Original Article

Clinicopathological Characteristics of *NRG1* Fusion–Positive Solid Tumors in Korean Patients

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Purpose Neuregulin 1 (*NRG1*) gene fusion is a potentially actionable oncogenic driver. The oncoprotein binds to ERBB3-ERBB2 heterodimers and activates downstream signaling, supporting a therapeutic approach for inhibiting ERBB3/ERBB2. However, the frequency and clinicopathological features of solid tumors harboring *NRG1* fusions in Korean patients remain largely unknown.

Materials and Methods We reviewed archival data from next-generation sequencing panel tests conducted at a single institution, specifically selecting patients with in-frame fusions that preserved the functional domain. The clinicopathological characteristics of patients harboring *NRG1* fusions were retrospectively reviewed.

Results Out of 8,148 patients, *NRG1* fusions were identified in 22 patients (0.27%). The average age of the patients was 59 years (range, 32 to 78 years), and the male-to-female ratio was 1:1.2. The lung was the most frequently observed primary site (n=13), followed by the pancreaticobiliary tract (n=3), gastrointestinal tract (n=2, stomach and rectum each), ovary (n=2), breast (n=1), and soft tissue (n=1). Histologically, all tumors demonstrated adenocarcinoma histology, with the exception of one case of sarcoma. *CD74* (n=8) and *SLC3A2* (n=4) were the most frequently identified fusion partners. Dominant features included the presence of fewer than three co-occurring genetic alterations, a low tumor mutation burden, and low programmed death-ligand 1 expression. Various clinical responses were observed in patients with *NRG1* fusions.

Conclusion Despite the rarity of *NRG1* fusions in Korean patients with solid tumors, identification through next-generation sequencing enables the possibility of new targeted therapies.

Key words *NRG1* fusion, Solid tumor, Pathology, Genomics, Targeted therapy

Introduction

Subsets of gene fusions in solid tumors have been identified as oncogenic drivers and potential therapeutic targets [1,2]. Among them, tyrosine kinases such as *ALK*, *ROS1*, *RET*, *NTRK*, and *FGFR* are frequently involved in oncogenic fusions. These fusions lead to the constitutive activation of downstream signaling pathways, promoting tumor cell proliferation and tumor progression [2].

Neuregulin 1 (NRG1), which is encoded by the *NRG1* gene, functions as an epidermal growth factor (EGF)–like ligand for members of the ERBB/epidermal growth factor receptor (EGFR) family, particularly human epidermal growth factor receptor 3 (HER3/ERBB3) [3]. This interaction results in the formation of heterodimers between HER3 and other ERBB family members, predominantly HER2 [4]. *NRG1* fusion was initially identified in the breast cancer cell line MDA-MB-175, where it manifested as a *DOC4-NRG1* transcript that was

specific to the tumor and played a role in promoting tumor cell proliferation [5,6]. Subsequently, *NRG1* fusion has been observed in various cancer types [7,8], with a notably high prevalence in pulmonary invasive mucinous adenocarcinoma (IMA) [9-11].

NRG1 fusion is a rare occurrence, reported to have an incidence of 0.2% based on a study involving 21,858 tumors with *CD74* being the most common partner [12]. Despite its rarity, *NRG1* fusion in solid tumors is now recognized as a target for drug therapy with evidence indicating a sustained response to targeted agents such as a pan-ErbB family inhibitor (afatinib) [13] and anti-HER2xHER3 bispecific antibody (zenocutuzumab) [14,15]. However, the frequency and clinicopathological features of solid tumors harboring *NRG1* fusions, particularly in Korean patients, remain largely unknown. In this study, we aimed to investigate the incidence and clinicopathological characteristics of *NRG1* fusion–positive solid tumors through a comprehensive molecular analysis.

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Materials and Methods

1. Patients

Between May 2017 and June 2022, a total of 8,148 patients with solid tumors underwent next-generation sequencing (NGS) analysis. Among them, 22 patients who exhibited *NRG1* fusions were included in this study. Clinical information and outcome data of the patients, including age at diagnosis, sex, primary tumor site, tumor stage, smoking status, pathological diagnosis, programmed death-ligand 1 (PD-L1) status, and survival data, were retrieved from electronic medical records. The best overall response to systemic therapy was evaluated based on the Response Evaluation Criteria in Solid Tumor ver. 1.1 criteria.

2. Histopathologic review

All available pathological slides stained with hematoxylin and eosin (H&E) were reviewed by two pathologists (Y.J.C. and H.S.S.). Their objective was to assess tumor differentiation and histological types. Since the majority of cases involved adenocarcinomas, the architectural features of the tumors were carefully examined, specifically focusing on the presence of tubule/cribriform formation, micropapillary clusters, and papillary configuration. Since previous studies have reported an association between *NRG1* fusion and mucinous adenocarcinoma [9,10], we examined the following cytologic features: cell morphology (especially columnar cells), intracellular or extracellular mucin, and cytoplasmic vacuoles. Lung adenocarcinomas were classified into two types based on cytological features and thyroid transcription factor-1 (TTF-1) expression: the terminal respiratory unit (TRU) type and the non-TRU type [16-18].

3. Immunohistochemistry and interpretation of PD-L1

Immunohistochemistry (IHC) was conducted using the following procedure. Tissue sections, 4-μm thick, were cut from formalin-fixed paraffin-embedded (FFPE) tissue blocks. The sections were deparaffinized with xylene and rehydrated using graded alcohol solutions. IHC staining was carried out utilizing a Ventana Discovery XT Automated Slide Stainer (Ventana Medical Systems, Tucson, AZ). Antigen retrieval was performed using Conditioning 1 buffer (citrate buffer, pH 6.0, Ventana Medical Systems). The slides were then incubated with primary antibodies against PD-L1 (prediluted, clone SP263, Ventana Medical System) and TTF-1 (1:150, 8G7G3/1, Agilent Dako, Santa Clara, CA). For PD-L1 (22C3), the PD-L1 IHC 22C3 pharmDx kit on the Dako Automated Link 48 platform (Dako, Carpenteria, CA) was utilized. Interpretation of the IHC results was conducted by two pathologists (Y.J.C. and H.S.S.). For PD-L1 IHC, the tumor proportion score (TPS) was assessed. TPS represents the percentage of viable tumor cells demonstrating partial or complete membrane staining at any intensity. Positive PD-L1 expression was defined as TPS ≥ 1% using the SP263 or 22C3 IHC assays. TTF-1 expression was considered positive when 10% or more of tumor cells exhibited nuclear staining, as described in previous studies [17,18].

4. NGS analysis

Targeted DNA and RNA sequencing was conducted using the TruSight Tumor 170 (TST170, Illumina, San Diego, CA) or TruSight Oncology 500 (TSO500, Illumina) panels. The TST170 panel is designed to identify 170 cancer-related genes, which encompass 151 genes associated with potential single-nucleotide variants (SNVs) and indels, 59 genes associated with potential amplifications, and 55 genes associated with RNA fusions and splice variants. The TSO500 panel comprises 523 cancer-related genes utilized for calculating tumor mutation burden (TMB), 130 regions for assessing microsatellite instability (MSI), and an equal number of genes as TST170 for detecting potential amplification, RNA fusions, and RNA splice variants.

Following the established protocol [19], DNA and RNA were extracted from 40 ng of FFPE tissue using the Qiagen AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany). Target enrichment was achieved through hybridization capture, and paired-end sequencing (2×150 bp) was conducted using a NextSeq sequencer (Illumina) following the manufacturer's instructions. In the analysis, variants meeting the following criteria were included: a total depth of at least 100× and a variant allele frequency of at least 3%. The interpretation of variants followed the recommendations provided by the Association for Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists [20].

5. RNA fusion detection and interpretation

RNA fusions were detected using the Manta fusion caller [21] integrated into the analysis pipeline of TST170 or TSO500 Local App ver. 2.0 or 2.2, respectively. For validating RNA fusions and interpretation with a visualized plot, Arriba RNA fusion caller (ver. 2.2.1) [22] was used. STAR (ver. 2.7.10a) [23] aligned bam with the option–chimOutType 'WithinBAM HardClip Junctions.' The detected fusion plots were generated using the R script draw_fusion.R, which visualizes the fusion partners, breakpoints, supporting reads, fusion type, frame status, and retained protein domains. For *NRG1* fusion to be considered functional, it needed to meet the following criteria: (1) predicted in-frame fusion, (2) located at the C-terminal region with a retained EGF-like domain, and (3) supporting split or discordant reads ≥ 5 [24].

6. Tumor mutation burden

The TMB scores were calculated using TSO500, which covered 1.33 megabases (Mb) coding regions. According to the TSO500 LocalApp manual, we included SNVs or insertions or deletions in the coding region with a variant allele frequency between ≥ 5% and < 90% and a read depth ≥ 50×. However, we excluded certain variants that were annotated with ≥ 50 counts in the COSMIC database, ≥ 10 counts in normal population databases (gnomAD exome/genome, 1000 Genome), or were present in the internal germline variant database.

Results

1. Characteristics of patients and prevalence of *NRG1* fusion

The clinicopathological characteristics of the patients are presented in Table 1. *NRG1* fusion was identified in 22 out of 8,148 patients (0.27%) with solid tumors. Among the *NRG1* fusion–positive patients, there were 10 males and 12 females, with a median age of 59 years (range, 39 to 78 years). Of these patients, 15 (68.2%) reported never smoking, while five were former smokers and two were current smokers (median pack-years: 15 and 21.5, respectively). The lung was the most common primary site of *NRG1* fusion–positive tumors (n=13, 59.1%). Following that, the pancreaticobiliary (PB) tract (n=3 [2 pancreatic cancer, 1 intrahepatic cholangiocarcinoma], 13.6%), gastrointestinal (GI) tract (n=2 [1 rectal cancer, 1 gastric cancer], 9.1%), ovary (n=2, 9.1%), breast (n=1, 4.6%), and soft tissue (n=1 [dedifferentiated liposarcoma], 4.6%) (Fig. 1). Regarding the primary tumor site, *NRG1* fusion was detected in 0.72% (13/1,814) of all lung cancer patients with available NGS reports, followed by breast (0.49%, 1 out of 205) and PB (0.39%, 3 out of 770) cancers (Fig. 2).

2. Pathologic features of *NRG1* fusion–positive tumors

Table 2 summarizes the pathological characteristics of the *NRG1* fusion–positive tumors. All of the *NRG1* fusion–positive tumors had adenocarcinoma histology, except for one case of dedifferentiated liposarcoma with spindle cell morphology (Fig. 3). Among the adenocarcinomas, non-TRU type lung adenocarcinoma, including mucinous adenocarcinoma, was the most common subtype (38.1%), followed by TRU type lung adenocarcinoma (23.8%) and GI and PB adenocarcinoma (23.8%). Regarding architectural features, all adenocarcinoma cases exhibited at least one typical pattern, with micropapillary patterns frequently observed (76.2%). Solid sheet patterns and undifferentiated areas were not prominently present. Among the adenocarcinoma cases, 61.9% displayed cancer cells with columnar cell morphology. Furthermore, the majority of (95.2%) showed at least intra-

Table 1. Characteristics of patients with *NRG1* fusion–positive tumors

Parameter	No. (%)
Sex	
Male	10 (45.5)
Female	12 (54.5)
Age (yr), median (range)	59 (39-78)
Smoking status	
Never	15 (68.2)
Former (median PY)	5 (22.7) (median, 15)
Current (median PY)	2 (9.1) (median, 21.5)
Stage at diagnosis	
I	3 (13.6)
II	4 (18.2)
III	6 (27.3)
IV	9 (40.9)
Primary site	
Lung	13 (59.1)
Pancreaticobiliary tract	3 (13.6)
Gastrointestinal tract	2 (9.1)
Ovary	2 (9.1)
Breast	1 (4.5)
Soft tissue	1 (4.5)

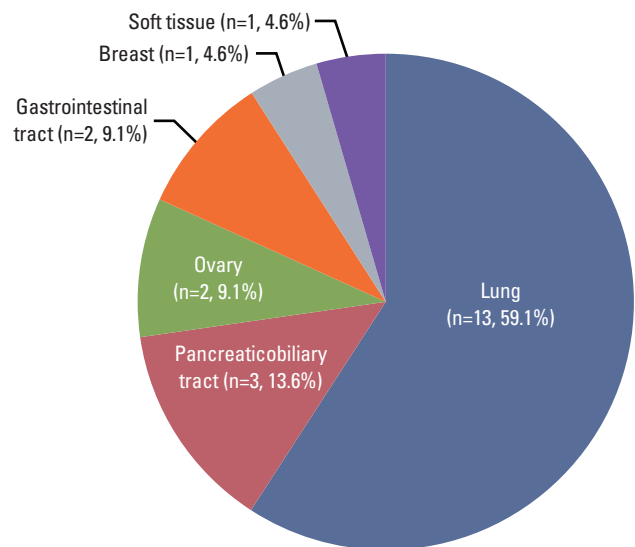NRG1, neuregulin 1; PY, pack-year.

Fig. 1. Pie chart with the prevalence of primary site of neuregulin 1 (*NRG1*) fusion–positive tumor.

cellular mucins or cytoplasmic vacuoles on H&E slides.

PD-L1 IHC was performed on 18 patients. Four patients (18.2%) were PD-L1–positive (three with lung adenocarcinoma and one with ovarian serous carcinoma). No high expres-
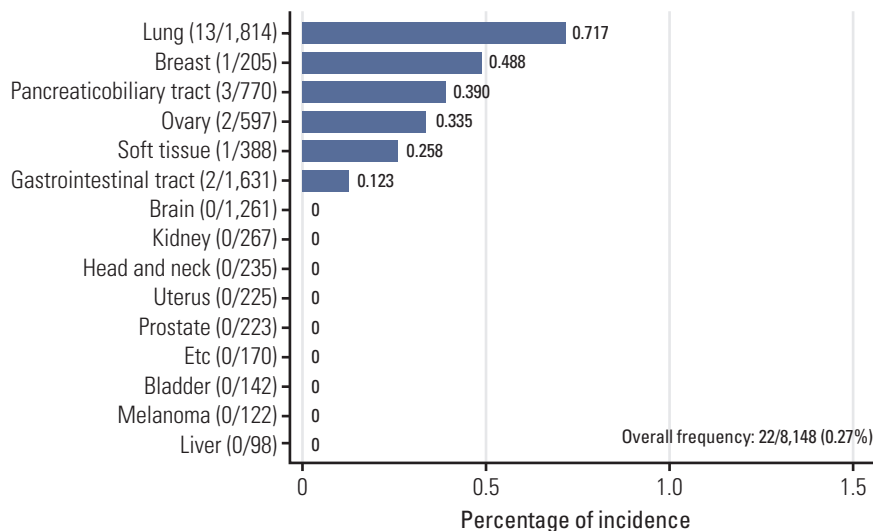
Fig. 2. Detection rate of neuregulin 1 (*NRG1*) fusion in all patients and in different primary sites. Etc (n=170) includes thyroid (n=50), thymus (n=38), lymphoma (n=28), malignancy of unknown primary (n=21), skin (n=17), testis (n=13), and adrenal gland (n=3).

sion (TPS ≥ 50%) was observed. Eight cases (61.5%) of lung adenocarcinoma (n=13) were TTF-1 IHC-negative.

3. Genomic features of *NRG1* fusion–positive tumors

Of the 22 cases, seven (31.8%) were tested using TST170, and the remaining 15 (81.8%) were tested using TSO500. Translocation (n=17, 77.3%) was the most common type of alteration, followed by inversion (n=4, 18.2%) and deletion (n=1, 4.5%). Among the *NRG1* fusion cases, the most frequent breakpoints occurred in exon 6 (n=13, 59.1%), and exon 2 (n=8, 36.4%). The most prevalent fusion partner was *CD74* (n=8, 36.4%), followed by *SLC3A2* (n=3, 13.6%). Both *CD74* and *SLC3A2* formed fusion transcripts with *NRG1* exon 6. The other fusion partners included *ATP1B1*, *CDH1*, *CLU*, *CRADD*, *FUT10*, *INCENP*, *KIF22*, *RBPMS*, *SLC20A2*, *VWA8*, and *XKR6*. Seventeen (77.3%) *NRG1* fusion–positive tumors had one or more co-occurring mutations: 1 (n=9, 40.9%), 2 (n=2, 9.1%), 3 (n=5, 22.7%), and 6 (n=1, 4.5%). All were *EGFR*/*ALK*/*ROS1*-wild type. *TP53* mutation was the most co-occurring mutation, present in 10 cases (54.5%). *KRAS* (G12S and G12D), *BRAF* (N581I), and *PIK3CA* (E453K) mutations were observed concurrently in four cases. TMB and MSI were calculated for 15 cases. The median value of TMB was 3.9/Mb (range, < 1.0 to 51.20/Mb), and the median value of MSI was 1.98% (range, < 1.0 to 5.0%). The genomic findings are summarized in Fig. 4.

4. Clinical outcome of *NRG1* fusion–positive tumors

Most patients were in an advanced stage at diagnosis, with nine stage IV patients (40.9%). Among the 13 patients diagnosed with stages I-III, 10 underwent surgical resection,

Table 2. Pathological features of *NRG1* fusion–positive tumors

Parameter	No. of available cases (%)
Histologic type	
Adenocarcinoma	21/22 (95.5)
Liposarcoma	1/22 (4.5)
Subclassification of adenocarcinoma	
Non-TRU type lung adenocarcinoma	8/21 (38.1)
TRU type lung adenocarcinoma	5/21 (23.8)
GI-PB adenocarcinoma	5/21 (23.8)
High-grade serous carcinoma of the ovary	2/21 (9.5)
Invasive ductal carcinoma of the breast	1/21 (4.8)
Architectural features	
Tubule/Cribriform	15/21 (71.4)
Micropapillary	16/21 (76.2)
Papillary	6/21 (28.6)
Cytologic features	
Columnar cell morphology	13/21 (61.9)
Intracellular mucin	12/21 (57.1)
Extracellular mucin	5/21 (23.8)
Cytoplasmic vacuoles	15/21 (71.4)
PD-L1 status	
Positive	4/22 (18.2)
Negative	14/22 (63.6)
Not available	4/22 (18.2)
TTF-1 expression (lung)	
Positive	5/13 (38.5)
Negative	8/13 (61.5)

GI, gastrointestinal tract; NRG1, neuregulin 1; PB, pancreatico-biliary tract; PD-L1, programmed death-ligand 1; TRU, terminal respiratory unit; TTF-1, thyroid transcription factor-1.
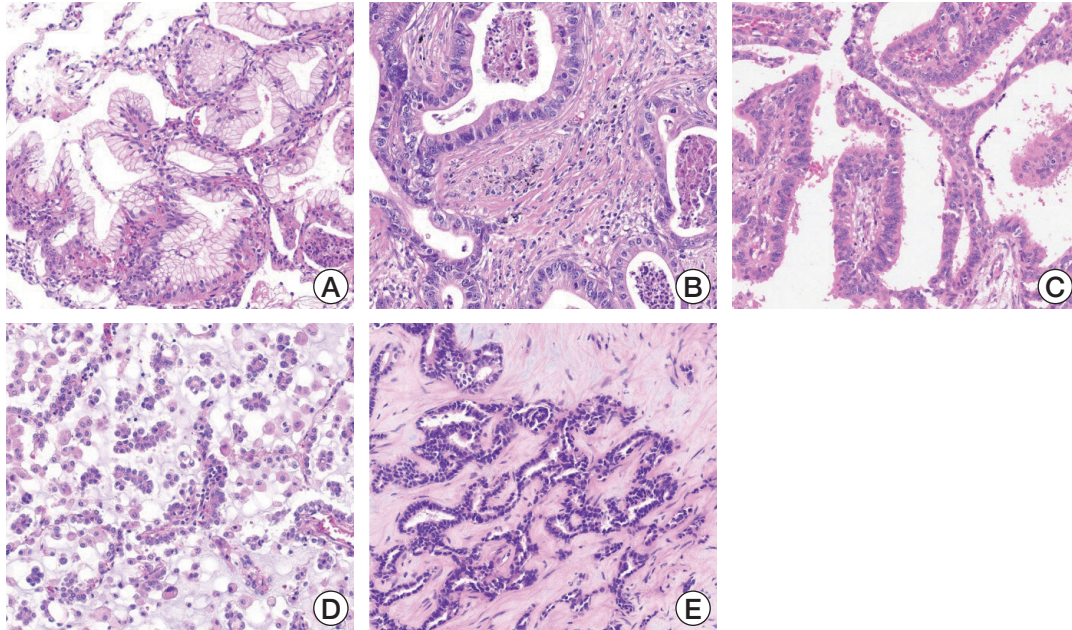
Fig. 3. Histologic features of neuregulin 1 (*NRG1*) fusion–positive tumors. (A) Typical histology of invasive mucinous adenocarcinoma (H&E, ×200). (B) Columnar tumor cells with intraluminal necrosis (H&E, ×200). (C) Columnar tumor cells in papillary architecture (H&E, ×200). (D) Micropapillary tumor clusters floating in the extracellular mucin pool (H&E, ×200). (E) Adenocarcinoma with tubular formation (H&E, ×200).

and all patients experienced recurrence or metastasis. One patient with liposarcoma received repeated radiotherapy as the sole treatment, while the remaining patients were treated with systemic chemotherapy. Although it is not appropriate to compare survival across different tumor types, the treatment sequence and survival after recurrence/metastasis in patients with *NRG1* fusion are depicted in Fig. 5. Among the 13 patients with lung cancer, four (P02, P10, P20, and P21) received *NRG1* fusion–targeting treatment (anti-HER2x-HER3 bispecific antibody zenocutuzumab) and were still undergoing treatment at the cut-off date with a durable response. However, one patient with pancreatic cancer (P06) was also treated with *NRG1* fusion–targeting treatment after poor responses to the two previous treatment lines but showed short progression-free survival (1 month) and died (overall survival, 7.2 months from initial diagnosis).

Discussion

In this study, we report 22 cases of *NRG1* fusion–positive solid tumors, accounting for 0.27% of the 8,148 cases of solid tumors with available NGS reports reviewed in a single tertiary institution. The study focuses on the clinical, pathological, and genetic characteristics of these cases. *NRG1* fusions were detected in various tumors and primary sites, occur-

ring at a rare incidence. The adenocarcinoma histology was predominant among the *NRG1* fusion–positive tumors. Most patients presented with advanced-stage disease and showed various responses to systemic treatment.

In this study, the prevalence of *NRG1* fusion was 0.27%, consistent with 0.2% in a previous report [12]. All but one patient demonstrated adenocarcinoma histology. This exceptional case exhibited sarcoma, consistent with a previous study that reported one case of sarcoma (0.2% frequency) [12]. In previous studies, *NRG1* fusion was most frequently detected in pulmonary IMA [9,12]. IMA has a columnar cell morphology, harboring intracytoplasmic mucin [25]. These findings (columnar cells or intracellular mucins) were observed in > 50% of patients in this cohort. In cases of lung adenocarcinoma, typical IMAs and adenocarcinomas with eosinophilic cytoplasm in columnar cell morphology, previously referred to as a subtype of non-TRU adenocarcinoma [18], were frequently encountered. In this study, all adenocarcinomas with these morphological features tested negative for TTF-1, leading to their collective classification as non-TRU type adenocarcinomas. Remarkably, the non-TRU type accounted for the largest proportion (61.5%) of lung adenocarcinomas, which partly agrees with a previous study where 57% of *NRG1* fusion–positive lung tumors were mucinous adenocarcinoma [11]. Despite occurring in the lungs, non-TRU type adenocarcinoma exhibits morpho-
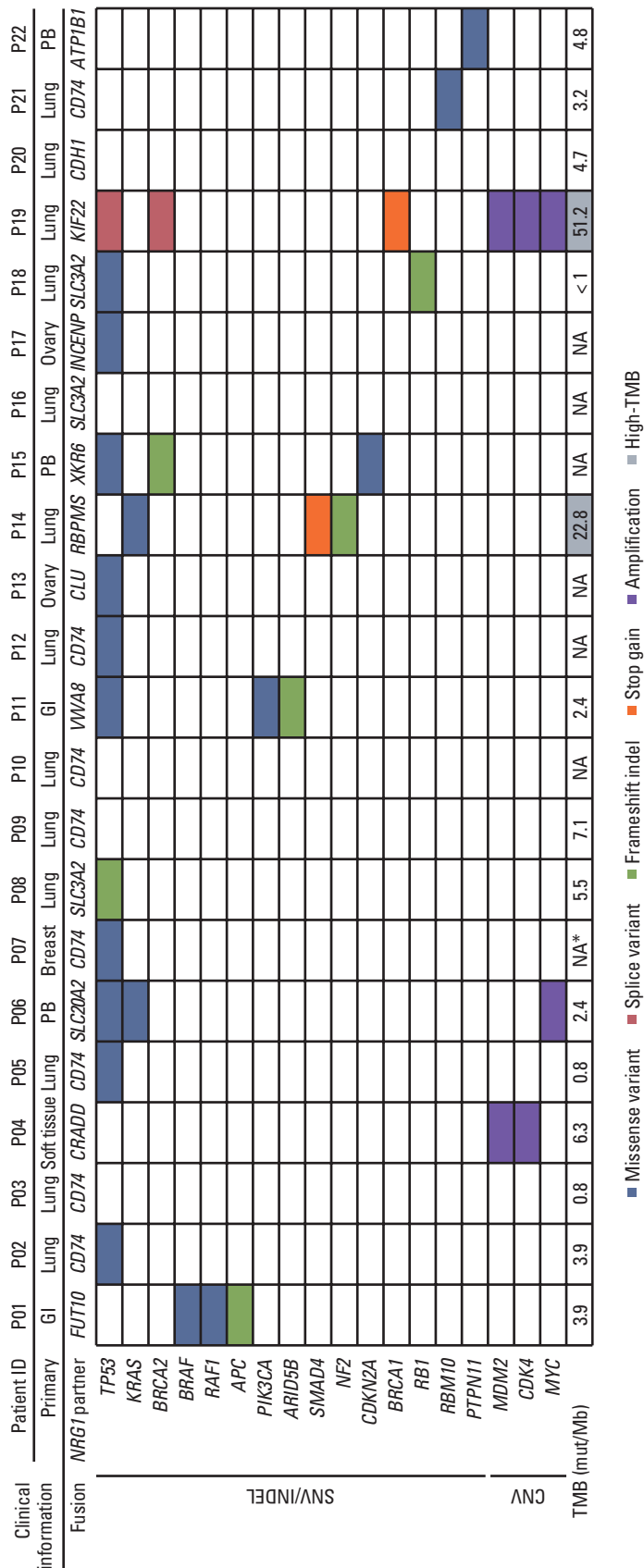
Fig. 4. Summary of co-occurring genomic alterations. CNV, copy number variation; GI, gastrointestinal; INDEL, insertion and deletion; NA, not available; PB, pancreaticobiliary; SNV, single-nucleotide variant; TTF-1, thyroid transcription factor-1.
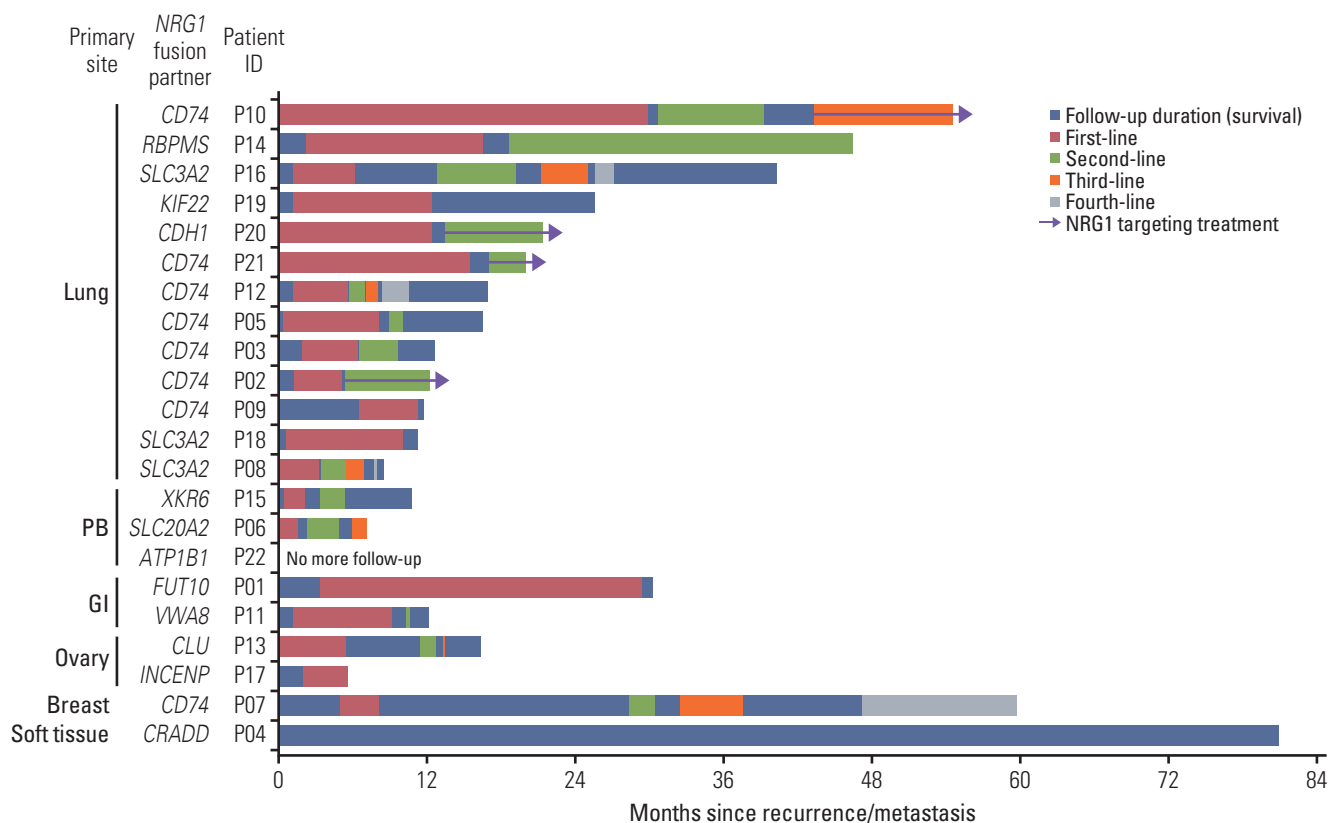
Fig. 5. Swimmer plot displaying treatment sequence and survival of patients with neuregulin 1 (*NRG1*) fusion. Swimmer plot showing treatment sequences (first-line and up to fourth-line of systemic treatment) with survival from diagnosis of recurrence/metastasis until the last follow-up. *NRG1* fusion partner genes and primary tumor sites are shown. The duration of NRG1 targeting treatment is also shown. Four patients with lung cancer (P02, P10, P20, and P21) are undergoing NRG1 targeting treatment at the cut-off date with a durable response. GI, gastrointestinal tract; PB, pancreaticobiliary tract.

logical and molecular characteristics similar to GI adenocarcinoma [26]. Thus, considering the total number of GI-PB adenocarcinomas identified in this study, 61.9% showed a gut phenotype. However, it is worth noting that *NRG1* fusion was not exclusively limited to these adenocarcinomas, as it was also detected in TTF-1–positive TRU-type adenocarcinomas or other types of carcinomas without mucin or columnar morphology. Hence, these findings indicate that *NRG1* fusion should not be ruled out solely based on histological findings. Interestingly, no solid sheet pattern was observed; however, a micropapillary pattern was present in 76.2% of the cases. Considering the well-established association between the micropapillary pattern and poor prognosis, it is reasonable to assume that this histological feature correlates with the frequent recurrence of *NRG1*-positive tumors observed in this study.

Of the 17 out of 22 cases with co-occurring mutations, most had three or fewer concurrent mutations. Interestingly, we found two co-occurring *KRAS* mutations in one lung IMA

(G12S) and one PB cancer (G12D). *KRAS* mutations have been reported to be mutually exclusive with *NRG1* fusions in pancreatic cancer [27]. However, concurrent *KRAS* or *EGFR* mutations have been reported in IMAs harboring *NRG1* fusion [11]. In a preclinical study using lung cancer cells, the coexistence of *KRAS* mutation and *NRG1* fusion synergistically activated tumor cell proliferation and conferred treatment resistance [28]. In this study, we observed a poor prognosis in a patient with PB carcinoma harboring co-occurring *KRAS* mutations.

All the patients showed no or low PD-L1 expression. No patient exhibited high PD-L1 expression. In addition, all cases, except for two, had a low TMB (less than 10/Mb). These findings suggest that *NRG1* fusion–positive tumors exhibit lower response rates to immune checkpoint inhibitors. The lower response rate observed in the eNRGy1 patient cohort supports this notion [11]. This indicates that alternative treatment strategies need to be explored for *NRG1* fusion–positive tumors to improve therapeutic outcomes.

Targeting the HER2/HER3 pathway is a treatment approach for *NRG1* fusion–positive tumors. Zenocutuzumab (Zeno, MCLA-128) is a bispecific HER2/HER3 antibody with enhanced antibody-dependent cytotoxicity. Zenocutuzumab binds to HER2 and interferes with the interaction between NRG1 and HER3, inhibiting HER2/HER3 heterodimerization [14,15,29]. Recently, a global multicenter phase I/II trial (the eNRGy trial) investigated the clinical efficacy and safety of the anti-HER2xHER3 bispecific antibody zenocutuzumab for NRG1 fusion–positive solid tumors and reported durable efficacy [30]. In our institution, four patients with lung cancer were treated with zenocutuzumab in the eNRGy trial and showed good responses and long treatment durations. However, as previously mentioned, one patient with pancreatic cancer did not respond to zenocutuzumab. Further trials are needed to validate the efficacy and safety of anti-NRG1 treatment in different tumor types.

This study had several limitations. Because of the rarity of *NRG1* fusion, although we examined over 8,000 tumors with NGS, only 22 cases were reviewed in the study, which might be insufficient to perform an appropriate statistical analysis. Except for the lungs, *NRG1* fusion was detected in fewer than three cases at each primary site, which is too few to determine the exact prevalence of different populations of tumors at different sites. As many of the specimens used to observe the histological findings were biopsy specimens, the morphological findings described above may not represent their entirety. In addition, most patients included in this study were treated with heterogeneous conventional systemic therapy depending on the primary site and tumor stage, making the appropriate evaluation of clinical outcomes complex and difficult.

In conclusion, we conducted a collective analysis of *NRG1* fusion–positive tumors at a single institution. *NRG1* fusion is a rare occurrence, and patients are frequently diagnosed at an advanced stage, predominantly with adenocarcinoma, showing diverse responses to therapeutic regimens. Despite its rarity, the detection of *NRG1* fusions holds significance as novel targeted agents are being developed. In patients with advanced-stage adenocarcinoma, NGS could serve as a valuable tool for screening various oncogenic driver gene alterations, including *NRG1* fusion.

Author Contributions
Conceived and designed the analysis: Cha YJ, Lee C, Lee CK, Shim HS.
Collected the data: Cha YJ, Lee C, Joo B, Kim KA, Lee CK, Shim HS.
Contributed data or analysis tools: Cha YJ, Lee C, Joo B, Kim KA, Lee CK, Shim HS.
Performed the analysis: Cha YJ, Lee C, Joo B, Kim KA, Lee CK, Shim HS.
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Conflicts of Interest
Conflict of interest relevant to this article was not reported.

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