a significantly worse OS than patients with the Gly388 allele. In transfected colon cancer cells, overexpression of Arg388 significantly increased cell proliferation and changes in epithelial to mesenchymal transition markers compared with cells overexpressing the Gly388 allele.

Conclusion
The Arg388 allele of FGFR4 may be a biomarker and a candidate target for adjuvant treatment of patients with resected colon cancer.

Key words
Adjuvant chemotherapy, Biomarkers, Colonic neoplasms, FGFR4, Polymorphism, Prognosis

Introduction
Surgery is the main treatment for colon cancer, although the recurrence rate is still high. The efficacy of adjuvant chemotherapy treatments has remained constant since introduction of a combination of oxaliplatin and fluorouracil (5-FU) to treat stage III colon cancer. Aside from pathological findings, the lack of biomarkers has made it difficult to identify high risk patients. Furthermore, palliative chemotherapy involving anti-epidermal growth factor or anti-vascular endothelial growth factor has failed to show a significant benefit in clinical trials. Therefore, it is essential to better characterize the molecular mechanisms of colon cancer to develop more effective treatment.

The tumor microenvironment provides the necessary signals for growth and survival of the primary tumor and enhance its invasion and dissemination to distant organs. Targeting tumor cells and the tumor microenvironment is thus crucial to the control and eradication of cancer. The
results of extensive studies have suggested that kinase inhibitors to multiple tyrosine residues that target rate-limiting steps in the metabolic pathways of tumor cells may be an effective treatment. Fibroblast growth factors constitute one class of possible targeting agents. These factors bind to four receptors (FGFR1-4) with tyrosine kinase activity involved in epithelial cell growth, migration/metastasis, and angiogenesis [1,2]. Among these receptors, FGFR4 has recently received a great deal of attention [1,3,4]. The overexpression of FGFR4 has been associated with cancer metastasis and poor survival outcome in gastric cancer, lung cancer, breast adenocarcinoma, and rhabdomyosarcoma [5-7]. The role of FGFR4 in colon cancer has been associated with enhancement of tumor cell proliferation, induction of the epithelial-mesenchymal transition (EMT) and resistance to chemotherapy [8-10]. A common polymorphism of FGFR4 involving conversion of guanine to adenine at position 1217 in exon 9 results in the substitution of arginine for glycine at codon 388 (Arg388) in the transmembrane domain, and this polymorphism has several clinical impacts on survival in breast cancer, high grade soft tissue sarcoma, head and neck cancer, and lung and colorectal cancer [10-13].

Thusbas et al. [6] reported poor disease-free survival (DFS) for breast cancer patients with the Arg388 allele of FGFR4 compared to patients with the Gly388 allele of FGFR4 who were treated with surgery followed by adjuvant chemotherapy without a difference in adjuvant endocrine therapy. Furthermore, our previous study reported that the Arg388 allele of FGFR4 was associated with a poor prognosis for esophageal cancer that was treated with chemoradiotherapy during its early stages (stage I-II), but not during its advanced stages (stage III-IV) [14]. Taken together, these results suggest that FGFR4 could be a crucial component in the early stages of cancer after curative resection or chemoradiotherapy.

Because of the increased need for effective colon cancer adjuvant treatments, we characterized the prognostic role of FGFR4 polymorphism after curative resection in colon cancer patients. The results suggested the molecular mechanism associated with the EMT, which is the rate-limiting step for tissue invasion during colon cancer progression [15].

2. Genotyping of FGFR4 in peripheral blood

Blood samples for genotyping were taken before surgery. Genomic DNA was extracted from peripheral blood using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) following the manufacturer’s protocols. Genotyping of the Gly388 allele of FGFR4 was performed by high resolution melting (HRM) analysis using a Rotor Gene 6000 (Corbett Research, Sydney, Australia). Polymerase chain reaction (PCR) primers were as follows: forward 5’-GGAGAGCTTCTGCACAGTGG-3’ and reverse 5’-CTTGCTGTGCTCT-GCT-3’. The reaction mixture for HRM included 200 nM PCR primers, 1 µM SYTO 9 fluorescent dye (Invitrogen, Carlsbad, CA), 0.5 units i-Taq polymerase and 40 ng genomic DNA in a 10 µL reaction volume. The cycling conditions included an initial 5 minutes hold at 95°C, followed by 40 cycles of 95°C for 5 seconds, 65°C for 30 seconds, and 72°C for 20 seconds, with melting temperatures increasing from 78°C to 92°C at 0.1°C/sec. The genotyping results were validated by direct sequencing (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems, Foster City, CA) of 16 samples (6%), and the results were 100% concordant. Appropriate positive/negative and internal controls were included.

1) Microsatellite instability testing

The pentaplex panel of mononucleotide repeats was used for microsatellite instability analysis. This panel is composed of five mononucleotide markers; BAT25, BAT26, NR21, NR22, and NR24. One primer in each pair was labeled with fluorescence (FAM, HEX) at the 5’ end. PCR for all markers was performed in 20 µL reaction volumes with 200 nM PCR primer, 0.5 U i-Taq polymerase, and 50 ng of genomic DNA. The PCR conditions were initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 40 seconds and 72°C for 30 seconds, and then final extension at 72°C for 5 minutes. The mixed PCR products with ROX standard were analyzed on an ABI 3130 x1 Genetic

Materials and Methods

1. Patients and samples

This investigation was conducted to determine the association of genetic polymorphisms and treatment outcomes in colon cancer. The study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (CUNH IRB-2014-016). All patients in this study were treated by curative resection for stage III colon adenocarcinoma (American Joint Committee on Cancer, sixth edition) for confirmed adenocarcinoma and gave informed consent for research use of their tissue and blood. Patients who died within 30 days after surgery with postoperative complications were excluded from the study. After surgery, patients received adjuvant chemotherapy based on their performance status or willingness under the current consensus guidelines. Data regarding a patient’s characteristics, history of adjuvant chemotherapy, DFS, and overall survival (OS) were obtained from medical records.
Analyzer using GeneScan Analysis software (Applied Biosystems).

2) Cell culture and transfection

Human colorectal cell line HCT 116 was cultured in Dulbecco’s modified Eagle medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (Gibco BRL, Rockville, MD) and 1% penicillin/streptomycin (Gibco BRL). To generate an FGFR4 overexpressing plasmid, approximately 2.4 kb of a PCR fragment corresponding to the full-length FGFR4 was amplified from HCT 116 cDNA using the following primers: forward 5’-CCCAACCTTGGATGCGCTGCTGGCCCTTTGG-3’ and reverse 5’-CCGCTGGAGTGTCTCGACCCAGACCCGAGGGGA-3’ (underlined sequences are the HindIII and XhoI restriction sites). A pcDNA6-FGFR4-Gly388 plasmid was constructed by cloning the PCR fragment into a pcDNA6 mammalian expression vector, which was verified by restriction endonuclease treatment and DNA sequencing. A mutant FGFR4 cDNA coding for arginine instead of glycine 388, pcDNA6-FGFR4-Arg388, was generated by PCR-mediated site-directed mutagenesis using the pcDNA6-FGFR4-Gly388 plasmid as a template, two mutagenic primers, and a QuikChange Site-directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s instructions. The mutagenic primers were as follows: forward 5’-GCTGCTCGCTCTGGCCAGGCTGTATCG-3’ and reverse 5’-GCCCTGGCCCTCGGATACACGCGCTGGCC-AGCACGAG-3’. The sequence of mutated FGFR4 was verified by DNA sequencing. Transfection was performed using Lipofectamine 2000 (Invitrogen). At 48-hour post-transfection, 5 µg/mL blasticidin (Sigma-Aldrich, St. Louis, MO) was added, and the live cells were selected as stably transfected cells.

3) Cell viability analysis

The cell viability was monitored using a RealTime-Glo MT cell Viability Assay kit (Promega, Madison, WI) following the manufacturer’s instructions.

4) Western blot analysis

Whole cell lysates were obtained with radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors (Thermo Fisher Scientific, Waltham, MA). The protein concentrations were measured using a BCA Protein Assay Kit (Pierce, Rockford, IL). The following antibodies were used: anti-FGFR4, anti-pFRS2α, anti-pSTAT3, anti-pAKT, anti-pERK, and anti-Snail from Cell Signaling Technology (Danvers, MA); anti-E-cadherin from BD Sciences (San Jose, CA); anti-vimentin from Santa Cruz Biotechnology (Santa Cruz, CA); anti-β-actin and anti-Twist from Abcam (Cambridge, UK); anti-CD133 from Miltenyi Biotec (Bergisch Gladbach, Germany); and anti-CD44 from R&D Systems (Minneapolis, MN).

5) Invasion and migration assay

The cell invasion assay was performed using Transwell filter chambers that were coated with 1 µg/mL Matrigel in culture media for 6 hours, then dried at room temperature. The cells were seeded at 2×10⁵ cells in 150 µL medium with 1% bovine serum albumin (BSA) into the upper chamber. Next, 600 µL of medium with 1% BSA and 20 µg/mL fibronectin (Calbiochem, La Jolla, CA) was loaded into the lower chamber. After 24 hours of incubation, cells that invaded to the bottom surface of the Transwell were fixed with 70% ethanol, stained with Diff-Quik solution (Sysmex, Kobe, Japan), and counted in five selected fields. The cell migration was measured using Culture-Inserts (Ibidi, Regensburg, Germany). Briefly, the Culture-Inserts were transferred into 6-well culture plates, after which cells were seeded at a density of 1×10⁵ cell/100 µL in each well of the Culture-Inserts. After 24 hours of incubation, the Culture-Inserts were removed, and cell-free gaps were created. Images of the closed gap were captured at the indicated incubation times.

6) Statistical analyses

Association analyses between genotypes and clinicopathological characteristics were performed using the chi-squared test and Fisher exact test. Survival curves were calculated using the Kaplan-Meier method, and curves were compared using the log-rank test. The DFS time was calculated from the time of diagnosis of disease to recurrence. The OS time was calculated from the diagnosis of disease to death from any cause, and patients who were alive at the last follow-up were recorded at that time. Univariate analyses were performed using the Kaplan-Meier method and the log-rank test. All variables from univariate analyses with p-values of < 0.1 were incorporated into the multivariate Cox hazard regression model with a stepwise forward procedure. All p-values were derived from a two-tailed statistical test with a 95% confidence interval for evaluation of the statistical significance between groups. All statistical analyses were performed using SPSS statistical software for Windows ver. 21.0 (IBM Corp., Armonk, NY), and a p < 0.05 was considered to indicate significance.
Table 1. Patient and clinicopathologic characteristics

<table>
<thead>
<tr>
<th>Patient demographics</th>
<th>Total (n=273)</th>
<th>FGFR4-388 genotype</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG (n=92, 34%)</td>
<td>AA/AG (n=181, 66%)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>70 (34-85)</td>
<td>70 (40-84)</td>
<td>70 (34-85)</td>
</tr>
<tr>
<td>&lt; 70</td>
<td>123</td>
<td>39 (42)</td>
<td>84 (46)</td>
</tr>
<tr>
<td>≥ 70</td>
<td>150</td>
<td>53 (58)</td>
<td>97 (54)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>152 (56)</td>
<td>57 (62)</td>
<td>95 (53)</td>
</tr>
<tr>
<td>Female</td>
<td>121 (44)</td>
<td>35 (38)</td>
<td>86 (47)</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>170 (62)</td>
<td>60 (65)</td>
<td>110 (61)</td>
</tr>
<tr>
<td>≥ 5</td>
<td>97 (36)</td>
<td>30 (33)</td>
<td>67 (37)</td>
</tr>
<tr>
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<td>6 (2)</td>
<td>2 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-2</td>
<td>25 (9)</td>
<td>9 (10)</td>
<td>16 (9)</td>
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<tr>
<td>T3-4</td>
<td>248 (91)</td>
<td>83 (90)</td>
<td>165 (91)</td>
</tr>
<tr>
<td>N stage</td>
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<td></td>
</tr>
<tr>
<td>N1</td>
<td>193 (71)</td>
<td>65 (71)</td>
<td>128 (71)</td>
</tr>
<tr>
<td>N2</td>
<td>80 (29)</td>
<td>27 (29)</td>
<td>53 (29)</td>
</tr>
<tr>
<td>LVI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77 (28)</td>
<td>67 (73)</td>
<td>129 (71)</td>
</tr>
<tr>
<td>No</td>
<td>196 (72)</td>
<td>25 (27)</td>
<td>52 (29)</td>
</tr>
<tr>
<td>PNI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>121 (44)</td>
<td>47 (51)</td>
<td>105 (58)</td>
</tr>
<tr>
<td>No</td>
<td>152 (56)</td>
<td>45 (49)</td>
<td>76 (42)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well to moderate</td>
<td>245 (90)</td>
<td>82 (89)</td>
<td>163 (90)</td>
</tr>
<tr>
<td>Poorly</td>
<td>28 (10)</td>
<td>10 (11)</td>
<td>18 (10)</td>
</tr>
<tr>
<td>Lymphocyte response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>267 (98)</td>
<td>89 (97)</td>
<td>178 (98)</td>
</tr>
<tr>
<td>Negative</td>
<td>6 (2)</td>
<td>3 (3)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Microsatellite status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSS</td>
<td>248 (91)</td>
<td>162 (65)</td>
<td>86 (94)</td>
</tr>
<tr>
<td>MSI</td>
<td>25 (9)</td>
<td>19 (76)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Tumor location\textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>112 (41)</td>
<td>40 (44)</td>
<td>72 (40)</td>
</tr>
<tr>
<td>Left</td>
<td>161 (59)</td>
<td>52 (56)</td>
<td>109 (60)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not done</td>
<td>23 (8)</td>
<td>9 (10)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>5-FU/LV</td>
<td>127 (47)</td>
<td>41 (44)</td>
<td>86 (47)</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>123 (45)</td>
<td>42 (46)</td>
<td>81 (45)</td>
</tr>
</tbody>
</table>

Values are presented as number (%). CEA, carcinoembryonic antigen; NA, not available; LVI, lymphovascular invasion; PNI, perineural invasion; MSS, microsatellite stable; MSI, microsatellite instable; 5-FU, fluorouracil; LV, leucovorin; FOLFOX, combination of folinic acid, 5-FU, and oxaliplatin. \textsuperscript{a}Right side cancers include ascending and transverse colon cancer and left side cancers include descending and sigmoid colon cancer.
Results

1. Study population

A total of 324 consecutive patients resected for stage III colon cancer between May 2004 and December 2011 were reviewed. Among the patients, 273 who met the inclusion criteria were enrolled in this study. Their median age was 70 years (range, 34 to 85 years). After surgery, 127 (47%) patients received 5-FU and leucovorin (FL) and 123 (45%) received a combination of folinic acid, 5-FU, and oxaliplatin (FOLFOX) as an adjuvant chemotherapy; 23 patients (8%) did not receive any adjuvant chemotherapy (Table 1). During follow-up (median, 41 months), 66 patients experienced disease recurrence, while 41 died from colon cancer. The rates of the 3-year DFS and 5-year OS were 74.2% (95% confidence interval [CI], 68.71 to 79.69) and 80.9% (95% CI, 75.22 to 85.58), respectively.

2. Incidence of FGFR4 Gly388Arg polymorphism

Out of 273 patients, 92 (34%) were homozygous for the Gly388 allele (GG), 146 were heterozygous (GA, 53%), and 35 were homozygous (AA, 13%) for the Arg388 allele. The percentage of patients with the Arg388 allele was higher than in some previous reports (50%-60%) [8], but consistent with another study reporting an incidence of 11.8% in colorectal cancer patients [9]. No significant association was found between clinical or histopathological tumor characteristics and the FGFR4 genotype (Table 1).

3. Treatment outcomes according to FGFR4 genotype

There was no significant difference in the DFS between patients heterozygous for the Gly388 and Arg388 alleles (p=0.500). However, the OS was significantly better in patients homozygous for the Gly388 allele than for heterozygous patients or patients with the Arg388 allele (p=0.017). Based on these results, further analyses were performed on the two groups of patients with the Gly388 allele and the Arg388 carrier patients (heterozygous and Arg388 allele) (Fig. 1). Using univariate analyses, N stage, perineural invasion (PNI), and tumor differentiation, the patients receiving adjuvant chemotherapy were significantly associated with the DFS. The OS, age, N stage, tumor differentiation, lymphocyte response, adjuvant chemotherapy and FGFR4 genotype were significantly associated with prognosis (Table 2). Multivariate analyses showed that the N stage (N2 vs. N1), presence of PNI, and absence of adjuvant chemotherapy were significant independent factors for the DFS. In addition, age (≥70 years), N stage (N2 vs. N1), poor differentiation, absence of a lymphocyte response, absence of adjuvant chemotherapy, and Arg388 carriers were significantly associated with poor prognoses for the OS (Table 3).

Fig. 1. (A, B) Survival outcomes according to FGFR4 genotype.
Table 2. Univariate analysis for survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Disease-free survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (≥ 70 yr)</td>
<td>1.337 (0.818-2.185)</td>
<td>0.246</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>1.079 (0.640-1.820)</td>
<td>0.770</td>
</tr>
<tr>
<td>CEA (≥ 5 ng/mL)</td>
<td>0.811 (0.190-3.459)</td>
<td>0.777</td>
</tr>
<tr>
<td>T stage (T3-4)</td>
<td>2.856 (0.663-12.300)</td>
<td>0.159</td>
</tr>
<tr>
<td>N stage (N2)</td>
<td>2.136 (1.249-3.651)</td>
<td>0.006</td>
</tr>
<tr>
<td>LVI (+)</td>
<td>1.005 (0.570-1.774)</td>
<td>0.985</td>
</tr>
<tr>
<td>PNI (+)</td>
<td>1.998 (1.222-3.266)</td>
<td>0.006</td>
</tr>
<tr>
<td>Differentiation (poorly)</td>
<td>2.067 (1.076-3.972)</td>
<td>0.029</td>
</tr>
<tr>
<td>Lymphocyte response (-)</td>
<td>1.746 (0.450-6.770)</td>
<td>0.420</td>
</tr>
<tr>
<td>Microsatellite status</td>
<td>1.541 (0.735-3.228)</td>
<td>0.278</td>
</tr>
<tr>
<td>Tumor site (right)</td>
<td>1.062 (0.648-1.741)</td>
<td>0.810</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU/LV</td>
<td>0.413 (0.203-0.840)</td>
<td>0.015</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>0.312 (0.149-0.653)</td>
<td>0.002</td>
</tr>
<tr>
<td>FGFR4 (AA or AG)</td>
<td>1.243 (0.735-2.102)</td>
<td>0.417</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; LVI, lymphovascular invasion; PNI, perineural invasion; 5-FU, fluorouracil; LV, leucovorin; FOLFOX, combination of folinic acid, 5-FU, and oxaliplatin; FGFR4, fibroblast growth factor receptor 4.

Table 3. Multivariate analysis for survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Disease-free survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (≥ 70 yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N stage (N2)</td>
<td>2.374 (1.444-3.903)</td>
<td>0.001</td>
</tr>
<tr>
<td>Differentiation (poorly)</td>
<td>1.845 (0.940-3.623)</td>
<td>0.075</td>
</tr>
<tr>
<td>PNI (+)</td>
<td>1.815 (1.100-2.996)</td>
<td>0.020</td>
</tr>
<tr>
<td>Lymphocyte response (+)</td>
<td></td>
<td>0.156 (0.041-0.592)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU/LV</td>
<td>0.431 (0.211-0.882)</td>
<td>0.021</td>
</tr>
<tr>
<td>FOLFOX</td>
<td>0.262 (0.139-0.608)</td>
<td>0.001</td>
</tr>
<tr>
<td>FGFR4 (AA or AG)</td>
<td>5.161 (2.062-12.916)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; PNI, perineural invasion; 5-FU, fluorouracil; LV, leucovorin; FOLFOX, combination of folinic acid, 5-FU, and oxaliplatin; FGFR4, fibroblast growth factor receptor 4.

4. The Gly388 allele of FGFR4 was an indicator of a good prognosis, regardless of adjuvant chemotherapy

As previously mentioned, the OS was significantly higher in patients with the Gly388 allele of FGFR4 than with Arg388 carriers. To evaluate the effects of adjuvant regimen, we analyzed the OS according to genotype, stratified by adjuvant chemotherapy. In the group without treatment, the 5-year OS percentages in patients with the Gly388 allele and Arg388 carriers were 88.9% and 30.0%, respectively (p=0.025). However, the difference in the 5-year OS percentages according to genotypes was attenuated in the FL-treated group compared with the no treatment group (p=0.06), and was comparable to the FOLFOX-treated group in patients with the Gly388 allele and Arg388 carrier patients (p=0.174). These results suggested that intensified adjuvant chemotherapy could overcome the poor prognosis of patients with the Arg388 allele in a similar manner to patients with the Gly388
allele. DFS and OS were analyzed according to adjuvant chemotherapy, stratified by the FGFR4 genotype, to determine the possible benefits of adjuvant chemotherapy for each genotype. Regarding the DFS and OS for Arg388 carriers, the FOLFOX-treated group had the most significant improvements when compared to the group without treatment. However, there was no significant difference in survival outcomes from the adjuvant chemotherapy regimen for Gly388 carriers with a good prognosis (Fig. 2).

5. The overexpression of the Arg388 and Gly388 alleles of FGFR4 promoted colon cancer cell proliferation

To determine if the presence of the Arg388 allele was associated with colorectal cancer cell progression, we conducted FGFR4 genotype analysis with cDNAs from eight colorectal cancer cell lines. The homozygous Gly388 allele was present in HCT 116 and SW480 cells, the heterozygous Gly388 allele was present in HT29, CaCo2, and KM12c cells,
Fig. 3. Fibroblast growth factor receptor 4 (FGFR4) dependent downstream signal and cell proliferation. (A) The expression of downstream signals of FGFR4, including pSTAT3, pAKT, and pERK, were increased more in overexpressed Arg388 than in overexpressed Gly388 cells. (B) However, the growth rate of overexpressed Arg388 cells was similar to that of overexpressed Gly388 cells. *p < 0.01 compared with vector control.

and the homozygous Arg388 allele was present in DLD1, DKO1, and HCT15 cells. Because the heterozygous and Arg388 alleles had poorer survival rates than the Gly388 allele, we selected the HCT 116 cell line, which did not contain the Arg388 polymorphism, to overexpress the Arg388 allele of FGFR4. The Arg388 and Gly388 stable expression plasmids were constructed and transfected into HCT 116 cells. An empty vector was also transfected into control cells. The overexpression of Arg388 and Gly388 of FGFR4 was confirmed by western blotting.

To determine the effects of Arg388 and Gly388 overexpression on downstream signaling, we analyzed the expression levels of FGFR4 downstream targets by western blotting. Phosphorylation of the primary FGFR target, FRS2α, was increased in overexpressed Arg388 and Gly388 cells compared with control cells. Although FRS2α was increased in both transfected cells, FRS2α was increased more in overexpressed Arg388 than in overexpressed Gly388 cells. Furthermore, the expression of downstream signals of FGFR4, including pSTAT3, pAKT, and pERK, was also further increased in overexpressed Arg388 than Gly388 cells (Fig. 3A).

The 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay was performed to assess the cell proliferation of overexpressed Arg388 and Gly388 cells. The cell growth rates increased in overexpressed Gly388 and Arg388 cells when compared with the control cells, but the growth rate of overexpressed Arg388 cells was similar to that of overexpressed Gly388 cells. These results indicated that FGFR4 is associated with increasing cell proliferation, but that there was no difference between cells overexpressing Gly388 and Arg388 (Fig. 3B).

6. The Arg388 allele of FGFR4 induced EMT signals and enhanced invasion and migration

The EMT involves profound changes in cell morphology and behavior. This process plays a crucial role in the early stages of cancer recurrence and metastasis. To study the role of the genotype in the EMT, we assessed the roles of the Arg388 allele compared with the Gly388 allele of FGFR4 during induction of EMT changes in stably transfected cells. Using western blot analyses, E-cadherin was significantly reduced in Arg388 overexpressed cells when compared with Gly388 overexpressed and control cells. In addition, there was increased expression of vimentin and Twist in Arg388 overexpressed cells compared with Gly388 overexpressed and control cells. These results suggested that the Arg388 allele of FGFR4 induces the EMT process (Fig. 4A). To investigate the functional properties of the EMT inducers, we conducted cell invasion and migration studies of FGFR4 transfected cells. Stably transfected Arg388 Gly388 cells, including the control HCT 116 cell, were seeded in Transwell filter chambers, and the cell motility towards human plasma fibronectin was determined. When compared with control cells, the Gly388- and Arg388-transfected cells showed sig-
Fig. 4. Effect of fibroblast growth factor receptor 4 (FGFR4) genotypes on epithelial-mesenchymal transition (EMT) signals, invasion, and migration. (A) The Arg388 allele of FGFR4 induces the EMT markers including, vimentin and Twist, while it decreased E-cadherin. (B) When compared with control cells, the Gly388- and Arg388-transfected cells showed significantly more cell invasion. In addition, Arg388-transfected cells were more invasive than Gly388-transfected cells. (C) Similar results were also seen using a wound healing assay. *p < 0.01.
significantly more cell invasion. Moreover, the Arg388-transfected cells were more invasive than the Gly388-transfected cells (Fig. 4B). Similar results were also seen using a wound healing assay (Fig. 4C). Together, these results suggested that both the Arg388-transfected and Gly388-transfected cells significantly increased the migration and invasion in colorectal cancer cells, but that overexpression of the Arg388 allele was more robust.

Discussion

The percentage of patients with recurrent or metastatic colon cancer who are cured is less than 10%; therefore, there have been extensive studies conducted to improve these prognoses. To improve adjuvant treatments, actual high-risk patients must be identified and adjuvant chemotherapies tailored to prevent recurrence developed. Therefore, the identification of biomarkers to select optimal patients and druggable targets of colon cancer would increase the efficacies of current treatments greatly.

Previous studies to define the functional role of FGFR4 have mainly characterized the overexpression of FGFR4 in cancer model systems. Recently, FGFR4 polymorphisms have been evaluated in several tumors, and the characteristics of the Arg388 allele of FGFR4 have been compared with the Gly388 allele, showing similar features of overexpression of FGFR4 [16,17]. In addition to in vitro studies, the Arg388 allele of FGFR4 has been associated with a poor prognosis after surgery in prostate and breast cancer patients [6,17]. It has been suggested that FGFR4 polymorphism can be a surrogate marker to reflect the abnormal FGFR4 pathway in tumors.

The results of the present study showed that the FGFR4 genotype has the highest odds ratio in the prediction of the OS. Contrary to previous studies of FGFR4 polymorphism using various tumor stages and treatment populations [6,7,11], the present study only characterized stage III colon cancer patients to clearly define the role of FGFR4 polymorphism in the OS after curative resection. Moreover, our results determined the role of adjuvant chemotherapy according to genotypes. Patients with the Gly388 allele of FGFR4 had a good prognosis, regardless of adjuvant chemotherapy. However, Arg388 allele carrier patients had a poor prognosis, although their OS was improved by treatment with an intensified chemotherapy regimen (FOLFOX > FL > no treatment). Based on these results, the Arg388 carriers are candidates for adjuvant chemotherapy, while patients with the Gly388 allele of FGFR4 are not, even though they have stage III colon cancer. Given the lack of effective biomarkers for colon cancer in an adjuvant setting, our results suggest that the FGFR4 genotype can be used to identify optimal treatment strategies and develop new treatment targets.

The FGFR4 genotype’s mechanism of action for aggressive tumors behavior reportedly involves receptor stability rather than a difference in protein expression [6,7]. In the prostatic cancer model, the presence of the Arg388 allele of FGFR4 increased receptor stability and sustained receptor activation following ligand binding when compared with the Gly388 allele. These changes were the result of increased activity of the SRF, AP, and ERK pathways [12,17]. A recent study reported that functional changes in FGFR4 polymorphisms originated from substitution of the conserved glycyne 388 residue to a charged arginine residue that altered the transmembrane spanning segment and exposed a membrane proximal STAT3 binding site [18]. These results explained the functional changes resulting from the genetic polymorphism during cancer progression. Therefore, genetic studies should characterize heterogeneous somatic mutations of tumors and genetic variation in patients to identify therapeutic targets.

To establish the biologic significance of FGFR4 polymorphism in colon cancer, we first evaluated protein expression according to various genotypes in colon cancer cell lines derived from patients and human tumor samples. Overall, the level of FGFR4 expression was not significantly different in each genotype (unpublished data). These results are similar to those of previous studies of breast and gastric cancers that reported no significant correlation with FGFR4 genotype and FGFR4 protein or mRNA expression [6,19]. Although the association of FGFR4 genotype and protein expression was not conclusive, gene polymorphism is an easier and more reproducible method than immunohistochemical staining because of the variability in expression from tumor heterogeneity or staining methods. Therefore, FGFR4 polymorphism could be a useful surrogate biomarker of FGFR4 function in cancer patients.

To validate the function of the FGFR4 genotype in the recurrence of cancer, its effects on the EMT were evaluated in transfected cell lines. The EMT is an early event that facilitates infiltration of surrounding tissue, ultimately resulting in metastasis to distant sites [20]. Several studies have reported that STAT3 may play an important role in establishing cell polarity during directed cell migration, which is essential to EMT and carcinoma metastasis using the insulin like growth factor-1 or the interleukin 1/STAT3 pathway [21,22]. Therefore, the present study was conducted to characterize induction of the EMT signal via STAT3 and pFRS2 activation according to the colon cancer genotype. Notably, the Arg388 allele of FGFR4 resulted in a stronger activation of pFRS2 and STAT3, which are known as downstream sig-
nals for FGFR4, as well as in stronger activation of EMT-associated proteins, such as vimentin, Twist, and the loss of E-cadherin, when compared with the Gly388 allele of FGFR4. We also showed that the Arg388 allele of FGFR4 had more invasive and migratory activities than the Gly388 allele. To date, few reports have associated the FGFR4 genotype with the STAT3 and EMT pathway during tumorigenesis. Our results support the use of FGFR4 as a therapeutic target in adjuvant treatment for colon cancer.

Small molecular inhibitors targeting the FGFR, especially FGFR1-3, have recently been developed for cancer therapy, including AZD4547, BGJ398, and dovitinib [23-25]. Although pan-FGFR inhibitors have also been developed [26-28], the exact mechanisms of action involving tumors are not fully understood, and it is still difficult to select optimal patients who can benefit from these agents. Furthermore, kinase inhibitors for multiple tyrosines can block many signal pathways and cause significant side effects involving their toxicities. Pan-EGFR inhibitors can induce dose-limiting toxicities in the clinic because of potent inhibition of FGFR1 and FGFR3, resulting in soft tissue mineralization and hyperphosphatemia [29]. To circumvent these problems, a close examination of the specific function of each type of FGFR is necessary. BLU9931 is the first selective FGFR4 inhibitor that achieved improvement of hepatocellular carcinoma prognosis [30]. In hepatocellular carcinoma, there is a well known association of FGFR4 with its β-klotho co-receptor and expression of its ligand, FGF19. However, there has been no report of a specific FGFR4 pathway inhibitor for colon cancer patients. Accordingly, further studies to validate FGFR4 polymorphism as a biomarker are needed in a larger population of colon cancer patients, including patients with stage II colon cancer. In addition, future studies should be directed toward the development of an effective FGFR4 pathway inhibitor or STAT3 inhibitor.

Conclusion

The present study employed FGFR4 polymorphism to help identify treatments for high risk patients with stage III colon cancer. By inhibiting the EMT pathway, FGFR4 can also be used as a new therapeutic target in adjuvant treatment. Based on these findings, the targeted therapy should involve both tumor-specific biology and the stage of the disease, and FGFR4 inhibitors could be used in clinical investigations to assess its efficacy on a subset of colon cancer patients according to their FGFR4 polymorphisms.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References


Effects and Mechanisms of Metformin on the Proliferation of Esophageal Cancer Cells *In Vitro* and *In Vivo*

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**Purpose**
The purpose of this study was to observe the effects of metformin on human esophageal cancer cell and to investigate its possible mechanisms.

**Materials and Methods**
Cell viability was detected by using a Cell Counting Kit-8, while cell cycle and apoptosis were assessed by flow cytometry and western blot was used to measure the expression of the related proteins. RNAi was used to knockout pyruvate kinase muscle isozyme 2 (PKM2). An Eca109 tumor model was established to evaluate the antitumor effect *in vivo*. Immunohistochemistry was determined based on the expression of PKM2 and Bim in tumor tissues. Tunnel was used to assess tumor cell apoptosis.

**Results**
Esophageal cancer cells viability was reduced after metformin treatment. The cell cycle was arrested in the G0/G1 phase, apoptosis was induced, caspase 3 was activated, caspase 9 was downregulated, and the pro-apoptotic protein Bim increased. Further study revealed that metformin could suppress the expression of insulin-like growth factor 1 receptor and its downstream proteins, phosphoinositide 3-kinase (PI3K), protein kinase B (AKT/PKB), phosphorylation of AKT (pAKT), mammalian target of rapamycin (mTOR), p70S6K, and PKM2. Insulin-like growth factor 1 partly reversed metformin-induced apoptosis and attenuated the repression effect of metformin to PI3K, pAKT, and PKM2. Knockout PKM2 resulted in the activation of caspase 3, down-regulation of caspase 9, and increased expression of Bim. In the Eca109 xenograft model, metformin significantly reduced tumor growth. Furthermore, we found that metformin treatment increased the rate of apoptosis, down-regulation of PKM2, and up-regulation of Bim in tumor tissues.

**Conclusion**
Metformin restrained esophageal cancer cell proliferation partly by suppressing the PI3K/AKT/mTOR pathway.

**Key words**
Metformin, Cell proliferation, Apoptosis, PKM2 protein, Bcl-2-interacting mediator of cell death

**Introduction**
Esophageal carcinoma is a common malignant tumor worldwide [1,2]. Indeed, more than 300,000 people worldwide died from esophageal cancer, of which 150,000 were Chinese in 2012 [3,4]. Current treatments for esophageal cancer include surgery, chemotherapy, and radio-chemotherapy; however, there all have limited effects on the disease. Therefore, it is essential to find new curative approaches to treatment of esophageal cancer.

Several studies [5,6] have shown a close link between type 2 diabetes and the risk of some solid cancers. Patients with type 2 diabetes show increased risk of developing a range of different cancers, including colorectal, breast, and liver cancer [6-9]. Hyperinsulinemia [10] plays a crucial role in increas-
ing cancer risk for people with type 2 diabetes. Metformin, an anti-diabetic drug, has been extensively employed for the treatment of type 2 diabetes to reduce blood glucose concentration and increase insulin sensitivity. These factors are likely to be associated with increased cancer risk. Accumulating evidence [11-13] has shown that diabetic patients treated with metformin display a reduced incidence of neoplastic disease and might improve cancer prognosis, including that of human esophageal cancer cell (ESCC), in clinical trials. Metformin [14-16] also shows evident inhibitory and pro-apoptotic effects in mice tumor models. However, the antitumor mechanisms of metformin remain elusive.

Previous reports [17,18] have shown that the antitumor mechanisms of metformin were partly attributed to activation of AMP-activated protein kinase (AMPK). AMPK can suppress the expression of mammalian target of rapamycin (mTOR), which can negatively regulate tumor growth and promote cancer cells apoptosis [19]. Furthermore, it has been argued [20] that metformin affects the insulin/insulin-like growth factor (I/IGF) pathway and decreases the expression of insulin-like growth factor 1 receptor (IGF-1R). IGF-1R [20,21], a receptor tyrosine kinase, is over-expressed in several types of cancer, including human ESCC. IGF-1R can activate the phosphoinositide 3-kinase (PI3K)/AKT/mTOR signaling pathway to regulate cell proliferation, survival, and angiogenesis. Moreover, IGF-1R can be used as an indicator and targets for cancer therapy. Pyruvate kinase muscle isozyme 2 (PKM2), which is a downstream molecule in the PI3K/AKT/mTOR pathway that plays a crucial role in the Warburg effect, is overexpressed in nearly all tumor cells. Studies [22-24] have shown that PKM2 contributes to tumor growth, which may partly be attributed to its involvement in cellular energy control and glucose metabolism. PKM2 depletion decreases the ability of human tumor cells to form tumors [25]. Inhibiting the PI3K/AKT/mTOR pathway may down-regulate PKM2 expression and alter cancer metabolism. Therefore, metformin could decrease the expression of IGF-1R to inhibit the PI3K/AKT/mTOR/PKM2 signaling pathway and suppress tumor growth, which may be a novel mechanism in human ESCC cancer.

In this study, we evaluated the antitumor activity of metformin in human ESCC cells. The results showed that metformin inhibited proliferation of ESCC cells, as well as induced cell cycle arrest and apoptosis. Furthermore, we found that metformin activated caspase 3, downregulated caspase 9, and increased the pro-apoptotic protein, Bim. To explore the underlying mechanisms, we observed the role of the PI3K/AKT/mTOR pathway in metformin mediated-antitumor response and found that it decreased the expression of IGF-1R, PI3K, AKT, pAKT, mTOR, and PKM2. IGF-1 partly reversed the antitumor effect of metformin in vitro. To explain determine if the down-regulation of PKM2 is associated with up-regulation of Bim, we depleted the PKM2 gene in ESCCs. We found that knockout PKM2 resulted in activation of caspase 3, downregulation of caspase 9 and increased expression of Bim. In addition, metformin reduced tumor burden in an ESCC xenograft animal model. In conclusion, our results supported that metformin has the ability to inhibit the proliferation of ESCCs, which may lead to suppression of the PI3K/AKT/mTOR signaling pathway.

Materials and Methods

1. Cell culture and regents

Eca109 and EC9706 were provided from American Type Culture Collection. Cells were maintained in 1640 media containing 10% fetal bovine serum in a humidified incubator at 37°C under 5% CO₂.

Metformin was purchased from Sigma (San Francisco, CA). Antibodies against caspase 3, cleaved caspase 3, caspase 9, Bcl-2, Bid, Bim, and PKM2 were purchased from Abcam (Cambridge, MA). IGF-IR, PI3K, AKT, pAKT, mTOR, and p70S6K were purchased from Cell Signaling Technology (Danvers, MA). β-Actin and rabbit secondary antibody were purchased from ZSGB-Bio (Beijing, China). A TUNEL staining In Situ Cell Death Detection Kit was obtained from Promega (Madison, WI). Cell Counting Kit-8 (CCK-8) was purchased from Nan Jing Jian-Cheng Bioengineering (Nanjing, China).

2. CCK-8 viability assay

The inhibitory effect of metformin was assessed by cell viability using CCK-8 assay. Cells were grown in 96-well plates at 5×10⁵ cells per well and cultured overnight at 37°C. Different concentrations of metformin were used to treat cells for 24 hours and 48 hours. The absorbance was determined at 450 nm using microplate reader.

3. Flow cytometric assay

Cells were collected and seeded into 6-well plates at 4×10⁶ cells per well, then maintained for 24 hours. Next, cells were treated with metformin (10 and 20 mM) for 24 hours. Cell cycle and apoptosis were determined by flow cytometry according to the manufacturer’s protocol (Boster, Wuhan, China). The results were indicated as the mean±standard deviation.
4. Western blot

Cells were collected and lysed using RIPA buffer (ZSG-Bio) containing phenylmethanesulfonylfluoride. Total protein was extracted and determined using a BCA Protein Assay Kit. Total protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, then transferred to polyvinylidene fluoride membrane. The first antibodies were added at the proper dilution and incubated at 4°C overnight, after which the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody was added at the appropriate dilution and incubated for 60 minutes. Finally, the bands were examined by enhanced chemiluminescence reagents.

5. Transfection with small interference RNA

The PKM2 small interference RNA (siRNA) and control were obtained from GenePharma (Shanghai, China). Eca109 or EC9706 cells were seeded in a 12-well plate (3×10⁶ cells/well) and transfected with 50 nM siRNA using Lipofectamine 2000 Transfection Reagent (Thermo Fisher Scientific, Waltham, MA). At 48 hours after transfection, the total cell extracts were collected for western blot, which was conducted using the siRNAs listed as follows: PKM2: siRNA 5’-

GGCUUCUUAAAGUUUATT-3’; negative control 5’-UUCUCGAACGUGUCAGUTT-3’.

6. Real-time fluorescent quantitative polymerase chain reaction

Trizol reagent (Bioo Scientific Co., Austin, TX) was used to extract the total RNA from cultured cells (Eca109 and EC9706), β-Actin and Bim mRNA were first reversed to generate a first-strand synthesis of DNA. Real-time fluorescent quantitative polymerase chain reaction (PCR) was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) with the following primers: Bim: forward 5’-TAAGTTCTGATGTGACCGA-GA-3’, reverse 5’-GCTCTGTCGAGGTAGG-3’; β-actin: forward 5’-ATGCCAACAGTGTCTGG-3’, reverse 5’-TACTCCTGCTTGCT ATCCACAT-3’.

7. Tumor xenograft model

Nude mice (6-week old) were used to establish an Eca109 tumor model. First, Eca109 cells were harvested, after which the mice were injected subcutaneously with 5×10⁶ tumor cells in the dorsal area. The mice were then randomly divided into control and treatment groups (5 mice per group) after 7 days.

![Fig. 1](attachment:image_url) Metformin inhibited the proliferation of esophageal cancer cells. Eca109 and EC9706 cells were treated with metformin (0, 5, 10, 20, and 40 mM) for 24 hours and 48 hours. Cell viability was determined by Cell Counting Kit-8. (A) Metformin reduced Eca109 cell viability. (B) Metformin decreased EC9706 cell viability. Data represent the mean±standard deviation of three independent experiments (*p < 0.05, **p < 0.01).
Fig. 2. Metformin induced cell cycle arrest and apoptosis in esophageal cancer cell cells. Eca109 and EC9706 Cells were treated with metformin (10 and 20 mM) for 24 hours. To investigate the cell cycle, cells were stained with propidium iodide (PI) according to the manufacturer’s instructions. For apoptosis determination, cells were analyzed by flow cytometry after staining with Annexin-V/PI according to the manufacturer’s instructions. (A) Typical flow cytometric graph of cell cycle. (B) The percentage of cells in each cell cycle phase. The results showed that metformin blocked cell arrest in the G0/G1 phrase in Eca109 and EC9706. (C) Typical flow cytometric graph of apoptosis. (Continued to the next page)
Next, mice in the treatment group were given metformin (300 mg/kg) via intraperitoneal injection, while those in the control group were administered normal saline (100 μL). Treatments were performed for 21 days, during which time tumor volumes (v=0.52×width²×length) were determined every 3 days.

8. Immunohistochemistry

Tumor tissues were embedded in paraffin, then sectioned (4 μm). Next, sections were deparaffinized, rehydrated with phosphate buffered saline containing H2O2 (3%). Immunostaining was then undertaken using anti-mouse PKM2 (1:100) and Bim (1:100) monoclonal antibody at 4°C. HRP-labeled second antibody was then used to combine with the primary antibody. An HRP-IgG antibody kit (Boster) was employed according to the manufacturer’s instructions and immunostaining results were quantified by counting cells positively stained with PKM2 and Bim in six consecutive and independent fields near the center of each section.

9. Quantitative assessment of apoptosis

Tumor tissues were fixed in 10% formalin, then embedded in paraffin, after which apoptosis was assessed using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; DeadEnd Fluorometric TUNEL System, Promega) according to the manufacturer’s instructions.

10. Statistical analysis

Data were analyzed by t tests conducted using SPSS ver. 17.0 (SPSS Inc., Chicago, IL). A p < 0.05 was considered to indicate significance.

Results

1. Metformin inhibited the proliferation of ESCCs

Eca109 and EC9706 cells were treated with different concentrations of metformin (0, 5, 10, 20, and 40 mM) for 24 hours and 48 hours. CCK-8 assay was used to determine the cell viability. The results showed that metformin inhibited the proliferation of ESCCs in a time and dose-dependent manner (Fig. 1A and B).
2. Metformin induced G0/G1 phase cell cycle arrest and apoptosis

To explore the possible mechanisms through which metformin suppressed the growth of ESCC, cell cycle and apoptosis were determined by flow cytometry after metformin treatment. When compared to the control, G0/G1 cells had accumulated (Fig. 2A and B) and apoptosis cells had increased in the experimental group (Fig. 2C and D).

The apoptosis-related proteins expression were detected
Fig. 4. IGF partly reversed the effect of metformin-induced apoptosis and attenuated the repression action of metformin toward PI3K, pAKT, mTOR, p70S6K, and PKM2 in esophageal cancer cells. Eca109 and EC9706 cells were pre-treated with IGF-1 (100 ng/mL) for 2 hours, then with metformin for 24 hours. Apoptosis was determined by flow cytometry. (A) Typical flow cytometric graph of apoptosis. (B) The average percentage of apoptosis in Eca109 and EC9706 cells after treatment with metformin. The results showed that metformin partly prevented the metformin-induced apoptosis. (C) The expression of PI3K, pAKT, mTOR, p70S6K, and PKM2 was detected by western blot. The outcome indicates that IGF-1 attenuates the repression action of metformin toward PI3K, pAKT, mTOR, p70S6K, and PKM2 in Eca109 and EC9706 cells (*p < 0.05 and **p < 0.01 compared to control, n=3). PI3K, phosphoinositide 3-kinase; pAKT, phosphorylation of AKT; PKM2, pyruvate kinase muscle isozyme 2; IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; PI, propidium iodide; Met, metformin; I+M, insulin-like growth factors+metformin; FITC, fluorescein isothiocyanate.
Fig. 5. Knockdown of PKM2 altered the expression of apoptosis-related proteins. Eca109 or EC9706 cells (3×10^3 cells/well) were plated in a 12-well plate. PKM2 was knocked down in Eca109 and EC9706 cells by siRNA (30 nM final concentration for both PKM2-specific and NC siRNA). At 48 hours after transfection, the whole cell extracts were collected for western blot. Total RNA was extracted from cultured cells (Eca109 and EC9706) using Trizol reagent according to the manufacturer’s instructions. β-Actin and Bim mRNA were quantified in duplicate by SYBR Green 2-step, real-time reverse transcription polymerase chain reaction. (A) Caspase 9, caspase 3, cleaved caspase 3, Bcl-2, and Bim were detected by western blot. The results showed that knockout PKM2 activated caspase 3 and downregulated caspase 9. Bim protein expression was evidently increased. (B) Bim mRNA level was increased in depletion PKM2 esophageal cancer cells. PKM2, pyruvate kinase muscle isozyme 2; NC, normal control.

by western blot. The results clearly showed that metformin activated caspase 3, down-regulated caspase 9, and up-regulated Bim. Bcl-2 and Bid remained unchanged (Fig. 2E).

3. Metformin suppressed PI3K/AKT/mTOR signaling pathway

To examine the potential molecular mechanisms for metformin antitumor effects, we detected the expression of IGF-IR, PI3K, AKT, pAKT, mTOR, p70s6k, and PKM2 by western blot. The results revealed that metformin reduced IGF-IR, PI3K, AKT, pAKT, mTOR, p70s6k, and PKM2 in EC109 and EC9706 cells (Fig. 3A and B).

4. IGF partly reversed the effect of metformin-induced apoptosis and attenuated the repression effect of metformin on the PI3K/AKT/mTOR/PKM2 pathway

To determine whether the effects of metformin mediated-apoptosis on Eca109 and EC9706 cells were associated with the IGF-IR/PI3K/AKT/mTOR pathway, insulin growth factor (IGF) treatment (100 ng/mL) was carried out 2 hours before metformin was added, after which apoptosis was detected by flow cytometry. The results showed that IGF partly reversed the effects of metformin-induced apoptosis in Eca109 and EC9706 (Fig. 4A and B). Furthermore, IGF attenuated the repression effect of metformin on PI3K, pAKT, mTOR, and PKM2 (Fig. 4C). These results suggest that the effects of metformin-induced apoptosis may be connected with the PI3K/AKT/mTOR/PKM2 signaling pathway.

5. Knockdown PKM2 altered apoptosis-related protein expression

To explain the relationship between the PI3K/AKT/mTOR/PKM2 signaling pathway and apoptosis, we depleted PKM2 to detect the expression of apoptosis-related proteins in Eca109 and EC9706. Knockdown of PKM2 activated
Fig. 6. Metformin inhibited tumor growth in vivo. Mice were treated with NS or metformin (300 mg/kg/day) for 21 days at the same time (n=5 per group). (A) Suppressed growth of tumor in mice. The results showed that metformin inhibited tumor growth (n=5, p < 0.01). Data are shown as the mean±standard deviation. (B) Representation of photos of tumors from mice bearing Eca109 receiving different treatments. Mice were sacrificed when they were moribund. (C) Typical graph of immunohistochemical staining of PKM2 and Bim in tumor tissues (×400). (D) Quantification of the number of PKM2 and Bim positive cells per field. Metformin treatment significantly decreased the number of PKM2-positive cells and increased the number of Bim-positive cells. (E) Typical sections from tumor tissue. (F) Apoptotic index within tissues. Metformin showed a significant increase of apoptotic cells in tumor tissues compared to the control (**p < 0.001). Data are expressed as the mean apoptotic index±standard deviation of cancer cells. NS, normal saline; PKM2, pyruvate kinase muscle isozyme 2; Met, metformin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.
caspase 3 and down-regulated the expression of caspase 9. Bim was notably upregulated and the Bim mRNA level was increased by PKM2-specific siRNA (Fig. 5). These results were consistent with the change in apoptosis-related proteins caused by metformin. Based on our findings, metformin increased the expression of the pro-apoptosis protein Bim, partly due to the suppression of the PI3K/AKT/mTOR/PKM2 signaling pathway.

6. Antitumor effect of metformin in vivo

To further assess the effects of metformin on ESCC proliferation in vivo, an Eca109 tumor model was established in nude mice. We found that metformin treatment decreased the tumor burden and that the inhibition rate reached up to 48.41% in the experimental group when compared with the control (Fig. 6A and B). Furthermore, we found that metformin reduced the expression of PKM2 and increased the expression of Bim in the tumor tissue (Fig. 6C and D). Moreover, evaluation of apoptosis by TUNEL staining revealed that there were more apoptotic cells in the experimental group than the control (Fig. 6E and F). Overall, these results indicate that increasing apoptosis may be related to the antitumor effect of metformin in the Eca109 model.

Discussion

In this study, we investigated the antitumor effects of metformin on ESCCs in vitro and in vivo. The results showed that metformin decreased the cell viability (Fig. 1) and induced cell cycle arrest and apoptosis (Fig. 2). Furthermore, metformin activated caspase 3, decreased caspase 9, and increased the expression of Bim. Potential mechanisms of antitumor activity have been investigated, and the results have shown that they may be associated with inhibition of the PI3K/AKT/mTOR/PKM2 signaling pathway (Fig. 3). IGF partly reversed the effects of metformin-mediated apoptosis, further confirming that metformin exerts its anti-tumor effects in connection with the PI3K/AKT/mTOR/PKM2 signaling pathway (Fig. 4). To explore the association between suppressing PI3K/AKT/mTOR/PKM2 and apoptosis, apoptosis-related proteins were detected after knockdown of PKM2 in Eca109 and EC9706 cells. We found that PKM2 depletion activated caspase 3 and reduced caspase 9. Bim protein expression was substantially increased and Bim mRNA level was up-regulated after PKM2 depletion (Fig. 5). In vivo, we found that metformin reduced tumor burden (Fig. 6A and B), and that this was accompanied with down-regulation of PKM2 and up-regulation of Bim in the tumor tissue (Fig. 6C and D). To the best of our knowledge, this is the first study to demonstrate that the PI3K/AKT/mTOR/PKM2 signaling pathway is a target of metformin in human ESCCs.

Although previous studies [26,27] showed that metformin inhibited proliferation of ESCC, the mechanisms responsible for this effect remained controversial. Currently, there are two main theories regarding that the mechanism through which metformin exerts its effects. In one, its effects are believed to occur through AMPK dependent and AMPK independent mechanisms under different cellular settings. It has been suggested [26,27] that metformin affects the AMP/ATP balance and active AMPK, which could negatively regulate mTOR signaling, further inhibiting protein synthesis and regulating the phosphorylation of different proteins. The other possibility [28,29] is that antitumor effects are linked to the ability of metformin to inhibit transcription of key gluconeogenesis genes in the liver and stimulate glucose uptake in muscle, increasing insulin sensitivity and reducing blood glucose while lowering the insulin level. A recent report [30] showed that metformin promoted autophagy and apoptosis in ESCC by down-regulating the STAT3 signaling pathway.

In the present study, we corroborate and extend the results of previous studies. We mainly investigated whether metformin affects the I/IGF pathway to inhibit the proliferation of ESCCs. Overexpression of IGF-1R [20,31] has been reported in human esophageal carcinoma cells. Specifically, overexpression can be activated by IGF-1 or IGF-2, which induces the PI3K/AKT/mTOR signaling pathway, resulting in survival and proliferation in various cancer cells. Thus, inhibition of IGF-1R [21] may be effectively block tumor growth in human ESCC. Our results verify that metformin down-regulates the expression of IGF-1R, which suppresses the PI3K/AKT/mTOR/PKM2 pathway in ESCCs. IGF can partly block metformin-induced anti-tumor effects and attenuate the repression action of metformin toward PI3K, pAKT, mTOR, p70S6K, and PKM2. PKM2, a downstream molecule of PI3K/Akt/mTOR signaling pathway, plays a crucial role in regulation of the metabolic fate of glycolytic intermediates for cancer cells [32]. Knockdown of PKM2 [33] caused increased apoptosis in many cancer cell lines. Inhibition of PI3K/AKT/mTOR down-regulates PKM2 expression and suppresses cancer metabolism, which has been confirmed in other various cancer cell lines. We found that metformin decreased the expression of PKM2 in esophageal cancer, suggesting that reducing PKM2 expression represents a general mechanism of metformin cytotoxicity, not limited for gastric cancer [34] and breast cancer [35]. In order to explain the association between PKM2 and apoptosis, we found knockdown PKM2 activated caspase 3 and down-regulated caspase 9. The pro-apoptosis protein Bim increased
after PKM2 depletion. Overall, our results revealed that induction of apoptosis by metformin may be associated with down-regulation of PKM2 in human ESCC cells.

We further confirmed that metformin decreased the tumor burden in vivo, showing that metformin treatment may be effective against ESCC. Following metformin treatment, down-regulation PKM2 and up-regulation Bim were consistent with the previous in vitro results. We also observed that metformin induced tumor cells apoptosis. Most importantly, there were no significant adverse effects in the metformin-treated mice. These findings indicate that metformin has potential antitumor effects in human ESCC.

However, it should be noted that the present study has some limitations. Specifically, we are not sure how metformin affects the expression of PKM2. It may bind PKM2 directly or indirectly influence the expression of PKM2; therefore, further study is needed. Moreover, the high dose of metformin used in this experimental setting may not be easy to transform to a clinical application. Accordingly, more clinical trials are needed to confirm the anti-tumor effects of metformin.

Conclusion

In conclusion, our results confirm that metformin inhibits the proliferation of ESCCs in vitro and vivo. These effects may be attributed to metformin inducing cell cycle arrest and apoptosis. The molecular mechanisms are connected with inhibition of the PI3K/AKT/mTOR/PKM2 signaling pathway. Our results support that metformin may become a novel and effective therapeutic agent for the treatment of esophageal cancer.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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p15^{ink4b} Loss of Expression by Promoter Hypermethylation Adds to Leukemogenesis and Confers a Poor Prognosis in Acute Promyelocytic Leukemia Patients

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Purpose
The p15^{ink4b} gene exerts its influence as an inhibitor of cyclin-dependent kinases and is frequently associated with hematological malignancies. Inactivation of this gene through DNA methylation has been found to be the most prevalent epigenetic alteration reported, with a high frequency in all French-American-British subtypes of acute myeloid leukemias, including acute promyelocytic leukemia (APL). In this study, we investigated the prognostic significance of p15 gene promoter hypermethylation and its expression in APL patients of Kashmir (North India).

Materials and Methods
p15 gene promoter hypermethylation was conducted by methylation-specific polymerase chain reaction, while its subsequent expression analysis was carried out by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR).

Results
Of the 37 patients, 16 (43.2%) were found to have methylated p15 genes. Of these 16 cases, seven (43.8%) were methylated partially and nine (56.2%) were found to have complete methylation. Moreover, nine of the 37 patients (24.3%) who presented with leukocytosis at their baseline had complete p15 gene methylation as well (p < 0.05). Semi-quantitative RT-PCR showed a complete loss of p15 expression in nine patients with complete methylation coupled with leukocytosis (p=0.031), while seven patients with partial methylation showed decreased p15 expression. Six patients relapsed during the maintenance phase of treatment and were found to have a completely methylated p15 gene and no p15 mRNA.

Conclusion
Complete methylation and loss of p15 gene expression causes susceptibility to relapse and decreased survival in APL patients. Thus, p15 promoter hypermethylation is a prospective prognostic indicator and a reliable clinical aid in assessment of patients with APL.

Key words
p15^{ink4b}, Acute promyelocytic leukemia, Arsenic tri-oxide, Leukocytosis, Promoter hypermethylation, Kashmir

Introduction

Acute promyelocytic leukemia (APL), which is known as acute myeloid leukemia-3, AML-3, or M3 according to the French-American-British (FAB) classification, is characterized by a predominance of malignant promyelocytes that carry a reciprocal translocation between the long arms of chromosomes 15 and 17: t(15;17)(q22;q11.2-q12) that results in the formation of a hybrid gene, PML-retinoic acid receptor α (RARα). The fusion protein PML-RARα has been shown to recruit methyltransferases on the CpG islands of the promoter region of the retinoic acid receptor RARα, resulting in the hypermethylation mediated silencing of RARα in APL cells [1]. In addition to the hypermethylation mediated silencing of the RARα gene, many tumor suppressor genes...
have been extensively reported in APL, particularly the p15 gene. p15\textsuperscript{ink4b} (p15) is a tumor suppressor gene located at 9p21, which encodes for p15 cyclin-dependent kinase inhibitor. The 9p21 chromosomal locus, which is referred to as the INK4/ARF locus, has been tightly linked to the formation of many types of tumors [2], p15 belongs to the INK4 kinase family of cyclin-dependent kinase inhibitors (which consists of p15, p16, p19, and p21). These inhibitors negatively regulate the cell cycle through competitive inhibition of the cyclin-dependent kinases 4 and 6 involved in retinoblastoma (Rb)-dependent cell cycle regulation. p15\textsuperscript{ink4b} expression has been shown to increase specifically during myeloid differentiation in vivo in both human bone marrow and peripheral blood cells [3]. The role of p15\textsuperscript{ink4b} during myeloid differentiation was further supported by in vitro studies of the M1 leukemia cell line. When terminal differentiation is induced in these cells, upregulation of p15\textsuperscript{ink4b} expression is accompanied by inhibition of CDK4 kinase activity and a decrease in levels of phosphorylated Rb [4]. Furthermore, overexpression of p15\textsuperscript{ink4b} in M1 cells causes cell cycle arrest in the G1 phase, providing additional evidence that the protein is involved in maturation and cell cycle inhibition of late stage progenitors [5]. This function is further supported by studies in human CD34\textsuperscript+ hematopoietic progenitor cells. When expression of p15\textsuperscript{ink4b} is triggered, higher levels of the protein are associated with transcriptional up regulation of genes known to induce myeloid differentiation. Studies conducted by Teofili et al. [6] suggested that p15 plays an important role in regulation of the proliferative activity of promyelocytes. Unlike other tumor suppressor genes, the p15 gene is preferentially hypermethylated at a 5\textquotesingle-CpG island, which has been shown to be associated with loss of transcription of this gene in leukemia cells [7,8].

In the present study, we attempted to evaluate the promotor hypermethylation of the p15 gene in newly diagnosed and relapsed APL patients treated with conventional chemotherapy protocols International Consortium on Acute Promyelocytic Leukemia-2006 (ICAPL-2006) and Arsenic Tri-Oxide (ATO) protocols. Moreover, mRNA expression of the p15 gene was analyzed using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) and correlated with p15 gene methylation and various clinico-pathological parameters.

Materials and Methods

A total of 37 APL cases were referred to the Department of Immunology and Molecular Medicine, Sher-I-Kashmir Insti-
tute of Medical Sciences (SKIMS), Srinagar (Jammu and Kashmir, India) from July 2013 to November 2015. We analyzed the course of all newly diagnosed and relapsed patients with APL treated consecutively on protocols (ICAPL-2006 and ATO) in the Department of Clinical Hematology and Medical Oncology at SKIMS. Patients were included in the study after approval from the ‘Institute Ethics Committee’ (IEC) of SKIMS and subjected to prospective evaluation of their response to conventional chemotherapy (ICAPL-2006 and ATO). All patients received baseline bone marrow analysis and were categorized against the morphologic criteria for the diagnosis of APL (AML-M3 or M3-variant) according to the FAB classification system [9]. About 5-6 mL of peripheral blood were collected from 37 newly diagnosed and relapsed patients into EDTA vials for methylation-specific polymerase chain reaction (MS-PCR) and RT-PCR after informed consent. The diagnosis was confirmed at our department by the presence of t(15;17) on peripheral blood cytogenetic studies, as well as detection of PML-RARA translocation by RT-PCR and quantitative PCR (q-PCR). Subsequently, blood samples were collected from healthy control subjects who did not harbor any hematological malignancies. The median age of the patients was 31 years (range, 6 to 92 years).

1. DNA extraction

Blood samples from each patient were diluted with equal volumes of RPMI-1640 cell culture media and subjected to density gradient centrifugation using Ficol Histopaque as density gradient material. Isolated white blood cells were equally distributed into two tubes for DNA and RNA extraction.

High molecular weight DNA was extracted from Ficol Histopaque isolated cells from patient samples and healthy control samples using the phenol-chloroform extraction protocol. The quality of extracted DNA was checked on 1% agarose gel and the concentration of the DNA obtained was measured in a spectrophotometer at 260 nm.

2. DNA methylation assay

MS-PCR was carried out according to the method described by Chim et al. [10]. Extracted DNA was subjected to bisulphite conversion using an EZ DNA Methylation kit from Zymo Research (Irvine, CA). MS-PCR analysis of P15 gene was carried out for its promoter using primer sets F: 5’-CGTGTTCTATTTTGGGTTT-3’ and R: 5’-CTAAGCCATTTCTTAGCCCC-3’ for methylated allele and F: 5’-TCTTGATTGCTGTCTTTTGGTT-3’ and R: 5’-CCATAAAACCCAAAACCC-3’ for unmethylated allele. Approximately 2.0 μL of the bisulphite converted DNA (nor-
4. cDNA Synthesis

A Maxima cDNA synthesis kit (Thermo Scientific, Waltham, MA) containing enzyme mix (M-MuLV RT enzyme and Ribolock RNase inhibitor), 5× reaction mix (reaction buffer, dNTPs, oligo(dt)20, random hexamer primers, and nuclease-free water) was used to perform the reverse transcriptase reaction.

Two micrograms of total cellular RNA were reverse transcribed to cDNA by incubation for 10 minutes at 25°C, 15 minutes at 50°C, and 5 minutes at 85°C in a total volume of 20 µL that contained 4 µL of 5× reaction mix, 2 µL of maxima enzyme mix and 12 µL of nuclease free water.

5. RT-PCR analysis

Multiplex PCR was carried out in a 25 µL reaction mixture using reverse and forward primers sets for p15 and β-actin. The primers used for p15 were F: 5′-TGGGG CGGAGGATGAG-3′ and R: 5′-AGGGGTGCCTTGGAAAT-3′, while for β-actin a primer set of F: 5′-TGACGGGTACCCACACTCT-3′ and R: 5′-CTAGAGCTTTGGTGACG-3′ was used. Briefly, 2 µL of cDNA was amplified in a 25 µL reaction containing (Taq polymerase, Taq buffer, MgCl2, and dNTPs). cDNA synthesized from the RNA of healthy control subjects were used as a positive control, water was used as a negative control and β-actin served as an internal control. Reaction times consisted of an initial denaturation of 94°C for 5 minutes, annealing at 54°C for 30 seconds, and elongation at 72°C for 7 minutes, followed by 35 additional cycles (30 seconds at 94°C, 30 seconds at 54°C, and 30 seconds at 72°C). Next, 6-8 µL of the PCR product was size-fractionated by electrophoresis in 2% agarose gel stained with ethidium bromide and visualized under a UV trans-illuminator (Flourchem, HD2-Cell Biosciences, Santa Clara, CA) at 365 nm. Primer sequences of p15 mRNA amplified a 450 bp product; whereas a product of 680 bp was amplified with the primer set for β-actin mRNA.

Results

The present study included a total of 37 APL patients comprising 22 males (59.5%) and 15 females (40.5%) ranging in age from 6 years to 91 years. Overall, 25 patients (67.6%) were <30 years of age and 12 (32.4%) were ≥30 years of age (Table 1). Based on risk stratification, patients were classified as low risk, high risk or intermediate risk according to the total leukocyte count and platelet counts at presentation. Nine of the 37 patients (24.3%) presented with leukocytosis
Table 1. Clinicopathological parameters of 37 APL patients at diagnosis according to the p15 methylation state

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
<th>Methylated</th>
<th>Methylated/Unmethylated</th>
<th>Unmethylated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>37 (100)</td>
<td>9 (24.3)</td>
<td>7 (18.9)</td>
<td>21 (56.7)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 30</td>
<td>12 (32.4)</td>
<td>1 (8.3)</td>
<td>2 (16.7)</td>
<td>9 (75.0)</td>
<td>0.741</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>25 (67.6)</td>
<td>8 (32.0)</td>
<td>5 (20.0)</td>
<td>12 (48.0)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22 (59.5)</td>
<td>6 (27.3)</td>
<td>3 (13.6)</td>
<td>13 (59.1)</td>
<td>0.021</td>
</tr>
<tr>
<td>Female</td>
<td>15 (40.5)</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
<td>8 (53.3)</td>
<td></td>
</tr>
<tr>
<td>TLC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1×10^11/L</td>
<td>9 (24.3)</td>
<td>9 (100)</td>
<td>0</td>
<td>0</td>
<td>0.031</td>
</tr>
<tr>
<td>≤ 1×10^11/L</td>
<td>28 (75.7)</td>
<td>0</td>
<td>7 (25.0)</td>
<td>21 (75.0)</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 4×10^10/L</td>
<td>7 (19.0)</td>
<td>1 (14.3)</td>
<td>2 (28.6)</td>
<td>4 (57.1)</td>
<td>0.689</td>
</tr>
<tr>
<td>&lt; 4×10^10/L</td>
<td>30 (81.0)</td>
<td>8 (26.7)</td>
<td>5 (16.6)</td>
<td>17 (56.7)</td>
<td></td>
</tr>
<tr>
<td>Promyelos in BM</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&gt; 60%</td>
<td>31 (83.8)</td>
<td>9 (29.0)</td>
<td>5 (16.1)</td>
<td>17 (54.9)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>≤ 60%</td>
<td>6 (16.2)</td>
<td>0</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Karyotyping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(15;17) positive</td>
<td>30 (81.0)</td>
<td>9 (30.0)</td>
<td>6 (20.0)</td>
<td>15 (50.0)</td>
<td>&gt; 0.990</td>
</tr>
<tr>
<td>t(15;17) negative</td>
<td>7 (19.0)</td>
<td>0</td>
<td>1 (14.3)</td>
<td>6 (85.7)</td>
<td></td>
</tr>
<tr>
<td>Transcript types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bcr-1</td>
<td>19 (51.4)</td>
<td>4 (21.0)</td>
<td>2 (10.5)</td>
<td>13 (68.5)</td>
<td>0.093</td>
</tr>
<tr>
<td>bcr-2</td>
<td>3 (8.1)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td></td>
</tr>
<tr>
<td>bcr-3</td>
<td>15 (40.5)</td>
<td>4 (26.7)</td>
<td>4 (26.7)</td>
<td>7 (46.6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). APL, acute promyelocytic leukemia; TLC, total leukocyte count; BM, bone marrow.

(> 1×10^11/L), whereas the remaining 28 patients (75.7%) had leucopenia (< 1×10^11/L). High platelet counts (> 4×10^10/L) were detected in seven patients (19.0%), while 30 patients (81.0%) presented with low platelet counts (< 4×10^10/L). Bone marrow examination revealed six patients (16.2%) with less than 60% promyelocytes and 30 (83.8%) with a promyelocyte count greater than 60% (Table 1).

Among 22 male patients, nine (41.0%) were found to have hypermethylation and 13 (59.1%) were unmethylated, while among 15 female patients, seven (46.7%) had p15 gene methylation and eight (53.3%) were unmethylated patients. Moreover, p15 methylation status differed significantly between males and females (p=0.021).

Overall, 16 patients were found to have methylated p15 genes in which seven patients were methylated partially and nine had complete methylation based on the presence of either two or a single band, respectively, upon MS-PCR analysis. The remaining 21 patients were observed to be negative for p15 gene methylation, as depicted by the absence of a methylated band upon MS-PCR analysis (Fig. 1). Moreover, the p15 gene methylation status in APL patients was found to be significantly associated (p=0.031). The discrimination of unmethylated and methylated DNA was explored by MS-PCR in healthy control subjects in which amplification of unmethylated DNA was only seen as depicted by the presence of a specific DNA band with unmethylated primers.

Interestingly, all nine of the 37 patients (24.3%) who presented with leukocytosis at their baseline (> 1×10^11/L) had complete p15 gene methylation, while among the remaining 28 patients with leucopenia (< 1×10^11/L), seven (18.9%) had partial methylation of the p15 gene at baseline. This difference between groups was significantly associated (p < 0.05). The other clinicopathological parameters and their correlation with p15 gene methylation status are given in Table 1.

This observation was further supported by the semi-quantitative expression of the p15 gene observed upon RT-PCR analysis of samples from the same series of 37 APL patients. We found that no p15 gene mRNA was expressed in nine patients who presented with leukocytosis coupled with complete p15 methylation (M group). In comparison, p15 mRNA was expressed in all patients in whom p15 alleles were either partially methylated (U/M group) or were unmethylated (U
Table 2. p15 mRNA expression by semi-quantitative RT-PCR in APL patients at diagnosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Methylated</th>
<th>Methylated/Unmethylated</th>
<th>Unmethylated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>p15 positive</td>
<td>28</td>
<td>0</td>
<td>7 (25.0)</td>
<td>21 (75.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>p15 negative</td>
<td>9</td>
<td>9 (100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as number (%). RT-PCR, reverse transcriptase polymerase chain reaction; APL, acute promyelocytic leukemia.

Fig. 2. Kaplan-Meier overall survival (A) and disease-free survival (B) plots of acute promyelocytic leukemia patients according to the methylation status of the p15 promoter region.

group). Similarly healthy control subjects showed a consistent pattern of unmethylation in the p15 gene and their mRNA was found to be intact Table 2. Thus, p15 gene silencing was found exclusively in patients with high leukocyte counts in addition to complete methylation of both p15 alleles (p=0.031).

All 37 patients were treated with all-trans retinoic acid in addition to conventional chemotherapy (ICAPl-2006). During the course of the study, eight patients expired. Among these, six patients (four from group M and two from group U) died during the induction phase of treatment, while two patients (from M group) relapsed and expired during the maintenance stage of chemotherapy.

Overall, 31 patients were followed during the course of our study. All patients achieved bone marrow remission by the end of the consolidation phase of chemotherapy as confirmed by their negative q-PCR and RT-PCR status for PML-RARA. However, during the maintenance stage of chemotherapy, six patients (four from M group and one each from the U/M and U groups) relapsed. High leukocyte count was observed in all six relapsed cases who were subsequently subjected to treatment with arsenic trioxide. Upon follow-up evaluation of the p15 methylation status and p15 mRNA expression during maintenance, we found that all six relapsed patients had complete p15 methylation, while two patients with complete methylation status belonged to the U and U/M groups at baseline. These findings indicate the poor prognostic implication of acquired complete methylation. The loss of p15 mRNA expression was subsequently seen in all six of the relapsed cases. The only remaining patient from M group and patients from U and U/M groups who achieved remission had no p15 methylation, and p15 mRNA was found intact in these patients. Of the six relapse patients, two complete methylation expired because of cerebral hemorrhage.

Multivariate analysis was performed to determine if complete p15 methylation is an independent poor prognosis factor in APL patients. We observed that the group M patients had an inferior survival (hazard ratio, 8.60; 95% confidence interval [CI], 1.7 to 43.0; p=0.009) compounded with an
increased risk of relapse (hazard ratio, 11.5; 95% CI, 1.9 to 135.4; p=0.050) relative to group U. In contrast, comparison of group M to group U/M revealed no significant difference in survival or risk of relapse. Therefore, the detection of complete p15 methylation status was found to be an independent poor prognosis factor for group M relative to group U that was not influenced by any other tested variables (e.g., age, sex, WBC count, platelet counts, treatment, and PML/RARα break point type).

Clinical outcome according to the pattern of p15 methylation and its mRNA expression was analyzed in APL patients in terms of their overall survival (OS) and disease-free survival (DFS). Patients were followed for a mean duration of 19.1 months ranging from 0 to 34 months and Kaplan-Meier survival analysis was performed to assess the OS of all 37 patients and the DFS of 31 patients who achieved clinical remission. Comparison of survival between patient groups was based on the log-rank test. The OS for groups M, U/M, and U patients was 38%, 100%, and 90%, respectively. Patients from group M showed the lowest OS, while those in group U/M showed a higher OS, but this difference was not significant (p=0.295) (Fig. 2A). We observed a significant difference in DFS (log-rank test, p=0.022), which was estimated to be 22%, 83%, and 93% in patients from groups M, M/U, and U, respectively (Fig. 2B). Moreover, patients from group M showed the lowest DFS (16.5 months).

An OS of 38% and 91% was observed in p15-negative and p15-positive patients, respectively. Patients who did not express p15 mRNA showed a lower mean OS of 19.2 months as compared to 30.3 months in patients who expressed p15 mRNA. The difference in OS between two groups was significant based on the log-rank value (p=0.031) (Fig. 3A). The DFS for p15-negative patients was lower than that of p15-positive patients (22% and 89%, respectively; log-rank test: p=0.004), with a mean DFS time of 29.6 months for p15-negative and 34.0 months for p15-positive patients (Fig. 3B).

**Discussion**

Promoter hypermethylation of p15\(^{15\text{th} 46}\) causes its silencing almost exclusively in cancers of the hematopoietic system, and is observed in acute leukemias of myeloid (AML) and lymphoid origins [11]. Aberrant hypermethylation occurs at the CpG islands of the gene, which extend throughout the promoter region, exon 1, and part of intron 1 [12]. Various studies have reported hypermethylation of p15 with a high frequency in all FAB subtypes of AMLs [13]. However, few studies have been conducted to specifically investigate p15 methylation in APL. In our study, which is first from India, we investigated the pattern of p15 promoter hypermethylation in APL patients and its subsequent expression by RT-PCR. The results revealed overall hypermethylation of the p15 gene in 45% of APL patients, which is marginally in agreement with the results of a previous study conducted by Teofili et al. [14], who reported that 52% of cases involved hypermethylation. Our results differ from another study by Chin et al. [15], who observed hypermethylation in 79% of cases. Aberrant methylation of p15\(^{15\text{th} 46}\) has been reported in up to 80% of patients with primary and secondary AML [16,17]. Although these studies vary slightly in terms of the
frequency of p15 hypermethylation, they all indicate that this gene is actively involved in the leukemogenesis of APL. Moreover, aberrant p15<sup>msh</sup> methylation levels have been associated with a generally poor prognosis in many forms of the disease. In contrast to Teofili et al. [14], who found no clinical variables showing any significant association with the pattern of methylation, our study noted a significant association of p15 hypermethylation with the gender of the APL patients (p=0.021) and leukocyte count at baseline (p=0.031). Interestingly, all nine patients (100%) who presented with leukocytosis at baseline (>1×10<sup>10</sup>/L) were found to harbor p15 gene methylation (p=0.031). These findings differ from those reported by Teofili et al. [14], who found p15 gene methylation in 58% of patients. Therefore, this study points towards a strong link between leukocytosis, which is generally considered a poor prognostic indicator in APL [18,19], and the pattern of p15 methylation in APL cases.

Apart from complete methylation (M group) and absence of methylation (U group), 19% of patients showed partial methylation of p15 (U/M group). A similar pattern was observed by Teofili et al. [14], although they reported a partial methylation pattern in more patients (41.0%) than in our study. Partial methylation of the p15 gene is expected to cause incomplete transcription of in which only the unmethylated part contributes to the expression or mRNA formation. Therefore, p15 mRNA expression was evaluated by semi-quantitative RT-PCR in all patients to explore the pattern of methylation and silencing of the p15 gene.

All the cases from group M patients, who reported complete methylation (9/9), showed a lack of p15 mRNA expression. Additionally, complete expression was observed in patients having fully unmethylated DNA. In the group U/M patients, who had partially methylated DNA, a differential pattern of p15 mRNA expression was observed as shown by the differential intensity of RT-PCR bands for mRNA. All seven patients with partial methylated DNA showed low intensity mRNA bands against those patients with no methylation and/or the control.

The loss of p15 expression in group M confirms the findings of the previous study by Teofili et al. [14]. All patients who were fully methylated showed loss of expression, which differs from the results of a study by Preisler et al. [20], who found that the presence of p15 methylation in leukemic cells is not always associated with the lack of p15 expression and that p15 expression is not always observed in the absence of methylation. p15 loss of expression was observed in nine patients with leukocytosis who had already been found with complete methylation (p=0.031), which is in stark contrast with the results of a previous study [14] in which leukocytosis did not show a significant relationship with methylation or expression of p15.

The prognostic and clinical significance of p15 promoter hypermethylation and subsequent gene silencing caused by it was evaluated in terms of the three year OS and DFS durations by the Kaplan-Meier method. Comparisons of the OS and DFS between patient groups were based on the log-rank test. There was no significant difference between the OS of M, U/M, and U groups patients (38%, 100%, and 90%, respectively; log-rank test p=0.295), with patients from group M showing the lowest survival of 19.2 months (Fig. 2A). Significant DFS (log-rank test p=0.022) (Fig. 2B) was associated with different patterns of p15 gene hypermethylation in patients who achieved clinical remission. Group M patients showed a lower DFS (22%) than groups M/U and U patients (83% and 93%, respectively). Similar results were reported by Teofili et al. [14], who found a significant 5 years DFS rate of 29%, 64%, and 79% for M, M/U, and U patients respectively, with OS values of 69%, 71%, and 85%, respectively. These findings are concordant with the OS values of 38% and 90% observed for groups M and U, respectively, in the present study. With regard to the U/M group, our OS was 100% when compared to the corresponding OS of 71% of Teofili et al. [14]. These findings can be attributed to the smaller sample size and lesser follow-up duration of our study. Further, our finding of decreased overall and DFS in APL patients with complete methylation is similar to the results reported by Chim et al. [15], who reported a high incidence of p15 methylation in patients with APL and demonstrated that the 5-year DFS of patients with abnormal methylation of p15 was significantly inferior to that of patients without p15 methylation.

A significant 3-year OS of 38% and 91% (log-rank p=0.031) (Fig. 3A) and DFS of 22% and 89%, respectively (log-rank p=0.004) was observed in p15-negative and p15-positive patients, respectively (Fig. 3B). These results are in agreement with those reported by Teofili et al. [14], who reported significant 5-year estimated DFS values (24% and 66%) for p15-negative and -positive patients, respectively. However, they found no significance difference in the OS of p15-negative and positive patients (OS, 74% and 85%, respectively). Our findings are also in agreement with many previous studies of patients with all AML FAB subtypes, in which individuals without p15<sup>msh</sup> hypermethylation at diagnosis had increased complete remission rates that also correlated with increased survival times [21,22]. While our study indicates p15 as a valid potential prognostic marker in APL, these findings need to be validated in larger series of samples.

We concluded that hypermethylation of p15 gene coupled with its loss of mRNA expression causes susceptibility to patients with APL which leads to their decreased survival. Thus, p15 promoter hypermethylation is a prospective prognostic indicator and a reliable clinical aid in assessment of patients with APL.
Conflicts of Interest

Conflict of interest relevant to this article was not reported.

References

Trends in Participation Rates for the National Cancer Screening Program in Korea, 2002-2012

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Purpose
The National Cancer Screening Program (NCSP) in Korea supports cancer screening for stomach, liver, colorectal, breast, and cervical cancer. This study was conducted to assess trends in participation rates among Korean men and women invited to undergo screening via the NCSP as part of an effort to guide future implementation of the program in Korea.

Materials and Methods
Data from the NCSP for 2002 to 2012 were used to calculate annual participation rates with 95% confidence intervals (CI) by sex, insurance status, and age group for stomach, liver, colorectal, breast, and cervical cancer screening.

Results
In 2012, participation rates for stomach, liver, colorectal, breast, and cervical cancer screening were 47.3%, 25.0%, 39.5%, 51.9%, and 40.9%, respectively. The participation rates increased annually by 4.3% (95% CI, 4.0 to 4.6) for stomach cancer, 3.3% (95% CI, 2.5 to 4.1) for liver cancer, 4.1% (95% CI, 3.2 to 5.0) for colorectal cancer, 4.6% (95% CI, 4.1 to 5.0) for breast cancer, and 0.9% (95% CI, −0.7 to 2.5) for cervical cancer from 2002 to 2012.

Conclusion
Participant rates for the NCSP for the five above-mentioned cancers increased annually from 2002 to 2012.

Key words
Neoplasms, Early detection of cancer, Mass screening

Introduction
Cancer is a leading cause of death, and cancer burden is expected to grow worldwide due to aging populations. For 2012, GLOBOCAN reported 14.1 million new cancer cases and 8.2 million deaths from cancer worldwide [1]. In Korea, cancer has remained the leading cause of death since 1983, when statistics first began to be collected [2]. Over the past 14 years, overall incidence rates have increased by 3.3% per year, and cancer is now responsible for nearly one in four deaths [3]. In 2012, a total of 224,177 cancer cases and 73,759 cancer deaths were reported in Korea, with stomach, liver, and colorectal cancers accounting for 45% of all newly diagnosed cancers in men, while stomach, liver, colorectal, breast, and cervical cancers accounted for 41% of all newly diagnosed cancers in women [3].

To reduce the cancer burden the World Health Organization (WHO) has suggested implementation of National Cancer Control Plans for prevention, early detection, diagnosis,
treatment, and palliation [4]. In 1996, the Korean government initiated a comprehensive “10-year National Cancer Control Plan (10-yr NCCP)” [5]. As part of this plan, the National Cancer Screening Program (NCSP) was launched in 1999. Currently, Korean men and women older than 40 years are eligible for stomach cancer screening via endoscopy or upper gastrointestinal series biennially. Liver cancer screening is only provided to people aged 40 years and over who are hepatitis B surface antigen or anti–hepatitis C virus (HCV) positive or have liver cirrhosis. An ultrasonographic examination and α-fetoprotein test is offered every 6 months for these high risk groups. Colorectal cancer screening is conducted for individuals aged 50 years and older, primarily via an annual fecal occult blood test (FOBT). People with positive results from the FOBT can choose to undergo either colonoscopy or a double-contrast barium enema test, as well as a histological examination if needed. Mammography is provided biannually to women aged 40 years or over. Every 2 years, a Pap smear is provided to women aged 30 years and over for cervical cancer screening [6].

Organized screening programs must be able to ensure high coverage and participation, as adequate participation in screening is essential to reducing cancer mortality [7]. Upon evaluation of the progress made by the first 10-yr NCCP from 1996 to 2005, the Korean Ministry of Health and Welfare developed plans for a second 10-yr NCCP for 2006-2015. The second 10-yr NCCP was designed to improve cancer screening rates among all Koreans by improving quality of screening and expanding support for cancer patients. The 10-year plan also sought to increase participation rates for the NCSP to 55% by 2015 [8]. Accordingly, the present study aimed to examine trends in participation rates in stomach, liver, colorectal, breast, and cervical cancer screening via the NCSP from 2002 to 2012. We also attempted to evaluate how NCSP policy changes affected these rates.

Materials and Methods

The data used in this study were collected from the NCSP database for 2002 to 2012. The NCSP database includes information on age, sex, and type of health insurance (Medical Aids, National Health Insurance) for individuals who were invited to undergo screening for stomach, liver, colorectal, breast, and cervical cancer via the NCSP. The database also includes information on screening date and screening results (negative, suspicious, highly suggestive of malignancy, or benign) for those who participated in the NCSP.

In the NCSP, all eligible men and women receive an invitation letter, along with information on screening methods and the locations of screening units, from the National Health Insurance Service (NHIS), beginning in January of each year. The NHIS selects eligible men and women for each cancer site according to the NCSP protocol (SI Table). For liver cancer screening, the NHIS defines high-risk individuals as those who have been tested or received medical care for hepatitis B virus or HCV infection, chronic hepatitis, chronic liver disease, or liver cirrhosis within the past 2 years.

In this study, participation rates were analyzed on a single-year basis between the years of 2002 and 2012, and assessed as the percentage of eligible people who underwent screening among those invited. The participation rates for each of the five major cancers were calculated as the percentage of people who participated in each cancer screening program among those invited by the NCSP to undergo screening according to the NCSP protocol (SI Table). Participation rates for each of the five cancers were also calculated according to sex, age, and health insurance status. We used health insurance status as a proxy for socioeconomic status. Insurance status was classified into one of three categories: medical aids program (MAP) recipients (extremely poor people who received livelihood assistance and were unable to pay for health care or insurance), NHIS beneficiaries of low-income status (target population for free-of-charge screening), and NHIS beneficiaries of high income status (target population for screening with a copayment).

To estimate changes in participation rates, we assessed the average annual percentage change (APC) by comparing rates for 2002 and 2012 as relative rates. These risks were reported as the average APC ([relative risk–1]×100/number of years) with 95% confidence intervals (CIs). All data were analyzed using the SAS statistical software ver. 9.3 (SAS Inc., Cary, NC). This study was approved by the Institutional Review Board of the National Cancer Center in Korea (approval number: NCCNCS-08-129).

Results

The trends in participation rates in the stomach, liver, colorectal, breast, and cervical cancer screening via the NCSP from 2002 to 2012 are shown in Fig. 1. The number of men and women invited to undergo stomach cancer screening increased from 9.8 million in 2002 to 12.6 million in 2012. During this period, the NCSP supported 37.6 million examinations for stomach cancer. Stomach cancer screening participation rates increased from 7.5% in 2002 to 47.3% in 2012 (Table 1). Individuals aged 60 to 69 years showed the highest APC (5.4%) in participation rates for stomach cancer screening, followed by those aged 70 to 79 (5.0%), 50 to 59 (4.3%),
and 40 to 49 years (3.8%). Furthermore, female participants (APC, 4.6%) and NHIS beneficiaries of higher socioeconomic status (APC, 4.9%) showed a higher APC than average for stomach cancer screening.

Table 2 lists the participation rates for liver cancer screening. From 2003 to 2012, a total of 7.7 million men and women at high risk for liver cancer were invited to undergo screening, and 2.3 million liver cancer examinations were conducted. Participant rates increased steadily from 13.2% in 2003 to 39.5% in 2012. Individuals aged 60 to 69 years showed the highest participation rates for liver cancer screening, followed by those aged 50 to 59 and 70 to 79 years. Individuals who were NHIS beneficiaries of low-income status showed higher participation rates and the highest APC.

Between 2004 and 2012, a total of 77.8 million men and women were invited to undergo screening for colorectal cancer, and 17.6 million examinations were conducted. Participation rates for colorectal cancer gradually increased from 7.3% in 2004 to 25.0% in 2012 (Table 3). The APC in participation rates between 2004 and 2012 was 3.3% (95% CI, 2.5 to 4.1). An increasing tendency was observed up to 2011, while the participation rates decreased by 7.9% from 2011 to 2012. Individuals aged 60 to 69 years showed the highest participation rates and APC during this period (4.2%), followed by those aged 70 to 79 (3.3%) and 50 to 59 (3.1%). Participation rates according to sex and health insurance status were similar.

Since inception of the breast cancer screening program, the NCSP has invited 66.3 million women to undergo breast cancer screening and supported more than 22.0 million screening tests for breast cancer. The number of women served increased from 0.4 million in the first year of the breast cancer screening program to more than 3.3 million in 2012 (Table 4). Among all cancer screening programs in the NCSP, participation rates for breast cancer screening increased the most. Specifically, participation rates for breast cancer screening reached 51.9% in 2012, up from 9.4% in 2002. Participation rates were highest among women aged 60 to 69 years, who showed the highest APC (5.8%), followed by women aged 50 to 59 years.

Between 2004 and 2012, a total of 77.4 million women were invited to undergo cervical cancer screening, and more than 24.6 million examinations were provided. Table 5 shows the participation rates for cervical cancer screening. Participation rates increased from 30.8% in 2002 to 40.9% in 2012, giving an annual increase of 0.9% over the 10-year period, which was the lowest among all cancer sites. Participation rates for cervical cancer screening were lower among MAP recipients than NHIS beneficiaries, although the MAP recipients showed a higher APC than the average. Women in their 30s showed a decreasing APC in participation rates between 2002 and 2012. Participation rates for women aged 80 years or older also decreased annually. In 2005 and 2006, there were significant decreases in participation rates for women aged 30 to 39 years.
Table 1. Cancer screening rates for stomach cancer in Korea, 2002-2012

<table>
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<tr>
<th>Variable</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
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<td>11.7</td>
<td>17.4</td>
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<td>47.3</td>
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<td>35.6</td>
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<td>13.1</td>
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<td>31.0</td>
<td>35.8</td>
<td>40.6</td>
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<td>48.3</td>
<td>51.4</td>
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<tr>
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<td>9.6</td>
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<td>31.3</td>
<td>35.2</td>
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<td>9.9</td>
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<td>26.5</td>
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<td>38.3</td>
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APC, annual percent change; CI, confidence interval; NHIS, National Health Insurance Service; MAP, Medical Aid Program.
Table 2. Cancer screening rates for liver cancer in Korea, 2003-2012

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<td>24.7</td>
<td>28.6</td>
<td>29.8</td>
<td>33.9</td>
<td>38.2</td>
<td>41.5</td>
<td>37.8</td>
<td>4.1 (3.2-5.0)</td>
</tr>
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<td>≥ 80</td>
<td>3.5</td>
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<td>11.3</td>
<td>12.4</td>
<td>16.0</td>
<td>16.6</td>
<td>16.3</td>
<td>17.6</td>
<td>20.0</td>
<td>19.5</td>
<td>2.0 (1.5-2.6)</td>
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</table>

APC, annual percent change; CI, confidence interval; NHIS, National Health Insurance Service; MAP, Medical Aid Program.
Table 3. Cancer screening rates for colorectal cancer in Korea, 2004-2012

<table>
<thead>
<tr>
<th>Variable</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
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<th>2011</th>
<th>2012</th>
<th>APC (95% CI)</th>
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</thead>
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<tr>
<td>No. of invitations</td>
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<td>6,320,500</td>
<td>7,421,263</td>
<td>7,440,959</td>
<td>8,260,582</td>
<td>8,483,437</td>
<td>9,075,852</td>
<td>9,271,231</td>
<td>15,537,702</td>
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<td>No. of participants</td>
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<td>1,392,268</td>
<td>1,832,333</td>
<td>2,281,444</td>
<td>2,794,663</td>
<td>3,049,112</td>
<td>3,884,839</td>
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</tr>
<tr>
<td>Screening rate (%)</td>
<td>7.3</td>
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<td>15.7</td>
<td>18.7</td>
<td>22.2</td>
<td>26.9</td>
<td>30.8</td>
<td>32.9</td>
<td>25.0</td>
<td>3.3 (2.5-4.1)</td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
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<td>Male</td>
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<td>12.2</td>
<td>14.8</td>
<td>17.9</td>
<td>21.0</td>
<td>25.2</td>
<td>29.2</td>
<td>30.9</td>
<td>24.1</td>
<td>3.1 (2.4-3.9)</td>
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<td>28.4</td>
<td>32.3</td>
<td>34.8</td>
<td>25.8</td>
<td>3.5 (2.6-4.4)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>NHIS (upper 50%)</td>
<td>5.5</td>
<td>9.2</td>
<td>15.7</td>
<td>19.7</td>
<td>23.3</td>
<td>28.2</td>
<td>31.3</td>
<td>32.2</td>
<td>24.4</td>
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<tr>
<td>NHIS (lower 50%)</td>
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<td>16.4</td>
<td>16.8</td>
<td>19.4</td>
<td>23.1</td>
<td>27.6</td>
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<td>50-59</td>
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<td>22.4</td>
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<td>29.8</td>
<td>31.5</td>
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<td>3.1 (2.1-4.0)</td>
</tr>
<tr>
<td>60-69</td>
<td>8.7</td>
<td>15.0</td>
<td>19.2</td>
<td>23.8</td>
<td>28.2</td>
<td>34.2</td>
<td>39.1</td>
<td>41.8</td>
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<td>70-79</td>
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<td>28.4</td>
<td>32.1</td>
<td>26.7</td>
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<td>≥ 80</td>
<td>1.2</td>
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<td>3.0</td>
<td>4.1</td>
<td>4.9</td>
<td>7.4</td>
<td>9.2</td>
<td>11.1</td>
<td>10.3</td>
<td>1.2 (1.0-1.4)</td>
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</tbody>
</table>

APC, annual percent change; CI, confidence interval; NHIS, National Health Insurance Service; MAP, Medical Aid Program.

Table 4. Cancer screening rates for breast cancer in Korea, 2002-2012

<table>
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<tr>
<th>Variable</th>
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<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>APC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of invitations</td>
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<td>5,023,019</td>
<td>5,245,222</td>
<td>5,478,592</td>
<td>6,229,050</td>
<td>6,215,697</td>
<td>6,779,458</td>
<td>6,891,289</td>
<td>6,328,749</td>
<td>6,838,809</td>
<td>6,459,450</td>
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<tr>
<td>No. of participants</td>
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<td>741,902</td>
<td>1,174,831</td>
<td>1,763,679</td>
<td>2,069,873</td>
<td>2,602,927</td>
<td>2,924,689</td>
<td>2,916,919</td>
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<td>Screening rate (%)</td>
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<td>14.1</td>
<td>21.4</td>
<td>28.3</td>
<td>33.3</td>
<td>38.4</td>
<td>42.4</td>
<td>46.1</td>
<td>49.0</td>
<td>51.9</td>
<td>4.6 (4.1-5.0)</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>NHIS (upper 50%)</td>
<td>6.4</td>
<td>10.3</td>
<td>12.0</td>
<td>18.1</td>
<td>31.2</td>
<td>37.5</td>
<td>41.9</td>
<td>46.5</td>
<td>49.1</td>
<td>48.3</td>
<td>54.7</td>
<td>5.2 (4.3-6.1)</td>
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<tr>
<td>NHIS (lower 50%)</td>
<td>13.2</td>
<td>17.9</td>
<td>19.5</td>
<td>25.8</td>
<td>28.0</td>
<td>32.3</td>
<td>38.1</td>
<td>41.2</td>
<td>46.4</td>
<td>51.1</td>
<td>50.8</td>
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<tr>
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<td>11.7</td>
<td>15.7</td>
<td>16.6</td>
<td>19.2</td>
<td>21.4</td>
<td>26.3</td>
<td>27.1</td>
<td>35.9</td>
<td>36.5</td>
<td>2.5 (1.9-3.2)</td>
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<tr>
<td>Age (yr)</td>
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<td></td>
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<tr>
<td>40-49</td>
<td>9.7</td>
<td>14.1</td>
<td>14.4</td>
<td>21.3</td>
<td>25.8</td>
<td>30.3</td>
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<td>40.2</td>
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<td>50-59</td>
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<td>38.9</td>
<td>44.2</td>
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<td>60-69</td>
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<td>15.0</td>
<td>23.6</td>
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<td>41.3</td>
<td>46.8</td>
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<td>7.5</td>
<td>12.9</td>
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<td>29.0</td>
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<td>37.3</td>
<td>40.3</td>
<td>43.3</td>
<td>47.7</td>
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<tr>
<td>≥ 80</td>
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<td>1.7</td>
<td>1.7</td>
<td>3.4</td>
<td>6.0</td>
<td>9.8</td>
<td>10.9</td>
<td>12.8</td>
<td>13.6</td>
<td>15.0</td>
<td>16.9</td>
<td>1.7 (1.5-2.0)</td>
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</tbody>
</table>

APC, annual percent change; CI, confidence interval; NHIS, National Health Insurance Service; MAP, Medical Aid Program.
Discussion

The Korean government launched the NCSP to provide cancer screening for stomach, breast, and cervical cancer to MAP recipients in 1999. The NCSP is supported financially by the central government, local governments, and the NHIS. Up to 2001, the NCSP only offered free cancer screening to medical aid beneficiaries. By 2002, the target population of the NCSP was expanded to include NHIS beneficiaries and free-of-charge screening was expanded to include NHIS beneficiaries in the lowest 20% income stratum in 2002. Screening for liver cancer was included in 2003, and colorectal cancer screening was included in 2004. Free-of-charge screening was further expanded to include those in the lowest 50% income stratum since 2005. Presently, MAP recipients and NHIS beneficiaries with a premium at 50% or lower are eligible for stomach, liver, colorectal, breast, and cervical cancer screening free-of-charge, while the remaining NHIS beneficiaries are eligible to undergo screening via the cancer screening program with a co-payment of 10% of the cost of the procedure [6] (S2 Table). During this period, participation rates in the NCSP for the five major cancers have increased. Participation rates showed annual increases of 4.3% for gastric cancer, 4.1% for liver cancer, 3.3% for colorectal cancer, 4.6% for breast cancer, and 0.9% for cervical cancer. The participation rates for all cancer types, except for colorectal cancer, exceeded 40% in 2011. Breast cancer screening showed the highest participation rates, exceeding 50% in 2012.

Despite the increasing trends in participation rates, these rates are still low relative to other nationwide cancer screening programs. In the UK, where nationwide organized cancer screening has been implemented, 73.4% of women aged 45-74 years underwent breast cancer screening by mammography in 2010-2011 [9], while 73.5% of women aged 25-49 years underwent cervical cancer screening by Pap smear in 2011-2012 [10]. There are many possible explanations for why less than half of the target population in Korea participated in the NCSP. Various studies have identified socioeconomic and health system-related characteristics as barriers to or facilitators of cancer screening [11-14]. As one such barrier, a lack of continuity in care might contribute to lower participation rates in Korea relative to that of the UK. Previous studies have reported that general practitioners or family doctors play major roles in increasing participation rates [15,16]. However, in the NCSP, any clinic, hospital, or specific screening facility can apply to be certified as a cancer screening unit, and individuals who are invited to undergo screening can visit any of these certified screening units. Thus, the potential lack of a close relationship or continuous connection between the physician at the cancer screening unit and the participant might have negatively affected the
participation rates.

The low participation rates in the NCSP might also stem from potential inconveniences associated with the screening tests. In the NCSP, participation rates for colorectal cancer screening were lowest (25% in 2012) among the five major cancer sites. Potential reasons for the low participation rate in colorectal cancer screening might be related to screening via FOBT, which is delivered as a primary screening test in the colorectal cancer screening program in Korea. Individuals who are invited to undergo colorectal cancer screening must collect stool samples by themselves at home, and then visit a colorectal cancer screening unit to submit the sampled stool within a stool container. This process usually requires participants to visit the screening unit twice, once to pick up a stool container and again to submit the sample. This process is inconvenient to participants, and may act as a barrier to colorectal cancer screening. Therefore, efforts to reduce barriers of FOBT, such as the delivery of a stool container by mail, need to be continued, and specialized strategies according to age, sex, and region are warranted.

Our study revealed a significant drop in participation rates for cervical cancer screening in 2005. This was likely because of policy changes in the cervical cancer screening program. From 1988 to 2004, cervical cancer screening was provided through a NHIS health checkup service. In 2005, the cervical cancer screening program was separated from the checkup service and included in the NCSP. This change likely generated confusion among women who had previously undergone cervical cancer screening through the NHIS health checkup service. Furthermore, in the NCSP, invitees must voluntarily decide whether to make a screening appointment or not, while the NHIS health checkup service strongly promoted and encouraged participation. Additionally, the target population for the cervical cancer screening program was expanded to all NHIS beneficiaries over the age of 30 years in 2011. Until 2010, only MAP recipients, NHIS beneficiaries insured through their employer, and the head of a household were invited to undergo cervical cancer screening at the age of 30 years. Other subscribers and dependents were invited to undergo cervical cancer screening from the age of 40 years. In the short term, these policy changes and expansion of the target population lowered the participation rates for cervical cancer screening. Nevertheless, participation rates of cervical cancer screening began to increase again in 2006 and surpassed the previous rates in 2011. Further, the actual number of participants increased more than two times compared to the number of participants in 2002. Accordingly, the policy changes should be sufficiently publicized prior to implementation to minimize confusion.

Finally, the current study revealed trends in changes in participation rates according to socio-demographic factors. Overall, participation rates were highest for individuals aged 60-69 years, and women showed higher participation rates than men. Participation rates were lowest in underserved groups, such as MAP recipients. Moreover, the APCs in participation rates were the lowest among MAP recipients for all cancer types, except for cervical cancer, for which the screening participation rates fluctuated among NHIS beneficiaries during the study period because of policy changes to the cervical cancer screening program. There were also no significant differences in participation rates for liver and colorectal cancer screening according to socio-economic status. However, participation rates for screening of these cancer sites were too low to reveal differences according to socio-economic status. According to previous studies, barriers to cancer screening faced by people of low socioeconomic status includes lack of time, lack of knowledge about cancer screening, physical disability or underlying disease, and logistic barriers [17]. It is possible that potential commonalities among MAP recipients (e.g., poverty and limited education) may underlie these barriers to screening. Thus, individually-targeted interventions in a health care setting are required, such as individualized in-person or telephone counseling, individualized letters and reminders, or other individually-targeted strategies, especially for people of lower socioeconomic status, to increase participation and reduce disparities in cancer screening.

It should be noted that our study has several limitations. The NCSP database lacks details regarding why people did not participate in the NCSP. Thus, we were unable to explore the influence of other important correlates, such as psychological factors (e.g., discomfort, concern about complications, or anxiety about the procedure) and health related factors (e.g., disability, health status, or health behaviors) that might be involved in adherence to screening. Moreover, although both organized and opportunistic cancer screenings are available in Korea, the current study used data from the NCSP database, which does not include information regarding opportunistic screening. Therefore, the results of this study should not be interpreted as reflecting overall screening rates, including both organized and opportunistic screening, for Korea. Further, the screening behaviors demonstrated in this study would not be generalizable to those for the entire Korean population.

### Conclusion

This study was conducted to investigate overall trends in participation rates for stomach, liver, colorectal and breast cancer screening via the NCSP according to sex, age, and health insurance type. Overall, participation rates for all
five cancer types continually increased from 2002 to 2012. Significant increasing trends were observed in participation rates for stomach, liver, colorectal, and breast cancer, but not for cervical cancer.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Acknowledgments

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References

CA19-9 or CEA Decline after the First Cycle of Treatment Predicts Survival in Advanced Biliary Tract Cancer Patients Treated with S-1 and CisplatinChemotherapy

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Purpose
While tumor markers (carbohydrate antigen 19-9 [CA 19-9] and carcinoembryonic antigen [CEA]) can aid in the diagnosis of biliary tract cancer, their prognostic role has not been clearly elucidated. Therefore, this study was conducted to evaluate the prognostic role of tumor markers and tumor marker change in patients with advanced biliary tract cancer.

Materials and Methods
Patients with pathologically proven metastatic or relapsed biliary tract cancer who were treated in a phase II trial of first-line S-1 and cisplatin chemotherapy were enrolled. Serum tumor markers were measured at baseline and after the first cycle of chemotherapy.

Results
Among a total of 104 patients, 80 (77%) had elevated baseline tumor markers (69 with CA 19-9 elevation and 40 with CEA). A decline ≥ 30% of the elevated tumor marker level after the first cycle of chemotherapy conferred an improved time to progression (TTP), overall survival (OS), and better chemotherapy response. Multivariate analysis revealed tumor marker decline as an independent positive prognostic factor of TTP (adjusted hazard ratio [HR], 0.44; p=0.003) and OS (adjusted HR, 0.37; p < 0.001). Subgroup analysis revealed similar results in each group of patients with CA 19-9 elevation and CEA elevation. In addition, elevated baseline CEA was associated with poor survival in both univariate and multivariate analysis.

Conclusion
Tumor marker decline was associated with improved survival in biliary tract cancer. Measuring tumor marker after the first cycle of chemotherapy can be used as an early assessment of treatment outcome.

Key words
Biliary tract neoplasms, Tumor biomarker, Carcinoembryonic antigen, CA 19-9 antigen, Antineoplastic agents
Introduction

Biliary tract cancer includes intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer. The incidence of biliary tract cancer is low in Western countries (2-3 persons/100,000 per year), but their incidence is relatively high in Asian countries (4-6 persons/100,000 per year) [1,2]. Complete resection is the only option to cure biliary tract cancer, but only 10% of patients are diagnosed at an early stage of the disease and are considered for curative resection [3]. For patients with unresectable or metastatic biliary tract cancer, chemotherapy has shown significant benefit compared with best supportive care alone [4,5]. In a randomized controlled phase III study, gemcitabine plus cisplatin showed improved overall survival (OS) and progression-free survival compared to gemcitabine alone [6]. Although gemcitabine plus cisplatin is considered as a standard of care in a first-line setting, this regimen has not been compared head to head with other fluoropyrimidine-based regimens in phase III studies [7]. In a phase II study, combination of S-1 and cisplatin showed comparable efficacy and favorable safety compared to gemcitabine plus cisplatin in patients with advanced biliary tract cancer [8]. Gemcitabine-based or fluoropyrimidine-based combinations are considered the standard first-line chemotherapy regimen for patients with advanced biliary tract cancer [6,9-11].

Despite the progress in chemotherapy regimen, prognosis of biliary tract cancer remains poor, with a median OS of 5-15 months, and only 15%-40% of patients show response to chemotherapy [12]. Selection of patients who might benefit from chemotherapy is important. In addition, early assessment of treatment efficacy can facilitate a physician’s clinical decision and prevent patients from unnecessary treatment. Metastatic disease, intrahepatic cholangiocarcinoma, liver metastasis, Eastern Cooperative Oncology Group (ECOG) performance status and alkaline phosphatase (ALP) level were identified as prognostic factors in patients with advanced biliary tract cancer [13]. However, the prognostic role of tumor markers including carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 has not yet been clearly elucidated. In patients with unresectable biliary tract cancer, pretreatment elevated CA 19-9 level was associated with poor overall survival, and CA 19-9 decline during chemotherapy showed prolonged survival in a subgroup of patients without biliary obstruction [14]. Recently, a 50% decline of CA 19-9 level after 10-12 weeks of chemotherapy showed improved survival in inoperable bile duct cancer patients [15]. The present study was conducted to evaluate the prognostic role of tumor markers (CA 19-9 and CEA) and changes in tumor markers in advanced biliary tract cancer patients. For early assessment of treatment efficacy by tumor marker change, tumor marker change was measured after the first cycle of chemotherapy (3 weeks after chemotherapy initiation). The study population was homogenous that all patients were prospectively enrolled in a phase II trial of first-line S-1 plus cisplatin chemotherapy. In this trial, combination chemotherapy was an effective outpatient-based regimen in patients with advanced biliary tract cancer [16].

Materials and Methods

1. Patients and treatment

Patients (n=104) with pathologically proven unresectable, metastatic, or relapsed biliary tract adenocarcinoma who participated in an expansion cohort of a phase II trial of S-1 and cisplatin were included [16]. Eligibility criteria included age over 20, ECOG performance status of 0 to 2, no prior chemotherapy or radiotherapy, adequate bone marrow, hepatic, and renal function. In addition, at least one measurable lesion according to the Response Evaluation Criteria in Solid tumors (RECIST) was required for inclusion. Written informed consent was obtained from each patient before enrollment and the protocol was approved by the Institutional Review Board of the Seoul National University Hospital, Seoul, Korea (0412-138-08).

S-1 was administered orally at a dose of 40 mg/m2 twice daily for 14 days, followed by a 7-day rest period. Cisplatin was given as a 90-minute infusion on day 1 of each cycle at a dose of 60 mg/m2. Treatment was repeated every 3 weeks until disease progression, unacceptable toxicity, or withdrawal of patient consent.

Best tumor response was assessed by computed tomography (CT) scans using the RECIST 1.0 criteria [17]. The overall response rate (RR) was defined as a proportion of patients having the best response of either complete response or partial response. CT scan was made at baseline and every two cycles (6 weeks) thereafter. Collection of medical history, physical examination, measurement of the CEA, CA 19-9 level, and toxicity evaluation was made on every cycle. Toxicity was measured according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver. 3.0. CEA level of 5 ng/mL and CA 19-9 level of 37 U/mL was defined as a cutoff value for normal level according to the historical data and manufacturer’s recommendation [18,19]. Baseline tumor marker level was measured on the day of the first cycle of chemotherapy (before chemotherapy injection) and follow up tumor marker was measured 3 weeks after the first cycle of chemotherapy (the day of second cycle chemotherapy administration, before chemotherapy injection).
Tumor marker change was compared between baseline and follow up tumor marker level. Tumor marker decline was defined as a 30% decrease in serum level of tumor marker after the first cycle of chemotherapy, which was measured 3 weeks after chemotherapy initiation (the day of second cycle chemotherapy administration).

2. Statistical analysis

The primary objective of the phase II study was to evaluate the RR of S-1 and cisplatin chemotherapy in patients with advanced biliary tract cancer. Time to progression (TTP) was defined as the interval between the first day of chemotherapy and the first day of documented progressive disease. Data from patients who were free of progression were censored at the date of the last tumor response evaluation. OS was defined as the date of the first chemotherapy to the date of death from any cause. Preplanned exploratory analysis of the correlation between serum tumor marker, tumor marker change, and treatment outcome of patients with advanced biliary tract cancer was performed in the prospective expansion cohort. The main focus of the present study was to evaluate the prognostic role of tumor marker change. Categorical variables were compared using a chi-square test. TTP and OS were calculated using the Kaplan-Meier method and comparisons were made using the log-rank tests. Hazard ratios (HR) were calculated using the Cox proportional hazard model. To adjust for the baseline characteristics, we used the Cox proportional hazard model in a forward stepwise manner. Prognostic factors found to have a probability value ≤ 0.20 upon univariate analysis were included in the multivariate analysis. Two-sided p-values < 0.05 were considered statistically significant. All analyses were performed with the SPSS ver. 18.0 (SPSS Inc., Chicago, IL).

Results

1. Patient characteristics

A total of 104 patients with advanced biliary tract cancer who were prospectively enrolled in a phase II trial of first line S-1 and cisplatin were included from January 2005 to December 2008. Baseline characteristics are summarized in Table 1. Tumor type was intrahepatic cholangiocarcinoma in 57, gallbladder cancer in 33, extrahepatic cholangiocarcinoma in 11, and ampulla of Vater cancer in three patients. Presentation of disease was initially metastatic in 71 patients, relapsed in 29 patients, and four patients had unresectable disease. Baseline serum ALP level (≥ 115 IU/L), CEA level (≥ 5 ng/mL), and CA 19-9 level (≥ 37 U/mL) was above normal level in 39 (37.1%), 40 (38.5%), and 69 (66.3%) patients. Eighty patients (77%) had either elevated CEA or CA 19-9 levels. Median levels of CEA and CA 19-9 were 3.0 ng/mL and 240 U/mL, respectively. Most patients had ECOG performance status 1 (85.6%). According to the inclusion criteria, none of the patients received prior systemic chemotherapy for metastatic disease.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients (%) (n=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median (range, yr)</strong></td>
<td></td>
</tr>
<tr>
<td>≥ 65 yr</td>
<td>29 (27.9)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60 (57.7)</td>
</tr>
<tr>
<td>Female</td>
<td>44 (42.3)</td>
</tr>
<tr>
<td><strong>Primary site</strong></td>
<td></td>
</tr>
<tr>
<td>Intrahepatic cholangiocarcinoma</td>
<td>57 (54.8)</td>
</tr>
<tr>
<td>Gallbladder cancer</td>
<td>33 (31.7)</td>
</tr>
<tr>
<td>Extrahepatic cholangiocarcinoma</td>
<td>11 (10.6)</td>
</tr>
<tr>
<td>Ampulla of Vater cancer</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td><strong>Initial presentation</strong></td>
<td></td>
</tr>
<tr>
<td>Relapsed</td>
<td>29 (27.9)</td>
</tr>
<tr>
<td>Locally advanced</td>
<td>4 (3.8)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>71 (68.3)</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>59 (56.7)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>53 (51.0)</td>
</tr>
<tr>
<td>Peritoneal seeding</td>
<td>20 (19.2)</td>
</tr>
<tr>
<td>Lung</td>
<td>9 (8.7)</td>
</tr>
<tr>
<td>Bone</td>
<td>6 (5.8)</td>
</tr>
<tr>
<td><strong>ECOG PS</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>11 (10.6)</td>
</tr>
<tr>
<td>1</td>
<td>89 (85.6)</td>
</tr>
<tr>
<td>2</td>
<td>4 (3.8)</td>
</tr>
<tr>
<td><strong>ALP (IU/L) (n=103)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 115</td>
<td>64 (62.1)</td>
</tr>
<tr>
<td>≥ 115</td>
<td>39 (37.9)</td>
</tr>
<tr>
<td><strong>CEA (ng/mL)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>64 (61.5)</td>
</tr>
<tr>
<td>≥ 5</td>
<td>40 (38.5)</td>
</tr>
<tr>
<td><strong>CA 19-9 (U/mL) (n=103)</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 37</td>
<td>34 (33.0)</td>
</tr>
<tr>
<td>≥ 37</td>
<td>69 (67.0)</td>
</tr>
<tr>
<td>≥ 370</td>
<td>46 (44.7)</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group; PS, performance status; ALP, alkaline phosphatase; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.
Table 2. Univariate analysis of TTP and OS

<table>
<thead>
<tr>
<th>Variable</th>
<th>TTP (95% CI)</th>
<th>p-value</th>
<th>OS (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65</td>
<td>5.0 (4.1-5.8)</td>
<td>0.54</td>
<td>9.3 (6.9-11.8)</td>
<td>0.72</td>
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<tr>
<td>≥ 65</td>
<td>6.8 (5.5-8.0)</td>
<td></td>
<td>7.5 (3.9-11.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.4 (3.7-7.2)</td>
<td>0.80</td>
<td>8.2 (7.0-9.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>Female</td>
<td>5.4 (3.8-7.1)</td>
<td></td>
<td>9.6 (6.2-13.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Primary site</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrahepatic</td>
<td>5.0 (4.0-5.9)</td>
<td>0.64</td>
<td>8.2 (7.0-9.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Others</td>
<td>6.3 (4.2-8.3)</td>
<td></td>
<td>10.6 (7.5-13.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Initial presentation</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic</td>
<td>5.0 (4.1-5.8)</td>
<td>0.24</td>
<td>8.2 (7.0-9.3)</td>
<td>0.020</td>
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<tr>
<td>Others</td>
<td>7.0 (4.9-9.0)</td>
<td></td>
<td>11.4 (8.7-14.1)</td>
<td></td>
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<tr>
<td><strong>Liver metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>5.4 (3.5-7.4)</td>
<td>0.50</td>
<td>8.6 (7.0-10.1)</td>
<td>0.22</td>
</tr>
<tr>
<td>Absent</td>
<td>5.4 (3.7-7.2)</td>
<td></td>
<td>8.6 (3.5-13.7)</td>
<td></td>
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<tr>
<td><strong>ECOG PS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9.8 (4.3-15.3)</td>
<td>0.40</td>
<td>18.6 (9.3-27.8)</td>
<td>0.065</td>
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<tr>
<td>1</td>
<td>5.3 (4.1-6.5)</td>
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<td>8.3 (6.8-9.8)</td>
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<tr>
<td>2</td>
<td>3.6 (0.0-8.6)</td>
<td></td>
<td>7.1 (1.2-13.1)</td>
<td></td>
</tr>
<tr>
<td><strong>ALP (IU/L)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≥ 115</td>
<td>3.7 (1.5-6.0)</td>
<td>0.039</td>
<td>7.3 (4.6-9.9)</td>
<td>0.006</td>
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<tr>
<td>&lt; 115</td>
<td>6.8 (5.9-7.7)</td>
<td></td>
<td>11.2 (8.6-13.9)</td>
<td></td>
</tr>
<tr>
<td><strong>CEA (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>4.7 (3.8-5.5)</td>
<td>0.015</td>
<td>7.1 (5.6-8.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>6.3 (4.5-8.1)</td>
<td></td>
<td>11.2 (8.9-13.5)</td>
<td></td>
</tr>
<tr>
<td><strong>CEA interquartile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th quarter</td>
<td>4.4 (2.7-6.0)</td>
<td>0.12</td>
<td>7.1 (5.6-8.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>3rd quarter</td>
<td>6.1 (4.0-8.1)</td>
<td></td>
<td>8.2 (5.1-11.2)</td>
<td></td>
</tr>
<tr>
<td>2nd quarter</td>
<td>6.2 (4.5-7.9)</td>
<td></td>
<td>10.9 (6.0-15.8)</td>
<td></td>
</tr>
<tr>
<td>1st quarter</td>
<td>5.4 (2.6-8.3)</td>
<td></td>
<td>12.0 (1.6-22.4)</td>
<td></td>
</tr>
<tr>
<td><strong>CA 19-9 (U/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 37</td>
<td>5.0 (3.7-6.3)</td>
<td>0.49</td>
<td>8.2 (7.0-9.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>&lt; 37</td>
<td>6.8 (4.6-9.0)</td>
<td></td>
<td>11.4 (9.2-13.6)</td>
<td></td>
</tr>
<tr>
<td><strong>CA 19-9 interquartile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th quarter</td>
<td>5.0 (3.5-6.5)</td>
<td>0.66</td>
<td>7.3 (5.7-8.8)</td>
<td>0.23</td>
</tr>
<tr>
<td>3rd quarter</td>
<td>6.1 (3.1-9.0)</td>
<td></td>
<td>8.6 (4.2-13.0)</td>
<td></td>
</tr>
<tr>
<td>2nd quarter</td>
<td>6.2 (3.6-8.8)</td>
<td></td>
<td>9.7 (5.3-14.2)</td>
<td></td>
</tr>
<tr>
<td>1st quarter</td>
<td>6.8 (3.0-10.5)</td>
<td></td>
<td>10.6 (6.3-14.9)</td>
<td></td>
</tr>
<tr>
<td><strong>CA 19-9 (U/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 370</td>
<td>5.3 (3.3-7.2)</td>
<td>0.51</td>
<td>7.5 (6.3-8.8)</td>
<td>0.043</td>
</tr>
<tr>
<td>&lt; 370</td>
<td>6.1 (4.0-8.2)</td>
<td></td>
<td>11.2 (8.4-14.0)</td>
<td></td>
</tr>
</tbody>
</table>

TTP, time to progression; OS, overall survival; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; PS, performance status; ALP, alkaline phosphatase; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

2. Efficacy of S-1 and cisplatin chemotherapy

This is the expanded result of the previously published phase II study [16]. After a median follow-up duration of 31.6 months, 90 death events and 80 progression events occurred. The median TTP was 5.4 months (95% confidence interval [CI], 4.2 to 6.7) and the median OS was 8.6 months (95% CI, 7.2 to 10.0). The overall RR was 27.9% and the disease control rate was 70.2%.
3. Prognostic impact of clinicopathologic variables and tumor marker

Baseline elevated CEA levels (4.7 months vs. 6.3 months, p=0.015) and elevated ALP levels (3.7 months vs. 6.8 months, p=0.039) were associated with poor TTP (Table 2). Interquartile ranges of CEA revealed a negative linear correlation between CEA level and patient overall survival. Liver metastasis and initial metastatic presentation was not associated with TTP. However, initial metastatic presentation was associated with poor OS (8.2 months vs. 11.4 months, p=0.020). Upon multivariate analysis using the Cox proportional hazard model, elevated baseline CEA level (adjusted HR, 1.78; p=0.016) was associated with poor TTP. There was no predictive role of baseline CEA or CA 19-9 level based on the best tumor response assessed by the RECIST criteria.

In terms of OS, initial metastatic presentation, poor ECOG performance status, elevated ALP level, elevated CEA level, and elevated CA 19-9 level above 370 U/mL were associated with poor prognosis upon univariate analysis (Table 2). Multivariate analysis revealed that elevated baseline CEA level (adjusted HR, 2.12; p=0.001), ALP level (adjusted HR, 1.88; p=0.005), and poor ECOG performance status (adjusted HR, 2.64; p=0.017) were independently associated with poor OS.

4. Tumor marker change and survival

We next evaluated whether decreases in serum tumor marker level after the first cycle of chemotherapy can predict treatment outcomes. Among 80 patients with elevated baseline tumor marker levels (CEA and/or CA 19-9), tumor markers after first cycle of chemotherapy were measured in 76 patients (95%). Tumor marker decline after the first cycle of chemotherapy (CEA and/or CA 19-9) was associated with favorable TTP (7.2 months vs. 3.7 months, p=0.004) and OS (12.3 months vs. 6.9 months, p<0.001) (Fig. 1). Multivariate analysis revealed that tumor marker decline after the first cycle of chemotherapy was an independent positive prognostic factor for both TTP (adjusted HR, 0.44; 95% CI, 0.25 to 0.75; p=0.003) and OS (adjusted HR, 0.37; 95% CI, 0.21 to 0.64; p<0.001). In addition, patients with tumor marker decline were associated with better responses to chemotherapy (Table 3). Patients with tumor marker decline showed an overall RR of 59% compared to 9% in patients without tumor marker decline (p<0.001). If we include the best response by RECIST criteria as a covariate factor in the multivariate analysis, tumor marker decline was an independent prognostic factor for OS (adjusted HR, 0.47; 95% CI, 0.23 to 0.95; p=0.035), but not for TTP.

5. CEA and CA 19-9 change and survival

Similar results were obtained in the subgroup of patients with elevated CA 19-9 level and CEA level. Among 69 patients with a baseline CA 19-9 level ≥ 37 U/mL, CA 19-9 level after the first cycle of chemotherapy was measured in 67 patients (97%). Among 40 patients (90%) who had elevated baseline CEA levels, the CEA level of 36 patients (90%) was measured after the first cycle of chemotherapy. In the 67 patients with baseline CA 19-9 levels ≥ 37 U/mL, CA 19-9 decline after the first cycle of chemotherapy was associated with favorable TTP (6.3 months vs. 3.6 months, p=0.015) and
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Values are presented as number (%). CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CR, complete response; PR, partial response; SD, stable
disease; PD, progressive disease. a)Either CA 19-9 or CEA.

< 0.001
1 (11.1)
7 (77.8)
1 (11.1)
0(
0(
0(
4 (14.8)
13 (48.1)
6 (22.2)
4 (14.8)
CR
PR
SD
PD
Not measured

1 (2.3)
3 (6.8)
22 (50.0)
9 (20.5)
9 (20.5)

1 (3.1)
18 (56.3)
9 (28.1)
3 (9.4)
1 (3.1)

< 0.001

1 (2.6)
2 (5.3)
19 (50.0)
8 (21.1)
8 (21.1)

0(
16 (55.2)
9 (31.0)
3 (10.3)
1 (3.4)

< 0.001

CEA
decrease
! 30%
CA 19-9
decrease
! 30%
CA 19-9
decrease
< 30%
p-value
Tumor marker Tumor marker
decrease
decrease
< 30%a)
! 30%a)
Response

Table 3. Tumor marker change and response rate in patients with elevated baseline tumor marker

p-value

CEA
decrease
< 30%

p-value

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OS (12.3 months vs. 6.5 months, p < 0.001) (Fig. 2). In 36
patients with elevated baseline CEA levels (! 5 ng/mL), CEA
decline after the first cycle of chemotherapy showed favorable TTP (7.4 months vs. 3.6 months, p=0.012) and OS (12.3
months vs. 6.5 months, p=0.024) (Fig. 3). Multivariate analysis revealed that CA 19-9 decline after the first cycle of
chemotherapy was an independent positive prognostic factor
for both TTP (adjusted HR, 0.48; 95% CI, 0.27 to 0.85; p=0.012)
and OS (adjusted HR, 0.35; 95% CI, 0.20 to 0.62; p < 0.001).
CEA decline was also an independent positive prognostic
factor for both TTP (adjusted HR, 0.34; 95% CI, 0.14 to 0.81;
p=0.015) and OS (adjusted HR, 0.38; 95% CI, 0.16 to 0.92;
p=0.032).

Discussion
We determined the prognostic role of tumor markers (CEA
and CA 19-9) and changes in tumor markers in advanced biliary tract cancer patients who were prospectively enrolled in
a phase II study of first line S-1 and cisplatin chemotherapy.
Biliary tract cancer is a rare cancer worldwide, and the prognostic factors have not been clearly defined or validated. Old
age, large tumor volume, metastatic disease, intrahepatic
cholangiocarcinoma, liver metastasis, ECOG performance
status and ALP level, CA19-9, and CA19-9 decline have been
proposed as prognostic factors in advanced biliary tract cancer [13-15,20]. Using our homogenous cohort of patients
treated with first-line S-1 plus cisplatin, we found that tumor
marker decline is associated with prolonged survival. In
addition, elevated baseline CEA was associated with poor
survival.
Among the variable tumor markers, CEA and CA 19-9
have been the most thoroughly investigated in patients with
biliary tract cancer. In patients with primary sclerosing
cholangitis, serum CEA and CA 19-9 play a role in the diagnosis of cholangiocarcinoma [21-23]. In addition, baseline
elevated CA 19-9 played a negative prognostic role in
advanced biliary tract cancer [14]. However, the prognostic
role of baseline CEA or their change has not been thoroughly
investigated. In the present study, patients with baseline
CEA ! 5 ng/mL had worse TTP and OS than those with CEA
< 5 ng/mL. Although CA 19-9 did not have a prognostic role
in terms of TTP, patients with CA 19-9 ! 370 U/mL had a
worse OS than those with CA 19-9 < 370 U/mL. Upon multivariate analysis, elevated CEA was associated with poorer
OS, but there was no prognostic role of CA 19-9.
We revealed that decreases in tumor markers after the first
cycle of chemotherapy can predict chemotherapy response
and patients prognosis. In patients with elevated baseline


Fig. 2. Carbohydrate antigen 19-9 (CA 19-9) change and survival. (A) CA 19-9 change and time to progression. (B) CA 19-9 change and overall survival.

Fig. 3. Carcinoembryonic antigen (CEA) change and survival. (A) CEA change and time to progression. (B) CEA change and overall survival.

tumor markers, tumor marker decline ≥ 30% after the first cycle of chemotherapy was associated with improved TTP and OS. In addition, tumor marker decline was associated with better tumor response. Similar results were obtained using CA 19-9 and CEA levels in patients with elevated baseline CA 19-9 and CEA levels, respectively. A recent study by Grunnet et al. [15] shows that CA 19-9 decline during chemotherapy is associated with improved survival in inoperable biliary tract cancer. However, CA 19-9 was measured 10-12 weeks after treatment and there were no data describing CEA. Tumor response assessment using the RECIST criteria is usually measured 6 to 8 weeks after the start of chemotherapy because earlier changes are seldom significant. Our results showed that tumor marker measurement after the first cycle of chemotherapy (3 weeks after the start of chemotherapy) could predict tumor response and survival in patients with advanced biliary tract cancer. Earlier prediction of treatment efficacy and prognosis based on tumor
markers can facilitate a physician’s decision and prevent patients from unnecessary, ineffective treatments. In addition, tumor marker changes can assist tumor response assessed by the RECIST criteria.

It should be noted that this study was limited in that only patients with measurable tumor lesions and those treated with first-line S-1 and cisplatin were included. As all patients in our study participated in a phase II trial of S-1 and cisplatin chemotherapy, we could not validate our findings in patients treated with a gemcitabine based regimen. However, chemotherapy regimen did not affect the prognostic role of CA 19-9 change in a study by Grunnet et al. [15]. Another limitation of the present study is that it was conducted in a relatively small number of patients so the study results should be interpreted with caution. However, as biliary tract cancer is a rare tumor, the data from 104 patients from a prospective phase II study are comparable to those of other studies performed in patients with biliary tract cancer. Finally, our findings need further validation in a larger independent cohort of patients that includes patients treated with gemcitabine based chemotherapy and those without measurable tumor lesions.

In conclusion, CA 19-9 or CEA decline ≥ 30% after the first cycle of chemotherapy can be used as an early measurement of treatment outcome in patients with advanced biliary tract cancer. Moreover, baseline elevated CEA level plays an independent negative prognostic role.

Conflicts of Interest

We thank to Jeil Pharmaceutical Co. Ltd. and Taiho Pharmaceutical Co. Ltd. for providing S-1.

Acknowledgments

We are grateful to Hyun Ju Ryu for her support as a clinical research coordinator.

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References


A Randomized Phase II Study of Leucovorin/5-Fluorouracil with or without Oxaliplatin (LV5FU2 vs. FOLFOX) for Curatively-Resected, Node-Positive Esophageal Squamous Cell Carcinoma

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Purpose
The optimal perioperative treatment for resectable esophageal squamous cell carcinoma (ESCC) remains controversial. We evaluated the efficacy and safety of leucovorin and 5-fluorouracil (LV5FU2) and LV5FU2 plus oxaliplatin (FOLFOX) combination chemotherapies administered adjuvantly for curatively-resected, node-positive ESCC.

Materials and Methods
Patients with pathologically node-positive esophageal cancer after curative R0 resection were enrolled and randomly assigned to receive LV5FU2 or FOLFOX biweekly for up to eight cycles. The primary endpoint was disease-free survival (DFS).

Results
Between 2011 and 2015, 62 patients were randomized into the two treatment groups (32 in the LV5FU2 arm and 30 in the FOLFOX arm). The median age was 60 years and both groups had similar pathologic characteristics in tumor, nodal status, and location. Treatment completion rates were similarly high in both groups. The DFS rate at 12 months was 67% in the LV5FU2 group and 63% in the FOLFOX group with a hazard ratio of 1.3 (95% confidence interval [CI], 0.66 to 2.62). After a median follow-up period of 27 months, the median DFS was 29.6 months (95% CI, 4.9 to 54.2) in the LV5FU2 arm and 16.8 months (95% CI, 7.5 to 26.1) in the FOLFOX arm (p=0.428), respectively, while the median overall survival was not reached in either arm. Grade 3 or 4 neutropenia was more frequent in patients in the FOLFOX arm than the LV5FU2 arm (20.0% vs. 3.1%).

Conclusion
The addition of oxaliplatin (FOLFOX) did not lead to better efficacy compared to LV5FU2 chemotherapy in an adjuvant setting in node-positive ESCC patients.

Key words
Esophageal neoplasms, Adjuvant chemotherapy, FOLFOX

Introduction

Esophageal cancer is the sixth most common cause of cancer death worldwide [1] and remains a significant unmet medical need in Korea [2] with an overall poor prognosis. Esophageal cancers are histologically classified as squamous cell carcinoma (SCC) or adenocarcinoma, and SCC, which has risk factors including smoking and alcohol abuse, is the most common form of esophageal cancer in Asian countries [3]. Surgery is the primary course of treatment for resectable disease, and lymph node metastasis has been shown to be a strong independent predictor of poor survival [4]. Although surgery is the mainstay of curative treatment, surgery alone has limited efficacy at improving long-term survival in locally advanced esophageal cancer, especially node-positive disease. Therefore, more effective multimodal treatment methods, including neoadjuvant or adjuvant chemotherapy,
radiotherapy and chemoradiotherapy (CRT) have been investigated for their potential to improve overall survival (OS).

Neoadjuvant chemotherapy or concurrent CRT followed by operation have been regarded as standard treatments for locally advanced esophageal cancer [5-8]. However, many patients have been found to have advanced, node-positive, esophageal cancer only after they received upfront surgery without neoadjuvant therapy because of the incomplete accuracy of preoperative tumor staging. For example, one report showed that 81 esophageal cancer patients were staged to have node-negative disease by positron emission tomography, chest computed tomography, and/or endoscopic ultrasonography and received upfront surgery without neoadjuvant therapy. However, 37 of these patients were found to have pathological node(s) in the surgical specimens [9].

For this population, adjuvant cisplatin and fluorouracil (FP) is recommended based on the improved disease-free survival (DFS) associated with adjuvant FP in patients with node-positive esophageal SCC [10]. However, most patients with esophageal cancer are elderly with poor nutritional status and become fragile after receiving major operations; therefore, adjuvant FP can be too toxic a regimen for this population [11]. Accordingly, it is necessary to identify an adjuvant chemotherapy regimen that is more tolerable but comparably efficient with FP. In this study, we prospectively compared the combination regimen of fluorouracil and leucovorin, and oxaliplatin (FOLFOX) with a non-platinum regimen of fluorouracil and leucovorin (LV5FU2) to evaluate whether FOLFOX can be an acceptable adjuvant regimen for locally advanced esophageal SCC.

Materials and Methods

1. Patients and study design

This study was a single-center, open-label, randomized phase II trial (NCT01467921) of patients aged 20 years or older with histologically confirmed SCC of the esophagus. All patients underwent curative esophagectomy and regional lymphadenectomy and had pathologically node-positive disease. Patients who received prior neoadjuvant chemotherapy, radiotherapy or concurrent chemoradiation (CCRT) before registration were excluded. Eligible patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ function. This study was approved by the Institutional Review Board of Samsung Medical Center. Written informed consent was acquired from each patient before enrollment. Patients with previous oxaliplatin use, severe co-morbid illnesses, and/or active infections were also excluded. All pathological staging of tumors for patients was based on the seventh edition of the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) classification guidelines.

2. Study treatment

Four to six weeks after surgery, eligible patients were randomized to receive (1:1) LV5FU2 or FOLFOX chemotherapy for up to eight cycles or until disease progression before completion of adjuvant treatment, unacceptable toxicity, or patient withdrawal. The LV5FU2 regimen consisted of 2-week cycles of 200 mg/m² leucovorin plus and a bolus injection of 5-fluorouracil (5-FU; 400 mg/m²) intravenously on day 1, followed by a 46-hour continuous infusion of 5-FU (2,400 mg/m²). In the FOLFOX arm, 2-week cycles of oxaliplatin 85 mg/m² were given intravenously on day 1 before leucovorin and 5-FU and the dose of 5-FU/leucovorin was the same as LV5FU2 regimen. If patients showed disease progression during or after the study treatment, further treatment could be administered at the discretion of the investigators.

3. Endpoints

The primary endpoint of the study was DFS, which was defined as the time from the date of registration to the date of recurrence, death, or last contact. Secondary endpoints included OS, safety and quality of life assessment. OS was calculated from the time of randomization to the date of death. These endpoints were measured in all registered patients (i.e., the intention-to-treat population). Recurrence and survival data were evaluated every four cycles during the study treatment and every 3 months after completion of the study treatment. Safety evaluation included all patients who received at least one dose of study drug and adverse events (AEs) were assessed according to the National Cancer Institute criteria Common Terminology Criteria for Adverse Events (CTCAE) ver. 3 [12].

4. Statistical analysis

Assuming a 12-month DFS rate of 60% for the LV5FU2 arm, this study required a total of 56 patients (28 per arm) with 90% power to detect a 20% difference in 12-month DFS using the two-sided log-rank test with an alpha level of 0.05. A 20% ineligible or non-assessable rate was assumed, resulting in an accrual goal of 68 patients (34 patients for each arm). For DFS and OS, survival functions were estimated using the Kaplan-Meier method and compared between
groups via the log-rank test. \( p < 0.05 \) were considered statistically significant. Cox regression analysis was used for univariate and multivariate analysis to identify significant prognostic factors for recurrence. All reported \( p \)-values are two-sided and were calculated using SPSS ver. 21 (IBM Corp., Armonk, NY).

Results

1. Study patients

Between January 2011 and March 2015, 62 patients were randomly assigned to receive either LV5FU2 (n=32) or FOLFOX (n=30) chemotherapy (Fig. 1). Among a total of 69 patients enrolled, one patient was excluded due to patient withdrawal and six were removed owing to preoperative treatment. The clinicopathologic characteristics of all patients are listed in Table 1. All patients in both arms had SCC of the esophagus and pathologically confirmed regional lymph node metastasis. The median age of all patients was 60 years (range, 43 to 77 years). Most were male (97%), and all patients had an ECOG performance status of 1. Both groups had similar pathological characteristics with regard to tumor, nodal status, and location. Approximately 70% of patients had stage III disease, and the upper esophagus tumors represented 20% of patients in the LV5FU2 group and 33% of the FOLFOX group.

2. Efficacy

At the data cutoff point (February 2016), the median follow-up duration was 27.0 months (range, 4.5 to 59.1 months). The DFS rate at 12 months, which was the primary endpoint in this study, was 67% (95% confidence interval [CI], 50.7 to 83.3) in the LV5FU2 group and 63% (95% CI, 45.7 to 80.3) in the FOLFOX group with a hazard ratio (HR) of 1.3 (95% CI, 0.66 to 2.62). Specifically, the median DFS was 29.6 months (95% CI, 4.9 to 54.2) in the LV5FU2 arm and 16.8 months (95% CI, 7.5 to 26.1) in the FOLFOX arm (\( p=0.428 \)) (Fig. 2A), respectively, while the median OS was not reached in either arm (\( p=0.904 \)) (Fig. 2B). The estimated 3-year DFS and OS were 46% and 57% in the LV5FU2 group and 39% and 59% in the FOLFOX group, respectively.

In total, 15 patients in the LV5FU2 group and 18 in the FOLFOX group recurred or progressed during follow-up. There were no significant differences in the pattern of recurrence between groups, although both local and distant recurrence were observed in six patients in the FOLFOX arm (Table 2). Univariate analysis revealed significant risk factors for tumor recurrence including advanced nodal status (N3, \( p=0.020 \)), tumor status (T3, \( p=0.005 \)), and stage III (\( p=0.019 \))
Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LV5FU2 (n=32)</th>
<th>FOLFOX (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>61.9±6.11</td>
<td>60.0±7.84</td>
</tr>
<tr>
<td>Range</td>
<td>46-73</td>
<td>43-77</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (96.9)</td>
<td>29 (96.7)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (3.1)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32 (100)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>32 (100)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>7 (21.9)</td>
<td>3 (10.0)</td>
</tr>
<tr>
<td>Moderate</td>
<td>22 (68.8)</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td>Poorly</td>
<td>2 (6.2)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>1 (3.1)</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Pathologic tumor status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>11 (34.4)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>T1a/T1b</td>
<td>1/8</td>
<td>0/8</td>
</tr>
<tr>
<td>T2</td>
<td>6 (18.8)</td>
<td>2 (6.7)</td>
</tr>
<tr>
<td>T3</td>
<td>15 (46.9)</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>Pathologic nodal status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>14 (43.8)</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>N2</td>
<td>13 (40.6)</td>
<td>9 (30.0)</td>
</tr>
<tr>
<td>N3</td>
<td>5 (15.6)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Pathologic staging</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>9 (28.1)</td>
<td>11 (36.7)</td>
</tr>
<tr>
<td>III</td>
<td>23 (71.9)</td>
<td>19 (63.3)</td>
</tr>
<tr>
<td>IIIA/IIIB/IIIC</td>
<td>10/8/5</td>
<td>8/7/4</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>6 (18.8)</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>Middle</td>
<td>11 (34.4)</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Lower</td>
<td>15 (46.9)</td>
<td>12 (26.7)</td>
</tr>
</tbody>
</table>

Values are presented as number (%). LV5FU2, leucovorin and 5-fluorouracil; FOLFOX, fluorouracil, leucovorin, and oxaliplatin; ECOG, Eastern Cooperative Oncology Group.

(Table 3). Middle esophageal cancers were associated with better DFS than upper esophageal cancers (p=0.032).

3. Toxicity

All enrolled patients were monitored for adverse effects. The overall incidence of grade ≥ 3 AEs was 9.4% (3 of 32 patients) in the LV5FU2 group and 23.3% (7 of 30 patients) in the FOLFOX group (Table 4). The most common AEs experienced in both treatment arms were neutropenia, thrombocytopenia, nausea, diarrhea, and anorexia. Grade 3 or higher neutropenia was more frequent in patients in the FOLFOX arm compared with the LV5FU2 arm (20.0% vs. 3.1%), but no patients exhibited febrile neutropenia. The addition of oxaliplatin resulted in more frequent fatigue and peripheral neuropathy. Grade 3 aspiration events occurred in one case in each arm but were not related to aspiration pneumonia.

4. Treatment and drug delivery

The median number of chemotherapy cycles administered was eight (range, 4 to 8) in the LV5FU2 group and eight (range, 2 to 8) in the FOLFOX group. Treatment completion rates were similarly high in both groups (84% in the LV5FU2 arm and 70% in the FOLFOX arm, p=0.230). The delivered
Table 2. Pattern of failure

<table>
<thead>
<tr>
<th>Site of recurrence</th>
<th>LV5FU2 (n=15)</th>
<th>FOLFOX (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loco-regional</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical lymph node</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mediastinal lymph node</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal lymph node</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node, others</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Distant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Bone</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Both local and distant</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

LV5FU2, leucovorin and 5-fluorouracil; FOLFOX, fluorouracil, leucovorin, and oxaliplatin.

3.1% of all patients in the LV5FU2 group and 26.7% in the FOLFOX group experienced delays in the assigned treatment.

Discussion

Prognosis is poor in most patients treated with curative resection of esophageal cancer who present with local and/or distant recurrence during follow-up. One Japanese study showed that two cycles of FP chemotherapy yielded a much greater survival benefit when administered before surgery than after in locally advanced esophageal SCC [13]. In addition, a recent meta-analysis showed that neoadjuvant CRT (HR, 0.8; p=0.002) and neoadjuvant chemotherapy (HR, 0.89; p=0.051) were associated with increased OS in locally advanced esophageal SCC [14]. Thus, neoadjuvant chemotherapy or CRT has attracted more interest clinically than adjuvant therapy. However, adjuvant FP chemotherapy has also been suggested to improve DFS, especially in patients with pathologically node-positive esophageal SCC, although this benefit did not extend to OS [10]. There are also many clinical situations in which chemotherapy or CRT cannot be administered before surgery and adjuvant therapy is
Table 3. Univariate analysis of the DFS and OS

<table>
<thead>
<tr>
<th>Predictive factor</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age (≥60 yr vs. &lt;60 yr)</td>
<td>0.88</td>
<td>0.44-1.76</td>
</tr>
<tr>
<td>Group (FOLFOX vs. LV5FU2)</td>
<td>1.32</td>
<td>0.66-2.62</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle vs. upper</td>
<td>0.35</td>
<td>0.14-0.92</td>
</tr>
<tr>
<td>Lower vs. upper</td>
<td>0.58</td>
<td>0.26-1.30</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate vs. well</td>
<td>0.57</td>
<td>0.24-1.35</td>
</tr>
<tr>
<td>Poorly vs. well</td>
<td>0.54</td>
<td>0.16-1.85</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 vs. T1</td>
<td>0.86</td>
<td>0.18-4.12</td>
</tr>
<tr>
<td>T3 vs. T1</td>
<td>3.21</td>
<td>1.42-7.25</td>
</tr>
<tr>
<td>N stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 vs. N1</td>
<td>1.55</td>
<td>0.71-3.37</td>
</tr>
<tr>
<td>N3 vs. N1</td>
<td>3.03</td>
<td>1.19-7.74</td>
</tr>
<tr>
<td>Stage (III vs. II)</td>
<td>2.91</td>
<td>1.19-7.14</td>
</tr>
</tbody>
</table>

DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; FOLFOX, fluorouracil, leucovorin, and oxaliplatin; LV5FU2, leucovorin and 5-fluorouracil.

Table 4. Toxicity profile

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>LV5FU2 (n=32)</th>
<th>FOLFOX (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any grade</td>
<td>G3-4</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>10 (31.2)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anemia</td>
<td>9 (28.1)</td>
<td>-</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>2 (6.2)</td>
<td>-</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7 (21.9)</td>
<td>-</td>
</tr>
<tr>
<td>Non-hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (43.8)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (15.6)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>12 (37.5)</td>
<td>-</td>
</tr>
<tr>
<td>Constipation</td>
<td>2 (6.2)</td>
<td>-</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>2 (6.2)</td>
<td>-</td>
</tr>
<tr>
<td>Mucositis</td>
<td>6 (18.8)</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>4 (12.5)</td>
<td>-</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1 (3.1)</td>
<td>-</td>
</tr>
<tr>
<td>Anorexia</td>
<td>12 (37.5)</td>
<td>-</td>
</tr>
<tr>
<td>Pruritus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hand-foot syndrome</td>
<td>1 (3.1)</td>
<td>-</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (12.5)</td>
<td>-</td>
</tr>
<tr>
<td>Insomnia</td>
<td>4 (12.5)</td>
<td>-</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>1 (3.1)</td>
<td>-</td>
</tr>
<tr>
<td>Skin rash</td>
<td>2 (6.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as number (%). LV5FU2, leucovorin and 5-fluorouracil; FOLFOX, fluorouracil, leucovorin, and oxaliplatin.
required after curative resection. Therefore, exploring which regimen is appropriate for adjuvant chemotherapy in patients undergoing curative resection for node-positive esophageal SCC could provide important clinical information.

The optimal chemotherapeutic agents and adequate number of cycles of adjuvant chemotherapy for node-positive esophageal SCC have not been established thus far, and the most commonly used FP regimen is associated with significant toxicity [11,15]. Given that many esophageal cancer patients are elderly and therefore sensitive to toxic chemotherapy after operation, we investigated the clinical role of an adjuvant FOLFOX regimen for esophageal SCC and compared it with a potentially less toxic regimen, 5-FU/leucovorin. In esophageal cancer, FOLFOX chemotherapy was introduced for use in patients with advanced or metastatic disease [16]. FOLFOX chemotherapy in the definitive CCRT was also recently shown to be more convenient and less toxic than a 5-FU plus cisplatin regimen [15].

However, in the present study, adjuvant FOLFOX chemotherapy did not improve DFS or OS relative to 5-FU/leucovorin chemotherapy but was instead related to a slightly increased incidence of neutropenia and dose reductions or delays. A possible explanation for this negative result could be that over 50% of the enrolled patients had advanced nodal disease (N2, N3); thus, adjuvant treatment with chemotherapy alone may be insufficient to control locoregional recurrence (66.7% for local recurrence, 33.3% for distant recurrence). Hsu et al. [17] suggested that postoperative radiotherapy could improve OS for patients with advanced nodal disease. Adjuvant 5-FU/leucovorin was also better at preventing disease recurrence than we expected, with 3-year DFS and OS rates of 46% and 57%, respectively, which were similar to the results of a previous trial using adjuvant FP [13,18]. Furthermore, many patients in the FOLFOX group did not complete the planned eight cycles of chemotherapy, and eight cycles of FOLFOX adjuvant chemotherapy might be insufficient to demonstrate the efficacy.

It should be noted that our study had some limitations. The LV5FU2 regimen used for the control arm is not a standard treatment. Although there is no consensus on optimal adjuvant chemotherapy for resected esophageal SCC, cisplatin-based combinations remain the most commonly used regimens. Recently, newer drugs such as taxane (paclitaxel, docetaxel) and platinum analogues (carboplatin) have been investigated for esophageal cancer in various settings. Neoadjuvant CCRT with paclitaxel plus carboplatin followed by surgery led to marked increases in OS in esophageal SCC [7].

Conclusion

In summary, the results of the present study suggest that postoperative chemotherapy with a FOLFOX regimen does not result in improved DFS compared with a LV5FU2 regimen for patients who undergo curative resection of pathologically node-positive esophageal SCC. Further prospective trials on adjuvant chemotherapy in a selected population undergoing esophageal cancer resection are needed.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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Impact of Resection Margin Distance on Survival of Pancreatic Cancer: A Systematic Review and Meta-Analysis

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Purpose
While curative resection is the only chance of cure in pancreatic cancer, controversies exist about the impact of surgical margin status on survival. Non-standardized pathologic report and different criteria on the R1 status made it difficult to implicate adjuvant therapy after resection based on the margin status. We evaluated the influence of resection margins on survival by meta-analysis.

Materials and Methods
We thoroughly searched electronic databases of PubMed, EMBASE, and Cochrane Library. We included studies reporting survival outcomes with different margin status: involved margin (R0 mm), margin clearance with ≤ 1 mm (R0-1 mm), and margin with > 1 mm (R>1 mm). Hazard ratio (HR) for overall survival was extracted, and a random-effects model was used for pooled analysis.

Results
A total of eight retrospective studies involving 1,932 patients were included. Pooled HR for overall survival showed that patients with R>1 mm had reduced risk of death than those with R0-1 mm (HR, 0.74; 95% confidence interval [CI], 0.61 to 0.88; p=0.001). In addition, patients with R0-1 mm had reduced risk of death than those with R0 mm (HR, 0.81; 95% CI, 0.72 to 0.91; p < 0.001). There was no heterogeneity between the included studies (I² index, 42% and 0%; p=0.10 and p=0.82, respectively).

Conclusion
Our results suggest that stratification of the patients based on margin status is warranted in the clinical trials assessing the role of adjuvant treatment for pancreatic cancer.

Key words
Meta-analysis, Pancreatic neoplasms, Resection margin, Systematic review

Introduction
Pancreatic ductal adenocarcinoma (PDAC) causes fourth leading cancer death in the United States in year 2014 [1]. Although only 10% to 20% has chance of resection, it is the only treatment that promises curing the disease [2]. Regarding the margin status after pancreatectoduodenectomy (PD) for PDAC, controversy exists about the impact of microscopic resection margin involvement (R1). Several studies have reported that it is an independent prognostic factor for poor long term survival [3-6], but not in other studies [7,8]. Main reason of this controversy partly originated from the issues of standardization of pathologic examination [9,10]. The standardization of pathological examination increased the rate of R1 resections after PD from 20% to 50% [11-13], and even to > 70% [14-17]. Moreover, there is ongoing debate concerning the definition of R1. According to the International Union Against Cancer (UICC) and the College of American Pathologists (CAP) reporting guidelines, R1 is...
defined as the microscopic presence of tumor cells at definite
resection margin [18,19]. However, the Royal College of
Pathologists (RCP) in the UK recommends that cases with
microscopic evidence of tumor extension to within 1 mm
from a circumferential margin or surface of the pancreatic
resection specimen should be classified as R1 [20].

Accurate assessment of R1 is clinically important, not only
because it provides prognostic information but stratification
within the setting of randomized controlled trials of adjuvant
therapy is based partly upon margin positivity. Appropriate
identification of those patients who would most benefit is
critical in the improvement of the management for PDAC.

Here, we conducted a systematic review and meta-analysis
to assess the impact of resection margin distance on the sur-
vival of the patients with PDAC. We intended to identify sur-
vival outcomes with different margin status: involved
margin (R0 mm), margin clearance with ≤ 1 mm (R0-1 mm),
and margin with > 1 mm (R>1 mm).

Materials and Methods

1. Data sources and search strategy

We performed a systematic literature review of published
articles and unpublished abstracts, which reported overall
survival of the patients with different surgical margin dis-
tance after resection of pancreatic cancer. Comprehensive
searches were performed in the databases of PubMed,
EMBASE, and Cochrane Library (last search update on 6
April 2015). The following key words with their correspon-
ding MeSH terms were used: combined to maximize sensi-
tivity: [(pancreatic cancer[MESH] OR (pancrea* AND
cancer) OR (pancrea* AND adenocarcinoma)][All Fields]
AND [margin][TIAB]. Additionally, the references cited in
retrieved articles were scrutinized by manual search.

2. Study selection

Two authors (K.S.K. and K.K.) independently reviewed
search results. Inclusion criteria were observational studies
that investigated survival outcomes according to different
resection margin distance following PD for PDAC: involved
margin (R0 mm), margin clearance with ≤ 1 mm (R0-1 mm),
and margin with > 1 mm (R>1 mm). To limit heterogeneity
across the studies and to get more clinically meaningful
results, we used following exclusion criteria: (1) studies that
included pancreatic malignancy other than adenocarcinoma,
(2) review articles or case reports, (3) studies that did not
report surgical margin status, and (4) studies that did not
provide sufficient data to acquire hazard ratio (HR) and its
95% confidence interval (CI) of different margin status for
overall survival (OS). Manual search for references of the
eligible studies was performed to minimize potential missing
data.

3. Data extraction

Data were extracted independently by two authors (K.S.K.
and K.K.), and discrepancies were resolved by consensus.
The following details were extracted: name of first author,
institution, country, study period, publication year, number
of participants, surgery type, T stage, N stage, adjuvant treat-
ment details, follow-up period, and pathologic examination
protocol.

4. Risk of bias assessment

Risk of bias was assessed by Risk of Bias Assessment tool
for Non-randomized Studies (RoBANS), which was vali-
dated for assessing the risk of bias for nonrandomized stud-
ies [21]. It contains six domains: selection of participants,
confounding variables, intervention measurement, blinding
of outcome assessment, incomplete outcome data, and selec-
tive outcome reporting. Two authors (K.S.K. and K.K.) inde-
dependently assessed and disagreements were resolved by
consensus.

5. Statistical analysis

The OS outcome was measured in terms of the time-
to-event HR of R0 mm compared with R0-1 mm and R>1
mm with R>1 mm. HR as well as its 95% CI was directly
extracted from the text or estimated using the published
Kaplan-Meier curves using the methods of Tierney et al. [22].
Pooled HR was calculated using the random-effects model
and presented with forest plots. Two-sided p-values less than
0.05 were considered statistically significant. A chi-square
statistic was used to test for statistical heterogeneity, and I²
statistic was also calculated to evaluate the extent of variabil-
ity attributable to statistical heterogeneity between trials. To
assess the publication bias, we applied funnel plot method
together with the Egger’s regression test. All statistical anal-
ysis was done using RevMan 5.3 analysis software (Cochrane
Collaboration, Copenhagen, Denmark).
Fig. 1. Study selection process.

Results

1. Selecting studies and characteristics of included studies

Two thousand eight hundred ninety-four studies were obtained from the searches of electronic database using our searching strategy. A total of 106 articles were reviewed in detail. Eight studies were finally selected into this meta-analysis [16,17,23-28]. All of studies were retrospective observational cohort studies reporting survival outcome of resected pancreatic cancer at single center. Two studies were presented in abstract form only [25,28]. The details of study selection are shown in Fig. 1. Two studies were reported from Unites States, two studies from UK, two studies from Japan, one study from Germany, and one study from Australia. The patients with R0 mm or R0-1 mm constitute 27.4% to 78.5%. Regarding surgical treatments, most of the patients underwent PD. In three studies, patients treated with distal pancreatectomy were included with the proportion of 19%, 15.3%, and 20.2%, respectively [23,26,27]. Only two studies described the proportion of the T and N stage according to the resection margin status [26,27]. In four studies, the percentage of the patients treated with adjuvant or neoadjuvant therapy was described. Basic characteristics of included studies are shown in Table 1. Details of pathologic evaluation of margin status are listed in Table 2. Details of pathologic examination protocol were described in six studies [14,20,29]. A summary of the risk of bias assessment is provided in Table 3.

2. Impact of resection margin distance on survival

We calculated overall pooled HR for OS with a random effects model. Chang et al. [23] reported disease-specific survival (DSS) instead of OS. Under the assumption that the DSS outcome might not differ from the OS, we pooled these data with the OS outcomes of the other seven studies. When we compared R>1 mm and R0-1 mm, R>1 mm had reduced risk of death than R0-1 mm (HR, 0.74; 95% CI, 0.61 to 0.88; p=0.001) (Fig. 2A). There was no heterogeneity between the included studies (I² index=42%, p=0.10). When we compared R0 mm with R0-1 mm, R0-1 mm had reduced risk of death (HR, 0.81; 95% CI, 0.72 to 0.91; p<0.001) (Fig. 2B). There was no heterogeneity among studies (I² index=0%, p=0.82).
### Table 1. Characteristics of included studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Institution</th>
<th>Study period</th>
<th>No. (%)</th>
<th>Surgical treatment</th>
<th>T stage</th>
<th>N+ (%)</th>
<th>Adjuvant treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al. (2009) [16]</td>
<td>Liverpool (UK)</td>
<td>1997-2007</td>
<td>71 (43.6) 57 (35.0) 35 (21.5)</td>
<td>PPPD (90.2%), Whipple (9.8%)</td>
<td>T3/4 (85.3%)</td>
<td>78.5</td>
<td>NA</td>
</tr>
<tr>
<td>Chang et al. (2009) [23]</td>
<td>Sydney (Australia)</td>
<td>1990-2007</td>
<td>132 (36.2) 56 (15.3) 177 (48.5)</td>
<td>Whipple (80.8%), left side pancreatectomy (19.2%)</td>
<td>&gt; 2 cm (77.0%)</td>
<td>59.5</td>
<td>Adjuvant CTx (26.3%), RT (5.8%)</td>
</tr>
<tr>
<td>Janot et al. (2012) [24]</td>
<td>Bochum (Germany)</td>
<td>2007-2009</td>
<td>5 (8.1) 12 (19.4) 45 (72.6)</td>
<td>Whipple (11.3%), PPPD (69.3%), TP (19.4%)</td>
<td>T3/4 (91.9%), &gt; 2.5 cm (66.1%)</td>
<td>79.0</td>
<td>NA</td>
</tr>
<tr>
<td>Thomay et al. (2012) [25]</td>
<td>Philadelphia (USA)</td>
<td>1991-2011</td>
<td>108 (36.4) 54 (18.2) 135 (45.5)</td>
<td>PD (100%)</td>
<td>NA</td>
<td>NA</td>
<td>Neoadjuvant CRT (34%)</td>
</tr>
<tr>
<td>Jamieson et al. (2013) [17]</td>
<td>Glasgow (UK)</td>
<td>1996-2011</td>
<td>111 (51.2) 46 (21.2) 60 (27.6)</td>
<td>PD (100%)</td>
<td>T3/4 (90.3%), &gt; 3 cm (50.7%)</td>
<td>80.2</td>
<td>Adjuvant therapy (47.0%), neoadjuvant CTx (0.9%)</td>
</tr>
<tr>
<td>Sugiura et al. (2013) [26]</td>
<td>Sizuoka (Japan)</td>
<td>2002-2010</td>
<td>34 (16.3) 40 (19.2) 134 (64.4)</td>
<td>PD (78.8%), DP (20.2%), TP (1.0%)</td>
<td>T3/4 (64.7%), &gt; 3 cm (47.1%)</td>
<td>69.2</td>
<td>Adjuvant CTx (84.6%), RT (11.5%)</td>
</tr>
<tr>
<td>Konstantinidis et al. (2013) [27]</td>
<td>MGH (USA)</td>
<td>1993-2001</td>
<td>157 (31.7) 169 (34.1) 170 (34.3)</td>
<td>PD (83.1%), DP (15.3%), TP (1.4%)</td>
<td>T3/4 (88.5%)</td>
<td>70.0</td>
<td>NA</td>
</tr>
<tr>
<td>Hashimoto et al. (2013) [28]</td>
<td>Wakayama (Japan)</td>
<td>2002-2012</td>
<td>30 (24.2) 38 (30.6) 56 (45.2)</td>
<td>PD (100%)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

R0 mm, involved margin; R0-1 mm, margin clearance with ≤ 1 mm; R>1 mm, margin with > 1 mm; PPPD, pylorus preserving pancreatectoduodenectomy; NA, not applicable; CTx, chemotherapy; RT, radiotherapy; TP, total pancreatectomy; PD, pancreatectoduodenectomy; CRT, chemoradiation; DP, distal pancreatectomy.
### Table 2. Pathologic examination protocol

<table>
<thead>
<tr>
<th>Study</th>
<th>Protocol</th>
<th>Evaluated margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al. (2009) [16]</td>
<td>RCP [20]</td>
<td>Pancreatic transection margin&lt;br&gt;Medial (or superior mesenteric vessel) margin&lt;br&gt;Posterior margin&lt;br&gt;Proximal duodenal (or gastric) margin&lt;br&gt;Distal duodenal margin&lt;br&gt;Common bile duct margin</td>
</tr>
<tr>
<td>Chang et al. (2009) [23]</td>
<td>Institutional</td>
<td>Pancreatic neck margin&lt;br&gt;Portal vein/superior mesenteric vein margin&lt;br&gt;Superior mesenteric artery / retroperitoneal (uncinate) margin&lt;br&gt;Bile duct margin&lt;br&gt;Proximal gastric/duodenal margin&lt;br&gt;Distal duodenal margin</td>
</tr>
<tr>
<td>Thomay et al. (2012) [25]</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jamieson et al. (2013) [17]</td>
<td>RCP [20,31]</td>
<td>Posterior margin&lt;br&gt;Anterior margin&lt;br&gt;Medial margin&lt;br&gt;Pancreatic transection margin</td>
</tr>
<tr>
<td>Konstantinidis et al. (2013) [27]</td>
<td>Staley et al. [29]</td>
<td>Common bile duct margin&lt;br&gt;Pancreatic transection (neck) margin&lt;br&gt;Posterior / retroperitoneal margin&lt;br&gt;Uncinate (superior mesenteric artery) margin</td>
</tr>
<tr>
<td>Hashimoto et al. (2013) [28]</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

RCP, Royal College of Pathologist; LEEPP, Leeds Pathology Protocol; NA, not applicable.

### 3. Publication bias

A funnel plot of the effect size for each subgroup category of the trial against the precision showed no asymmetry (Fig. 3). Egger’s regression test for potential publication bias yielded no potential unpublished studies. (Egger’s test, p=0.373 for between R>1 mm and R0-1 mm, p=0.852 for between R0-1 mm and R0 mm, respectively).

### Discussion

The reported R1 rates after PD for PDAC showed a high variation ranging from 17% to 85%. Previous studies which reported low R1 resection rates of less than 20% had local recurrence rate of 60%-80% [7,30,31]. These findings indicated a considerable underestimation of the true R1 status. Lack of a standardized pathological examination protocol and different definitions of resection margin are probably the main reasons for the high variation in reported R1 rates. In this meta-analysis, six studies explained details of standardized pathological examination. Eventually, when ‘1 mm rule’ was applied, R1 rates were greater than 35.6% except a study by Janot et al. [24] which had low number of patients.

Controversy exists over the anterior surface of PD specimens as to whether it should be regarded as part of the resection margin. Anterior surface as a resection margin was recommended in Japan [32,33] and in Europe [14]. Because the surgeon does not transect any tissues in this area, however, anterior surface was not regarded a true resection mar-
Table 3. A summary of risk of bias assessment using the Risk of Bias Assessment Tool for Non-randomized Studies (RoBANS)

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Confounding variables</th>
<th>Performance</th>
<th>Detection</th>
<th>Attrition</th>
<th>Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campbell et al. (2009) [16]</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Chang et al. (2009) [23]</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Janot et al. (2012) [24]</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Thomay et al. (2012) [25]</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
<tr>
<td>Jamieson et al. (2013) [17]</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Sugiuara et al. (2013) [26]</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Konstantinidis et al. (2013) [27]</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Low</td>
</tr>
<tr>
<td>Hashimoto et al. (2013) [28]</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

gin. Some authors proposed that assessment of this margin should be excluded from a standardized pathological examination protocol [15], or that the “0 mm” clearance rule should be used [9,34]. While most common involved margin in the pancreatic cancer is the medial or posterior resection margin [15], Jamieson et al. [35] reported that R1 at anterior surface made up 12.8% of the R1 cases and that these patients presented favorable outcome than those with R1 at medial or transection margin. In this meta-analysis, anterior surface was considered a resection margin in only two studies [17,24].

The ‘1 mm rule’ has been adopted from the association between the circumferential margin status and local recurrence of the rectal cancer. Verbeke et al. [36] reported that tumor growth in pancreatic head cancers is more dispersed than in rectal cancer, claiming that 1 mm definition needs to be considered. Single institutional studies including encompassed ones in this meta-analysis reported the association of the margin clearance with OS. Chang et al. [23] and Jamieson et al. [17] demonstrated that margin clearance by at least 1.5 mm identified a subgroup of patients which may potentially achieve long-term survival. Gebauer et al. [37] reported that margin clearance of 2 mm or greater as an independent prognostic factor for OS. However, because each study had limited number of patients, any conclusive result could not be drawn. Through the pooled HR of current meta-analysis including 1,932 patients, we could verify that R=1 mm had reduced risk of death than R0=1 mm, and R0=1 mm also had reduced risk of death than R0 mm.

While adjuvant chemotherapy is currently the standard treatment for patients following a potentially curative PD for PDAC in Europe, chemoradiotherapy as an adjuvant treatment is considered based on the margin status. Two recent meta-analyses have suggested that patients with R1 status appear to benefit from postoperative chemoradiotherapy [8,38]. Chang et al. [23] noted that patients with close resection margins (<1.5 mm) may have a better response to adjuvant radiotherapy compared with involved margins (R0 mm) as a result of the probable low volume of residual local disease, and potentially constitute a subgroup that is most likely to have the greatest benefit. In conjunction with these results, our results could be used in identifying a subgroup that will benefit from radiotherapy after PD for PDAC.

Several studies examined the effect of neoadjuvant treatment on resection margin status [7,39-42]. Katz et al. [40] reported that patients who received chemoradiation had longer superior mesenteric artery margin distances than those who did not. In the study by Delpero et al. [42], neoadjuvant treatment was correlated with a reduced risk for a positive posterior margin. In contrary, Raut et al. [7] reported that neoadjuvant therapy was not a statistically significant predictor of margin status. In one study by Thomay et al. [25] included in this meta-analysis, neoadjuvant treatment was given to 34% of the patients. The patients with R0=1 mm had similar risk of death compared to R=1 mm, and 34% reduction of death compared to R0 mm in that study. One might argue that high proportion of neoadjuvant treatment than other studies might explain the result. However, the hypothesis that neoadjuvant treatment could decrease the adverse effect of R1 is not evidenced by randomized trials. Further studies to investigate the role of neoadjuvant treatment using a standardized pathological examination protocol are warranted.

Major limitation of our study is that included studies did not provide adequate information on the distribution of prognostic factors according to margin status. Given that most of the patients were of T3-4 and/or lymph node involvement, stratification according to resection margin sta-
Fig. 2. Forest plot for HR of the R>1 mm and R0-1 mm margin (A) or R0-1 mm and R0 mm margin (B). R0 mm, involved margin; R0-1 mm, margin clearance with ≤1 mm; R>1 mm, margin with >1 mm; HR, hazard ratio; CI, confidence interval.
Fig. 3. Funnel plot of the included studies regarding R>1 mm and R0-1 mm margin (A) or R0-1 mm and R0 mm margin (B).

Conclusion

While existing controversy about R1 status in the resected pancreatic cancer, our meta-analysis suggests that patients with resection margin with 0-1 mm had reduced risk of death than those with involved margin status, and greater risk of death than those with > 1 mm margin. Based on these result, stratification of patients based on margin distance with standardized pathological examination should be implicated in the future clinical trial of adjuvant therapy for pancreatic cancer.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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37. Gebauer F, Tacheczy M, Vashist YK, Marx AH, Yekubes E, Izbicki JR, et al. Resection margin clearance in pancreatic can-
Surrogate Endpoints in Second-Line Trials of Targeted Agents in Metastatic Colorectal Cancer: A Literature-Based Systematic Review and Meta-Analysis

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Purpose
The purpose of this study was to evaluate progression-free survival (PFS) and objective response rate (ORR) as surrogate endpoints of overall survival (OS) in modern clinical trials investigating the efficacy of targeted agents in the second-line treatment of metastatic colorectal cancer (mCRC).

Materials and Methods
A systematic search of literature pertaining to randomized phase II and III trials evaluating targeted agents as second-line treatments for mCRC was performed. The strength of the correlation between both PFS and ORR and OS was assessed based on the Pearson’s correlation coefficient (R) and the coefficient of determination (R²).

Results
Twenty trials, including a total of 7,571 patients, met the search criteria. The median duration of post-progression survival (PPS) was 7.6 months. The median differences between experimental and control arms were 0.65 months (range, −2.4 to 3.4) for the median PFS and 0.7 months (range, −5.8 to 3.9) for the median OS. PFS and ORR showed moderate (R=0.734, R²=0.539, p < 0.001) and poor correlation (R=0.169, R²=0.029, p=0.476) with OS, respectively. No differences between anti-angiogenic agents and other drugs were evident.

Conclusion
Targeted agents investigated in the second-line treatment of mCRC provided minimal PFS gains translating into modest OS improvements. Considering both the moderate correlation between PFS and OS and the short duration of PPS, the OS should remain the preferred primary endpoint for randomized clinical trials in the second-line treatment of mCRC.

Key words
Colorectal neoplasms, Biomarkers, Molecular targeted therapy
Introduction

The choice of the primary endpoint is essential to the design of clinical trials. While overall survival (OS) actually reflects the ultimate goal of cancer treatments, and is therefore regarded as a preferred choice in the metastatic setting, the surrogacy of other endpoints was investigated in different malignancies. The identification of valuable surrogate endpoints, which are potentially reachable in a shorter time and with a lower number of patients, would allow notable decreases in trial duration, thus expediting drug development and making new options more rapidly available for cancer patients.

With regard to metastatic colorectal cancer (mCRC), the reliability of response parameters and progression-free survival (PFS) during first-line treatments as surrogate endpoints of OS has previously been evaluated. While surrogacy for OS has not been formally proven for the objective response rate (ORR) [1,2], nor for the new parameter of early tumor shrinkage [3], PFS was shown to achieve strong surrogacy for OS in trials conducted before the introduction of targeted agents [2,4]. In a recently published literature-based analysis of surrogate endpoints in second-line treatment for mCRC, PFS was considered a reliable surrogate for OS [5]. However, about half of the clinical trials included in that systematic review compared chemotherapy only regimens, without targeted agents. In recent years, the adoption of new drugs with different mechanisms of action and the availability of multiple effective treatments after progression has enabled extension of post-progression survival (PPS), and is challenging the role of PFS as a surrogate of OS. Even though a previous analysis suggested that in modern trials OS could be better associated with PPS than with PFS [6], significant surrogacy for PFS was confirmed, justifying its adoption as a primary endpoint in first-line studies in mCRC [7-9]. However, in a systematic review and meta-analysis of 101 randomized controlled trials conducted in advanced colorectal cancer, none of the surrogate endpoints considered (ORR, PFS, time to progression) achieved the level of evidence required to qualify correlation levels as high or excellent by means of common surrogate evaluation tools [10].

In the last few years, several targeted agents have been tested in second- and further-lines of treatment and shown to produce significant, although only incremental, gains in OS. Today, as previously shown for first-line treatments, the effectiveness of new drugs in third and later lines might dilute the impact of second-line regimens on OS. Moreover, the frequent adoption of cross-over designs, especially in clinical settings with no other effective options, deeply influences OS findings, making the choice of earlier endpoints extremely appealing.

The present literature-based analysis was conducted to evaluate the correlation of both PFS and ORR with OS in modern clinical trials investigating the efficacy of targeted agents in the second-line treatment of mCRC. Since the relevance of surrogate endpoints may differ according to the mechanisms of action of investigated drugs, this analysis also separately evaluated the correlation of PFS and ORR with OS for anti-angiogenic agents relative to drugs with other mechanisms of action.

Materials and Methods

1. Literature search

A literature search was performed in October of 2015 to identify all randomized phase II and phase III trials evaluating molecular-targeted agents as second-line treatments for advanced colorectal cancer. The literature search was performed using PubMed, and the following keywords: “(colorectal cancer) AND (pretreated OR “previously treated" OR “second line") AND random". Following a comment by a reviewer, a second search “(colorectal cancer) AND (pretreated OR “previously treated" OR "second line") AND randomized controlled trial [Publication type]" was performed to verify that all records included in the latter search had already been included in the former search. References of the selected articles were also checked to identify further eligible trials. Moreover, the proceedings of the American Society of Clinical Oncology (ASCO) annual meeting and European Society of Medical Oncology meeting were searched from 2012 onwards for relevant abstracts. When more than one report describing the results of the same trial was available, the most recent information (corresponding to a longer follow-up and a higher number of events) was utilized. Trials randomizing patients to receive or not receive an anti-epidermal growth factor receptor monoclonal antibody were included only if results in the RAS (or at least KRAS) wild-type subgroup were available.

2. Data abstraction

For each eligible trial, the following data were collected, if available:
- Study phase (II or III).
- Details of study treatment: control arm; experimental arm (or arms if more than one experimental treatment). Control arms were identified based on the null hypothesis of the statistical design underlying each single trial as reported in full manuscripts or presented abstracts.
Details regarding cross-over (administration of experimental treatment to patients assigned to the control arm after disease progression).
- Study primary endpoint.
- Patients’ enrolment: number of enrolled patients, number of patients assigned to control arm, number of patients assigned to experimental arm.
- ORR: proportion of objective responses in the control arm, proportion of objective responses in the experimental arm; relative risk of response (calculated as the ratio between the response rate in the experimental arm and in the control arm).
- PFS: median PFS in the control arm, median PFS in the experimental arm, hazard ratio (HR) with 95% confidence interval, p-value.
- OS: median OS in the control arm, median OS in the experimental arm, HR with 95% confidence interval, p-value.
- PPS: absolute PPS was calculated as the difference between median OS and median PFS; relative PPS was calculated as the ratio between median PFS and median OS. For instance, in a treatment arm with a median PFS of 4 months and a median OS of 10 months, absolute PPS was 6 months (10−4) and relative PPS was 60% (6/10).

For trials with more than two treatment arms, multiple records were completed, one for each comparison.

Two investigators independently abstracted the data from the publications, and subsequently compared their results. All data were checked for internal consistency, and disagreements were resolved by discussions among the investigators.

3. Statistical analysis

To analyze the correlation between PFS and OS, two different regression analyses were performed: (1) correlation between the HR for PFS and HR for OS and (2) correlation between the difference in median PFS and the difference in median OS between arms. Similarly, to analyze the correlation between ORR and OS, two different regression analyses were performed: (1) correlation between the relative risk of response and HR for OS and (2) correlation between the difference in ORR between arms and the difference in median OS between arms.

All analyses were weighted by the sample size of each comparison. In the case of trials with two experimental arms and a single control arm [11-14], two separate comparisons were analyzed (each experimental arm versus the control arm). However, to avoid double-counting of the patients enrolled in the control arm and the risk of clustered data, each comparison was given a lower weight that was obtained by equally dividing the total number of patients of the control arm between the two comparisons.

In each analysis, the strength of the correlation was evaluated by calculating the Pearson’s correlation coefficient (R) and the coefficient of determination (R²). Pearson’s R is a simple measure of the linear correlation between two variables, giving a value between 1 and −1, where 1 is a total positive correlation, 0 is the absence of correlation, and −1 is a total negative correlation. The coefficient of determination is such that 0 ≤ R² ≤ 1. Although there are no specific cut-offs to define a moderate or strong correlation, a higher R² score indicates a stronger association.

Correlations were graphically described by bubble plots, where each bubble represents a comparison between one experimental arm and one control arm, with bubble size proportional to the sample size of each comparison. As all analyses were weighted by the sample size of each trial/comparison, weighted least-squares regression lines were calculated and reported in each graph.

Exploratory subgroup analyses were performed according to the type of experimental drug tested (anti-angiogenic drugs vs. other drugs).

Statistical analyses were conducted using SPlus (S-PLUS 6.0 Professional, release 1, Insightful Corporation, Seattle, WA) and SPSS ver. 22.0 (IBM Corp., Armonk, NY). Graphs were realized using SigmaPlot (Systat Software, San Jose, CA). For all analyses, a p-value of < 0.05 was considered statistically significant.

Results

1. Trial characteristics

Overall, 20 trials were identified (Fig. 1), nine phase III trials and 11 randomized phase II trials (Table 1) [11-30]. A total of 7,571 patients were enrolled in these trials, and the median number of enrolled patients was 197 (range, 75 to 1,226). The primary endpoint was PFS in 12 trials (60%) [11-15,18,19, 21-24,30], OS in six trials (30%) [16,20,26-29] and ORR in one trial (5%) [17]. In one trial (5%), PFS and OS were co-primary endpoints [25]. Four trials had three treatment arms, with two comparisons between each of the two experimental arms and the single control arm [11-14]. In one trial, there were four arms (two experimental arms and two control arms) with two separate comparisons [15]. Overall, 25 comparisons were recorded (Table 1).

Information regarding cross-over was not available for most reports (19 out of 25 comparisons). In the six reports with details about subsequent administration of experimental drugs (or drugs with the same mechanism of action) in patients assigned to control arms, cross-over was quite neg-
eligable (median proportion, 3.5%; range, 0% to 32%).

2. Outcomes

Based on all comparisons with available information, the median value of the OS in the 25 experimental arms was 13.1 months (range, 9.6 to 21.4), and the median value of the OS in the control arms was 13.9 (range, 8.8 to 19.8). The median difference between experimental and control arms was equal to 0.7 months (range, −5.8 to 3.9). In the 21 comparisons with available information, the median HR for OS was 0.90 (range, 0.69 to 1.57).

Based on all comparisons and available information, the median value of the PFS in the 24 experimental arms was 6.4 (range, 2.1 to 8.5), and the median value of the median PFS in the control arms was 5.4 (range, 2.4 to 9.0). The difference in median values between the experimental and control arms was equal to 0.65 months (range, −2.4 to 3.4). In the 23 comparisons with available information, the median HR for PFS was 0.85 (range, 0.61 to 1.45).

Based on all available information regarding the median OS and median PFS, the median absolute PPS in the experimental arms was 7.6 months (range, 4.4 to 14.6). The relative PPS (expressed as a proportion of OS) ranged from 43.4% to 82.3%, with a median value of 55.7%. In the control arms, the median absolute PPS was 7.6 months (range, 3.6 to 14.3) and
Table 1. Main characteristics of the included trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of patients</th>
<th>Treatments</th>
<th>Primary endpoint</th>
<th>Anti-angio (Y/N)</th>
<th>HR (PFS)</th>
<th>Delta PFS (mo)</th>
<th>HR (OS)</th>
<th>Delta OS (mo)</th>
<th>RR response</th>
<th>Delta ORR (%)</th>
<th>PPS/OS [exp arm–ctr arm] (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bendell et al. [15]</td>
<td>36</td>
<td>Exp arm</td>
<td>FOLFOX + axitinib</td>
<td>Y</td>
<td>1.04 (Y)</td>
<td>1.2</td>
<td>0.69 (3)</td>
<td>0.97 (–0.6)</td>
<td>55.6 (54.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bendell et al. [15]</td>
<td>49</td>
<td>Ctr arm</td>
<td>FOLFOX + bevacizumab</td>
<td>Y</td>
<td>1.27 (–1.2)</td>
<td>1.36 (–2.8)</td>
<td>1.04 (1)</td>
<td></td>
<td>55.8 (56.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bennouna et al. [16]</td>
<td>409</td>
<td>Exp arm</td>
<td>Chemo + bevacizumab</td>
<td>Y</td>
<td>0.68 (1.6)</td>
<td>0.81 (1.4)</td>
<td>1.25 (1)</td>
<td></td>
<td>49.1 (58.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cao et al. [17]</td>
<td>65</td>
<td>Exp arm</td>
<td>FOLFOX + bevacizumab</td>
<td>N</td>
<td>1.01 (0.8)</td>
<td>1.27 (1.4)</td>
<td>1.53 (8.9)</td>
<td>0.70 (12)</td>
<td>63.7 (61.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciardiello et al. [18]</td>
<td>39</td>
<td>Ctr arm</td>
<td>FOLFOX + cetuximab</td>
<td>Y</td>
<td>0.63 (0.1)</td>
<td>0.58 (0.9)</td>
<td>0.78 (3)</td>
<td></td>
<td>5.9 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohn et al. [11]</td>
<td>50</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>0.95 (8)</td>
<td>0.80 (12)</td>
<td>0.99 (3)</td>
<td></td>
<td>9.4 (9.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cunningham et al. [12]</td>
<td>71</td>
<td>Exp arm</td>
<td>FOLFOX + cediranib</td>
<td>Y</td>
<td>1.17 (0.1)</td>
<td>1.00 (2.8)</td>
<td>0.70 (8.1)</td>
<td></td>
<td>57.1 (60.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cunningham et al. [12]</td>
<td>73</td>
<td>Exp arm</td>
<td>FOLFOX + cediranib</td>
<td>Y</td>
<td>0.95 (3)</td>
<td>1.08 (7)</td>
<td>1.00 (0)</td>
<td></td>
<td>62.4 (62.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eng et al. [19]</td>
<td>60</td>
<td>Ctr arm</td>
<td>Cetuximab + anti-</td>
<td>N</td>
<td>0.85 (1)</td>
<td>0.70 (2.9)</td>
<td>1.35 (11.7)</td>
<td></td>
<td>58.1 (56.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gianantonio et al. [20]</td>
<td>286</td>
<td>Exp arm</td>
<td>FOLFOX + bevacizumab</td>
<td>Y</td>
<td>1.24 (2.1)</td>
<td>1.15 (–2)</td>
<td>1.09 (2)</td>
<td></td>
<td>46.1 (53.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hecht et al. [13]</td>
<td>85</td>
<td>Exp arm</td>
<td>FOLFOX + irinotecan</td>
<td>Y</td>
<td>0.61 (2.6)</td>
<td>0.75 (2.1)</td>
<td>2.64 (14.1)</td>
<td></td>
<td>43.4 (56.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hecht et al. [13]</td>
<td>84</td>
<td>Exp arm</td>
<td>FOLFOX + irinotecan</td>
<td>N</td>
<td>0.59 (–0.4)</td>
<td>1.50 (–5.8)</td>
<td>0.59 (–4.1)</td>
<td></td>
<td>48.6 (64.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mehta et al. [21]</td>
<td>50</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>1.17 (0.7)</td>
<td>1.05 (2.8)</td>
<td>1.00 (0)</td>
<td></td>
<td>57.1 (60.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iwamoto et al. [22]</td>
<td>187</td>
<td>Exp arm</td>
<td>FOLFOX + bevacizumab</td>
<td>Y</td>
<td>1.17 (0.1)</td>
<td>1.00 (2.8)</td>
<td>0.70 (8.1)</td>
<td></td>
<td>57.1 (60.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masi et al. [23]</td>
<td>92</td>
<td>Exp arm</td>
<td>FOLFOX / FOLFOX</td>
<td>Y</td>
<td>0.97 (1.8)</td>
<td>0.77 (–1.4)</td>
<td>1.24 (4)</td>
<td></td>
<td>51.8 (67.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O’Neill et al. [14]</td>
<td>50</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>N</td>
<td>0.70 (–0.1)</td>
<td>1.15 (–1.3)</td>
<td>0.56 (–12.3)</td>
<td></td>
<td>53.0 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peeters et al. [24]</td>
<td>95</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>1.23 (–1.7)</td>
<td>0.90 (3.1)</td>
<td>1.24 (14)</td>
<td></td>
<td>70.6 (40.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peeters et al. [25]</td>
<td>205</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>0.70 (1.8)</td>
<td>0.81 (2.3)</td>
<td>2.10 (31)</td>
<td></td>
<td>60.6 (66.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seymour et al. [26]</td>
<td>230</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>0.70 (1.8)</td>
<td>0.81 (2.3)</td>
<td>4.10 (31)</td>
<td></td>
<td>60.6 (66.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tabernero et al. [27]</td>
<td>536</td>
<td>Exp arm</td>
<td>FOLFOX + irinotecan</td>
<td>Y</td>
<td>0.79 (1.2)</td>
<td>0.84 (1.6)</td>
<td>1.07 (0.9)</td>
<td></td>
<td>57.1 (61.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Cutsem et al. [28]</td>
<td>426</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>0.83 (1.4)</td>
<td>1.00 (1.2)</td>
<td>1.06 (1)</td>
<td></td>
<td>57.2 (64.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Cutsem et al. [29]</td>
<td>612</td>
<td>Exp arm</td>
<td>FOLFOX + panitumab</td>
<td>Y</td>
<td>0.76 (2.2)</td>
<td>0.82 (1.4)</td>
<td>1.78 (8.7)</td>
<td></td>
<td>48.9 (61.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vieitez et al. [30]</td>
<td>38</td>
<td>Exp arm</td>
<td>Raltitrexed + gefitinib</td>
<td>Y</td>
<td>1.49 (2.6)</td>
<td>2.3 (8.2)</td>
<td>82.3 (5)</td>
<td></td>
<td>82.3 (5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exp arm, experimental arm; Ctr arm, control arm; Anti-angio, anti-angiogenic agent; HR, hazard ratio; PFS, progression-free survival; OS, overall survival; RR, relative risk; ORR, objective response rate; PPS, post-progression survival; FOLFOX, oxaliplatin, 5-fluorouracil, and leucovorin; FOLFIRI, irinotecan, folic acid, and 5-fluorouracil; Chemo, chemotherapy, combination of fluoropyrimidine and oxaliplatin or irinotecan. aIn the case of trials with two experimental arms and a single control arm [11-14], two separate comparisons were analyzed (each experimental arm versus the control arm), bCoprimary endpoints. cIn KRAS codon 12-13-61 wild-type tumors.
expressed as a proportion of OS, while the relative PPS ranged from 40.9% to 75.0%, with a median value of 60.7% (Table 2). Fig. 2 describes the median PFS and median PPS based on all comparisons included in the analysis, scattered by the type of experimental drug (anti-angiogenics vs. other drugs).

Based on all available information, the median ORR in the 25 experimental arms was 19% (range, 5% to 48%), and the median ORR in the control arms was 12% (range, 0% to 35%). The median difference in the ORR between experimental and control arms was equal to 2.6% (range, –12.3% to 31%). The median relative risk of response was 1.24 (range, 0.59 to 7.00).

3. Association between PFS and OS

Information regarding HRs for PFS and OS was available for 21 trials. Overall, there was a moderate correlation (R=0.734, R²=0.539, p<0.001) (Table 3, Fig. 3A). The slope of the regression line (0.739) suggests that a 0.1 improvement in PFS HR corresponds to a 0.074 improvement in OS HR. The correlation between HRs for PFS and OS was significant for the 13 comparisons investigating anti-angiogenic drugs (R=0.655, R²=0.429, p=0.015) and the eight comparisons investigating other drugs (R=0.857, R²=0.734, p=0.007) (Table 3, Fig. 3B and C). There was no significant interaction between drug categories and the correlation between HRs for PFS and OS (p=0.775) (Table 3).

Similar results were observed when the correlation between PFS and OS was analyzed for both endpoints based on the difference in median values between study arms. This information was available for 24 comparisons (Table 3, S1 Fig. A). Overall, there was a moderate correlation between PFS and OS (R=0.632, R²=0.399, p<0.001). The slope of the regression line (1.065) suggests that a one month increase in the difference in median PFS corresponds to a 1.06 month increase in the difference in median OS. The correlation between PFS and OS based on the difference in median values between study arms was significant for both the 16 comparisons evaluating anti-angiogenic drugs (R=0.651, R²=0.423, p=0.006) and the eight comparisons evaluating other drugs (R=0.724, R²=0.525, p=0.042) (Table 3, S1 Fig. B and C). The interaction between drug categories and the correlation between PFS and OS was not significant (p=0.110) (Table 3).

4. Association between ORR and OS

Information regarding the relative risk of objective response and HR for OS was available for 20 comparisons. Overall, there was a weak correlation that was not statistically significant (R=0.169, R²=0.029, p=0.476) (Table 4, Fig. 4A). The correlation between relative risks of response and HRs for OS was not significant for the 12 comparisons.
evaluating anti-angiogenic drugs ($R=0.361$, $R^2=0.131$, $p=0.249$) or the eight comparisons evaluating other drugs ($R=0.441$, $R^2=0.195$, $p=0.274$) (Table 4, Fig. 4B and C). There was no significant interaction between drug categories and the association between the relative risk of response and the HR for OS ($p=0.654$) (Table 4).

Information regarding the difference in ORR and the median OS between study arms was available for 25 comparisons. Based on these parameters, a weak correlation was found ($R=0.345$, $R^2=0.119$, $p=0.092$) (Table 4, S2 Fig. A). The correlation between response and OS considering the difference in ORR and in the median OS between study arms was weak to moderate for the 16 comparisons of anti-angiogenic drugs ($R=0.522$, $R^2=0.272$, $p=0.038$) and the nine comparisons investigating other drugs ($R=0.632$, $R^2=0.399$, $p=0.068$) (Table 4, S2 Fig. B and C). The interaction between this correlation and drug categories was not significant ($p=0.904$) (Table 4).

**Discussion**

Different targeted agents recently gained approval for the second-line treatment of mCRC based on relatively small absolute gains in OS. Nevertheless, the impact of these treatments on the overall prognosis of mCRC patients is rather limited [31], and the improvements achieved with novel treatments are below the expectations. Overall, the results from the 20 second-line trials included in the present analysis indicate that the median PFS accounts for 44% and 39% of the median OS in the experimental and control arms, respectively. Although PFS will probably increase in the future, the median absolute duration of PPS in our series was quite short (7.6 months) due to the availability of new effective options in later lines. These findings demonstrate that, at least for the timeframe in which the trials included in this analysis were conducted, the impact of third- and further-line treatments on mCRC patients’ prognosis was rather modest.

We systematically reviewed the inherent literature to focus on clinical trials investigating the efficacy of targeted agents in the second-line treatment of mCRC to assess the correlation of earlier endpoints, PFS and ORR, with OS, and to
Table 3. Correlation between PFS and OS in all comparisons and scattered by the type of experimental drugs.

<table>
<thead>
<tr>
<th>Type of Drugs</th>
<th>No. of comparisons</th>
<th>R</th>
<th>R²</th>
<th>p-value</th>
<th>p for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>All comparisons</td>
<td>21</td>
<td>0.734</td>
<td>0.539</td>
<td>&lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Anti-angiogenic drugs</td>
<td>13</td>
<td>0.655</td>
<td>0.429</td>
<td>0.015</td>
<td>0.775</td>
</tr>
<tr>
<td>Other drugs</td>
<td>8</td>
<td>0.857</td>
<td>0.734</td>
<td>0.007</td>
<td>-</td>
</tr>
</tbody>
</table>

PFS, progression-free survival; OS, overall survival.

Fig. 3. Correlation between overall survival (OS) and progression-free survival (PFS). (A) Correlation between hazard ratios in all comparisons with available information (n=21). (B) Correlation between hazard ratios in all comparisons with available information pertaining to anti-angiogenic drugs (n=13). (C) Correlation between hazard ratios in all comparisons with available information with other drugs (n=8).
Table 4. Correlation between ORR and OS in all comparisons and scattered by the type of experimental drugs.

<table>
<thead>
<tr>
<th>All comparisons</th>
<th>Anti-angiogenics (n=12)</th>
<th>Other drugs (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of comparisons</strong></td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td><strong>ORR objective response rate; OS, overall survival.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Correlation between relative risks and hazard ratios</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Correlation between differences in ORR and in median OS values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Correlation between relative risks and hazard ratios</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Correlation between differences in ORR and in median OS values</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Correlation between objective response rate and overall survival (OS). (A) Correlation between relative risks and hazard ratios in all comparisons with available information (n=20). (B) Correlation between relative risks and hazard ratios in all comparisons with available information with anti-angiogenic drugs (n=12). (C) Correlation between relative risks and hazard ratios in all comparisons with available information with other drugs (n=8).
analyze their surrogacy for OS. While a similar approach was previously pursued by other groups [5], we chose to restrict our analysis to modern trials of targeted agents to put our results in the context of ongoing and future studies in this setting. In fact, previous studies have clearly shown that the reliability of surrogate endpoints must be properly verified within the context in which these endpoints should be subsequently adopted. Namely, out of the 23 trials included in the systematic review by Giessen et al. [5], as many as nine trials compared chemotherapy-only treatment regimens without targeted agents. Furthermore, those authors emphasized that a re-analysis according to the different mechanisms of drug activity should be conducted as soon as a larger set of trials was available. Therefore, we conducted an exploratory subgroup analysis to assess potential differences in surrogacy according to the targeted agents’ mechanisms of action (mainly anti-angiogenic versus directed against other cellular targets), as already suggested in first-line studies [7]. This exploratory subgroup analysis did not produce clear evidence of an interaction between the mechanism of action and surrogacy for the endpoints considered. A clear limitation of this study is that, while the anti-angiogenic group is clearly defined, the “other drugs” group includes agents with heterogeneous mechanisms of action.

Although our analysis has several limitations, we observed a moderate correlation between PFS and OS, while a poor correlation between ORR and OS was reported, with no relevant differences according to the drugs’ mechanisms of action. It should be noted that, after demonstrating a similar moderate correlation between PFS and OS, other authors concluded that PFS may be considered an appropriate surrogate endpoint in second-line treatments for mCRC [5]. However, when specifically focused on targeted agents, our results can affirm that OS remains the preferred primary endpoint for randomized clinical trials in this setting. However, the following considerations should be taken into account to justify this interpretation. First, only small median absolute gains in PFS were reported in statistically positive trials, making it rather difficult to translate these results into clinically relevant improvements in OS. According to the ASCO perspective, improvements of at least three months in median OS (primary endpoint) or median PFS (secondary endpoint) should be regarded as meaningful for mCRC patients experiencing disease progression with all prior therapies, or not eligible for standard options [32]. However, the slope of the regression line in our analysis suggests that small benefits in PFS, on average, are going to translate into modest OS differences. These achievements can only be regarded as clinically relevant if supported by solid improvements in quality of life, which were rarely assessed in the available literature. While the lack of molecular criteria able to positively select patients more likely to benefit from targeted agents may explain the present findings, the introduction of “precision medicine” principles into clinical research will likely change the present scenario.

Secondly, since the duration of PPS is quite short, the adoption of PFS instead of OS as a primary endpoint would not lead to a dramatic decrease in the duration, sample size, and financial costs of trials, or to a considerable acceleration of a drug’s development. However, the recent availability of new effective drugs in later lines of treatment, i.e., after failure of second-line agents, will probably prolong the duration of PPS. Moreover, only 30%-40% of patients included in second-line clinical trials actually receive treatments after progression. Hopefully, this percentage will increase in response to the introduction of highly effective targeted strategies in earlier lines of treatment. Both of these aspects may further weaken the correlation between the PFS and OS and lead to reconsideration of the surrogacy of second-line PFS in currently ongoing and future trials.

In other settings, cross-over has been shown to play a relevant role in the correlation between PFS and OS. As expected, if a high proportion of patients assigned to the control arm receive the experimental drug after disease progression (or a drug with the same mechanism of action), the difference between treatment arms might be significantly decreased [33]. In the present analysis, information regarding the possibility of cross-over according to study protocol and the proportion of patients actually receiving cross-over was not available in most trials; however, as detailed in the Results, this proportion was quite low in all trials for which this information was available.

A limitation of the present meta-analysis is that it is not based on individual patient data, but rather on data extracted from the publications (or, in some cases, from meeting presentations); therefore, we could only estimate trial-level, but not individual patient-level surrogacy. However, even if analysis of the individual patient-level association can lead to an estimation of how much the endpoints are likely to be causally linked to each other, the trial-level analysis remains useful to show the proportion of the OS effect captured by surrogate endpoints [34]. Although intrinsically limited, this information could facilitate the interpretation of trial results and design of future trials in this specific setting.

In conclusion, caution is needed when assessing the surrogacy of potentially useful endpoints and supporting their adoption in phase III clinical trials. Notably, only five out of 36 drugs approved by the U.S. Food and Drug Administration on the basis of surrogate endpoints were able to provide an OS benefit in subsequent trials [35]. Based on our data, OS should be the primary endpoint for registrative phase III trials in the second line treatment of mCRC. Given its moderate surrogacy for OS, PFS may be adopted in earlier steps of drug development.
Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (http://www.e-crt.org).

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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A Rare Case of Phyllodes Tumor Metastasis to the Stomach Presenting as Anemia

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Metastasis of a phyllodes tumor to the stomach is an extremely rare condition with important clinical implications. A 44-year-old woman was initially diagnosed with a phyllodes tumor in her right breast in 2008, and subsequently presented to an outpatient clinic with dizziness on December 16, 2013. We found that she had severe anemia (hemoglobin levels, 6.7 g/dL), and we quickly performed esophagogastroduodenoscopy to identify the cause. This procedure revealed large ulcerating masses with active bleeding in the stomach. Histopathological examination revealed that the masses were consistent with phyllodes tumor metastases. In patients with a metastatic phyllodes tumor presenting as anemia, gastric metastasis should be considered as one of the differential diagnoses because overlooking the possibility might have dire consequences if cytotoxic chemotherapy were administered.

Key words
Phyllodes tumor, Stomach, Neoplasm metastasis, Anemia

Introduction
Phyllodes tumors, which originate from the epithelium and interstitium of the terminal duct-lobular unit in breasts, are rare neoplasms that account for 0.3%-1.0% of all breast tumors, 2.5% of fibroepithelial breast tumors, and 0.3% of benign breast tumors [1]. The term “phyllodes tumor” was established in 1838 by Muller, and this lesion was initially believed to be benign with no potential for distant metastasis [2]. However, in 1931, Lee and Pack [3] reported a case of phyllodes tumor metastasis to the lungs, which revealed that these tumors could exhibit malignant behavior. Many cases of metastatic phyllodes tumors have subsequently been reported, with most metastases being discovered in the lungs [3], although a few cases have involved metastases to the kidney [2], duodenum [4], and pancreas [5].

Ordinary breast cancer with invasive ductal or lobular pathology commonly spreads to the skeleton, lungs, and/or liver. Although not frequent, some reports have described...
metastasis to the stomach from invasive ductal and/or invasive lobular breast carcinoma [6]. However, phyllodes tumor of the breast rarely metastasizes to the gastrointestinal tract, and reported cases are scarce. Therefore, we report here a rare case of phyllodes tumor metastasis to the stomach that presented as anemia and describe the clinical implications of this case.

**Case Report**

A 44-year-old woman visited our out-patient clinic with dizziness in December 2013. She had previously been diagnosed with a 5 cm phyllodes tumor in her right breast and underwent right lumpectomy with axillary lymph node dissection in August 2008 (Fig. 1), which was followed by radiotherapy. In July 2011, she underwent right total mastectomy because of local recurrence of a 2.5 cm phyllodes tumor in the treated breast. In August 2013, at 5 years after the initial diagnosis, chest computed tomography (CT) revealed a 4.7 cm lung mass. Right lower lobectomy was performed, and the pathology results revealed a metastatic phyllodes tumor. As the risk of another recurrence was considered high, adjuvant chemotherapy was recommended, but the patient refused to undergo this treatment. Four months later, multiple liver nodules were found upon chest CT, and a liver biopsy revealed metastatic phyllodes tumor cells identical to those from the primary breast phyllodes tumor. After confirming the liver metastasis, we recommended palliative chemotherapy using an ifosfamide-containing regimen to the patient, but she refused this treatment and selected only palliative symptomatic care.

![Fig. 1. Initial histopathological findings from 2008: a phyllodes tumor in the right breast (H&E staining, ×100).](image1)

While receiving the symptomatic care, she visited our out-patient clinic because of dizziness and was admitted for further evaluation. Blood tests revealed severe anemia (hemoglobin levels of 6.7 g/dL), and we subsequently performed esophagogastroduodenoscopic evaluation, which identified a large gastric mass (approximately 7 cm in diameter) with active bleeding and several related masses (Fig. 2). Therefore, based on an initial impression of multiple metastatic gastric tumors, we performed endoscopic hemostasis with cautery and biopsy. The biopsy confirmed that the tumors were metastases from the breast phyllodes tumor (Fig. 3). Two days later, we performed endoscopic hemostasis again for re-bleeding at the site of the gastric metastases. After the bleeding had been stopped, we considered total gastrectomy for complete bleeding control. However, we judged the patient as having a very high perio-

![Fig. 2. Esophagogastroduodenoscopic findings: a large ulcerofungating mass in the lower body-angle (A) and the cardia side of the gastroesophageal junction on the lesser curvature (B).](image2)
the clinical behavior of phyllodes tumors is difficult. For example, Hines et al. [8] reported that rapid tumor growth and size could not predict malignant behavior, while other studies have suggested that a tumor size of >7 cm was a predisposing factor for both malignant behavior and poor prognosis [8]. In addition, it has been reported that younger patients are more likely to have a benign phyllodes tumor [9]. Furthermore, poor prognosis may be related to mixed mesenchymal components, such as osteosarcomatosis or chondrosarcomatosis [10]. Other studies have found it controversial that tumor size was related to distant metastasis [10], although positive surgical margins and a large tumor size may be significant factors for local recurrence [7].

The only curative therapy for a phyllodes tumor is complete surgical removal, including the surrounding normal tissues, and it is unclear whether chemotherapy, hormonal agents, or radiotherapy have a suitable therapeutic effect [7]. However, adjuvant radiotherapy might be useful in patients with unfavorable characteristics, such as a large tumor, high nuclear polymorphism, high mitotic index, absence of necrosis, and increased vascularity [11]. Unfortunately, there is no standard therapy for metastatic phyllodes tumors, and ifosfamide is considered the most active agent for this indication [12], although there is no evidence regarding the efficacy of hormonal therapy. Mitus et al. [13] reported an improved median survival using a combination of doxorubicin plus cisplatin, cyclophosphamide, or ifosfamide in their retrospective series of 37 patients with metastatic phyllodes tumors. Another potential treatment is sunitinib, which is an oral inhibitor of type 1 and 2 vascular endothelial growth factor receptors, platelet-derived growth factor receptors (PDGF-α, β), c-kit, FMS-like tyrosine kinase-3, and RET kinase. Sunitinib provided a major response in patients with a metastatic phyllodes tumor [14], and therefore merits further evaluation.

In the present case, a 5 cm malignant phyllodes tumor in the right breast was diagnosed in August 2008, and local recurrence was observed at approximately 3 years after surgical removal and radiotherapy. Metastases to the lung, liver, and stomach were subsequently observed during August–December 2013. The stomach metastasis was located in the lesser curvature of the stomach, and consisted of a 7 cm ulcerofungating mass with multiple related nodules that presented as anemia (Fig. 2). The biopsy specimen from the stomach lesion exhibited sarcoma-like features that consisted of short spindle cells (Fig. 3), which are characteristic of a phyllodes tumor, and histopathological features that were similar to those of the primary breast specimen (Fig. 1).

The causes of anemia in patients with cancer are diverse, and include intrinsic or iatrogenic blood loss, nutritional deficiencies (primarily in iron or folic acid), hemolysis, bone marrow failure from various etiologies, infection, inflamma-

Discussion

Phyllodes tumor, which are very rare (0.3%-1.0% of all breast neoplasms), originate from the fibroepithelial connective tissue of the breast [11]. A phyllodes tumor can be classified as benign, borderline, or malignant, and is diagnosed according to the histopathological manifestations of stromal hypercellularity, cellular pleomorphism, mitotic count, the shape of the margin and the stromal pattern. The median age of onset for phyllodes tumors is 45 years [7]. Prediction of
tion, or the cancer burden itself [15]. In the present case, at the time of liver biopsy performed to evaluate the multiple liver nodules, the hemoglobin level was 11.0 g/dL (August 2013). However, we did not perform additional evaluation of the anemia, as it was mild and asymptomatic. Nevertheless, based on the large size of the gastric mass, we assume that the stomach metastasis was present before she presented with anemia at our outpatient clinic (December 2013).

This case has two clinical implications. First, patients with a metastatic malignant phyllodes tumor and anemia should be carefully evaluated for the possible causes of anemia, which may include gastric metastasis. Second, when considering chemotherapy and/or oral sunitinib to manage a metastatic phyllodes tumor, clinicians should carefully search for any bleeding foci, as chemotherapy and/or sunitinib can increase the risk of bleeding. In the present case, the patient refused chemotherapy, although it is possible that this treatment might have had dire consequences if we administered it before identifying the gastric metastasis.

In conclusion, we report here a rare and unusual case of phyllodes tumor metastasis to the stomach, which presented as anemia. In patients with a metastatic phyllodes tumor presenting as anemia, gastric metastasis should be considered as one of the differential diagnoses. In addition, clinicians should be aware of the risk of bleeding from a hidden focus, including the stomach, when considering chemotherapy and/or targeted therapy.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

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References

## Prescribing Information

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**위치**

- 2016년 5월 19일
- 타그리스(TAGRISSO)
- BREAK THROUGH THE T790M RESISTANCE BARRIER

이전에 EGFR-TKI로 치료 받은 적이 있는 T790M 변이 양성 국소 진행성 또는 전이성 비소세포암 환자의 치료

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키트루다의 혜능 효과
비소세포폐암: 진행성 비소세포폐암의 치료
PD-L1 양성으로, 백금 기반 화학요법제 치료 도중 또는 이후에 진행이 확인된 환자에게 두여한다.
다만 EGFR 또는 ALK 변이가 확인된 환자는 이 약을 두여하기 전에 이러한 변이에 대한 승인된 치료제를 두여한 후에도 질병의 진행이 확인된 경우에만 한다.

흑색종: 수술이 불가능하거나 전이성이 흑색종의 치료

주요안전성정보

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