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Clinical Utility of Next-generation Sequencing in Real-world Cases: A Single-institution Study of Nine Cases

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Abstract. *Background/Aim: Targeted next-generation sequencing (NGS) is a well-established technique to detect pathogenic alterations in tumors. Indeed, it is the cornerstone of targeted therapy in precision medicine. We investigated the clinical utility of next-generation sequencing in real-world cases. Patients and Methods: We retrospectively selected six representative cancer cases, wherein targeted NGS played a pivotal role in the diagnosis and treatment of patients. Additionally, we analyzed three cases with rare, unusual pathogenic alterations. Results: Our NGS analysis revealed that four patients had TPR-ROS1, EGFR-RAD51, and NCOA4-RET fusions and MET exon 14 skipping mutation, respectively, which can be treated with targeted therapy. Furthermore, we used NGS as a diagnostic tool to confirm the origin of unknown primary malignant tumors in two cases. Interestingly, NGS also helped us identify the following cases: patients exhibiting BRCA1 and TP53 mutations that exhibited histological and immunohistochemical characteristics consistent with endometrioid carcinoma, patients with high-grade serous carcinoma not possessing a TP53 mutation, and patients with small cell lung cancer with a ERBB2 mutation and displaying no loss of RB1. Conclusion: We recommend targeted NGS for the diagnoses and targeted therapy of cancer patients.*

Cancer is a genetic disease that is caused by mutations in genes involved in the cell cycle and in cell signaling, cell growth, proliferation, survival, cancer invasion, and immunity (1, 2). In the era of precision oncology, molecular analysis of patient samples is essential for tumor classification, treatment planning, and prognostic stratification (3). Currently, several pathology labs follow the "one-gene one-test" approach, wherein they perform techniques such as polymerase chain reaction (PCR), Sanger sequencing, and pyrosequencing to detect targetable gene alterations, such as those in *EGFR, BRAF, KRAS, ALK, ROS, MET, and PIK3CA*. However, all these tests require a substantial amount of template DNA and are only applicable to the hotspot regions of targetable genes (4-7).

Remarkably, next-generation sequencing (NGS), massive parallel sequencing, simultaneously detect hundreds of genes (8, 9). Although whole exome sequencing and whole genome sequencing are widely used for research purposes, they have limited clinical applications owing to their low coverage depth and high cost (10). Thus, targeted NGS is the most widely employed sequencing approach for molecular analysis in clinical practice (11, 12).

Conventionally, cancer is diagnosed by histological evaluation. Indeed, a direct visual inspection of the patient's sample by light microscopy provides considerable information regarding diagnosis, prognosis, and a treatment plan (13-15). Despite determining the overall pathological landscape of a patient sample, this approach does not reveal the underlying genetic alterations in cancer. Additionally, interobserver discrepancy and "gray zone" are some other limitations of histologically classifying cancers (16-19). Alternatively, immunohistochemistry and molecular techniques, such as PCR and fluorescence *in situ* hybridization, have also been used as ancillary tests for enhancing diagnostic efficiency and for establishing a treatment plan (20-28). However, targeted NGS can simultaneously identify multiple genetic events and transform patient care in this era of precision medicine (3, 12, 29, 30).

In this study, we retrospectively investigated the cases wherein NGS played a critical role in cancer diagnosis and treatment planning.

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Key Words: Next-generation sequencing, immunohistochemistry, histology, malignancy.

Table I. *Clinicopathologic characteristics of the patient cohort.*

Case number	Age at diagnosis	Sex	Pathological diagnosis	Tumor origin	Sampling site	Stage
Case 1	55	M	ADC	Lung	Lung	IVB
Case 2	57	F	ADC	Lung	Pleural fluid	IVB
Case 3	71	M	ATC (no PTC component)	Thyroid	Thyroid	IVB
Case 4	73	M	Sarcomatoid Ca	Lung	Small intestine	IVB
Case 5	63	F	ADC	Unknown	Uterus, Pancreas	IV
Case 6	48	F	ADC	Unknown	Lung, LN	IVB
Case 7	45	F	Endometrioid Ca	Ovary	Ovary	IA
Case 8	65	F	HGSC	Ovary	Ovary	IIIB
Case 9	72	M	SmCC	Lung	Liver	IVB

ADC, Adenocarcinoma; ATC, anaplastic thyroid carcinoma; Ca, carcinoma; F, female; HGSC, high-grade serous carcinoma; LN, lymph node; M, male; PTC, papillary thyroid carcinoma; SmCC, small-cell carcinoma.

Patients and Methods

Study population. Among 125 cancer cases that had been subjected to targeted NGS from 2021 to 2022 in the Department of Pathology at Kyungpook National University Chilgok Hospital, we retrospectively studied nine cases. Four of these cases were selected because NGS played a critical role in finding rare targetable genetic alterations that had been undetected in other molecular methods. On the other hand, we chose two cases where NGS had been used as a diagnostic tool. The remaining three cases that were selected were dilemma cases, as they exhibited significant discrepancies between the NGS, histological, and immunohistochemical data. The clinicopathological data, including age, sex, histological diagnosis, and tumor stage, of the selected cases were retrieved from the electronic medical records of the hospital. The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Daegu Joint Institutional Review Board (No. DGIRB 2021-12-004-001). The requirement for written informed consent from the patients was waived because of the retrospective nature of the study.

Pathological evaluation. Surgical specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. The entire tumor specimen was microscopically examined if the tumors were smaller than 3 cm in diameter. For each selected case, at least three representative tumor sections (one section per 1 cm of the tumor) were submitted for pathologic evaluation. These sections were cut into 4-μm thick sections and stained with hematoxylin and eosin to obtain formalin-fixed, paraffin-embedded (FFPE) tissue blocks. Two pathologists experienced in surgical pathology (MK and JYJ) reviewed all these stained sections. Thereafter, the tumors were diagnosed and classified according to the fifth edition of the World Health Organization Classification of Tumors.

Immunohistochemistry. The FFPE sections were deparaffinized and rehydrated with xylene and alcohol. Thereafter, these sections were incubated with antibodies against ALK (rabbit monoclonal, clone D5F3, 1:50; Cell Signaling Technology, Danvers, MA, USA), CD56 (mouse monoclonal, clone CD564, 1:100; Novocastra, Newcastle, UK), chromogranin A (mouse monoclonal, clone DAK-A3, 1:500; Dako, Glostrup, Denmark), cytokeratin AE1/AE3 (mouse monoclonal, clone AE1/AE3, 1:600; Agilent, Santa Clara, CA, USA), ER (mouse monoclonal, clone GF11, 1:750; Novocastra), ki-67 (mouse monoclonal, clone MB-1, 1:150; Dako), p16 (mouse monoclonal, clone E6H4, prediluted; Ventana Medical Systems, Oro Valley, AZ, USA), p53 (mouse monoclonal, clone DO7, 1:300; Novocastra), PAX2 (rabbit monoclonal, clone EP3251, 1:300; Abcam, Cambridge, UK), PAX8 (rabbit polyclonal, 1:50; Cell Marque, Rocklin, CA, USA), PR (mouse monoclonal, clone PGR 312, 1:150; Novocastra), ROS1 (rabbit monoclonal, clone D4D6, prediluted; Cell Signaling Technology), synaptophysin (mouse monoclonal, clone DAK-SYNAP, 1:900; Dako), SMAD4 (mouse monoclonal, clone B-8, 1:50; Santa Cruz Biotechnology, Dallas, TX, USA), TTF-1 (mouse monoclonal, clone 8G7G3/1, 1:150; Dako), vimentin (mouse monoclonal, vim 384, 1:200; Dako), and WT 1 (mouse monoclonal; clone 6F-H2, 1:200; Cell Marque). The sections were chromogenically visualized using an ultraView Universal DAB Detection Kit (Ventana Medical Systems) or EnVision FLEX/HRP (Dako) and were subsequently counterstained with hematoxylin. These stained slides were analyzed by MK and JYJ.

PCR analysis for EGFR and ROS1 fusion. A representative section comprising the largest tumor volume (at least 30%) was subjected to molecular analysis in each case. To detect *EGFR* mutations, we performed peptide nucleic acid (PNA)-mediated real-time PCR using the PNAClamp EGFR Mutation Detection Kit (Panagene, Daejeon, Korea) or the PANAMutyper EGFR Kit (Panagene) according to the manufacturer's instructions. Additionally, to detect *ROS1* rearrangements, we performed real-time PCR using the ROS1 Gene Fusions Detection Kit (AmoyDx, Xiamen, PR China).

Targeted NGS. We detected cancer-related genetic alterations by performing targeted NGS using a customized cancer panel (ONCOaccuPanel, NGeneBio, Seoul, Republic of Korea) as per the manufacturer's instructions. The ONCOaccuPanel was designed to identify potential single nucleotide variants (SNVs) in 323 cancer-related genes. This panel also identified insertions/deletions (indels) and copy number variations (CNVs) and potential fusion variants of six genes. To conduct targeted NGS, we first extracted DNA from the FFPE sections using the QIAGEN AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany). Following this, 100 ng of the extracted DNA was subjected to hybridization capture-based target enrichment and paired-end sequencing (2×150 bp) performed using a MiSeq sequencer (Illumina, San Diego, CA, USA) according to the

Table II. *Cancer-related genomic alterations identified in this study.*

Case number	Gene	Mutation type	HGVSc	Amino acid change
Case 1	*TPR-ROS1*	Fusion	NM_003292.3-NM_002944.2	Not applicable
Case 2	*EGFR-RAD51*	Fusion	NM_005228.5-NM_002875.5	Not applicable
Case 3	*NCOA4-RET*	Fusion	NM_005437.3-NM_0020975.6	Not applicable
	TP53	Missense	NM_000546.5:c.713G>A	p.Cys238Tyr
	TERT	Missense	NM_198253.2:c.-124G>A (C228T)	Not applicable
Case 4	*MET*	Splicing	NM_000245.2:c.2888-17_2953del	Not applicable
	KRAS	Missense	NM_033360.2:c.182A>T	p.Gln61Leu
Case 5	*KRAS*	Missense	NM_033360.2:c.35G>T	p.Gly12Val
	TP53	Missense	NM_000546.5:c.785G>T	p.Gly262Val
	SMAD4	Nonsense	NM_005359.5:c.399C>G	p.Tyr133*
	CDKN2A	Frameshift	NM_000077.4:c.143del	p.Pro48Argfs*5
Case 6	*KRAS*	Missense	NM_033360.2:c.35G>T	p.Gly12Val
	STK11	Missense	NM_000455.4:c.580G>T	p.Asp194Tyr
	SMARCA4	Nonsense	NM_003072.3:c.4226C>A	p.Ser1409*
Case 7	*BRCA1*	Nonsense	NM_007294.3:c.5445G>A	p.Trp1815*
	TP53	Frameshift	NM_000546.5:c.766del	p.Thr256Hisfs*89
	TSC2	Frameshift	NM_000548.3:c.4568_4569del	p.Glu1523Valfs*5
Case 8	*BRCA2*	Frameshift	NM_000059.3:c.6724_6725del	p.Asp2242Phefs*2
	ARID1A	Nonsense	NM_006015.4:c.1669C>T	p.Gln557*
	PARP1	Nonsense	NM_001618.3:c.43A>T	p.Lys15*
Case 9	*ERBB2*	Missense	NM_004448.2:c.2686C>G	p.Arg896Gly
	TP53	Missense	NM_000546.5:c.743G>T	p.Arg248Leu
	CREBBP	Missense	NM_004380.2:c.4337G>T	p.Arg1446Leu
	RUNX1	Missense	NM_001754.4:c.485G>A	p.Arg162Lys
	MAP3K1	Nonsense	NM_005921.1:c.3379G>T	p.Glu1127*
	FANCL	Nonsense	NM_001114636.1:c.223C>T	p.Gln75*

manufacturer's instructions. Furthermore, Burrows–Wheeler Aligner and Genome Analysis Tool Kit were used to analyze the SNVs, indels, and CNVs in the samples. Variants with a total depth of at least 100× and allele frequencies of at least 3% were included in the variant analysis; STAR-Fusion and FusionCatcher were used to evaluate the fusion variants. Thereafter, we conducted variant interpretation and annotation based on the recommendations of the Association for Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists (31).

Results

Patient cohort and the genomic landscape of the selected cases. Table I presents the clinicopathological characteristics of nine cancer cases evaluated in this study, designated as cases 1-9. The median age of nine patients, four men and five women, was found to be 63 years (range=45-73 years). Furthermore, we identified four advanced lung adenocarcinoma cases, two ovarian cancer cases and one anaplastic thyroid carcinoma case. However, the remaining two cases were malignancies of unknown origins. Additionally, all patients had stage IV cancer except for the case 7 patient who had stage 1A cancer.

Next, we performed targeted NGS at a mean depth of 373.3× (range=243.9×-444.7×) and assessed the mean number of pathogenic mutations to be 2.8 (range=1-6) (Table II). Mean number of variants harboring mutations of uncertain significance was 5.3 (range=3-9).

Cases 1-4. NGS played a critical role in finding rare targetable alterations. Case 1 was diagnosed as lung adenocarcinoma (Figure 1), and the patient had multiple lymph node and liver metastases. Furthermore, our immunohistochemical analysis revealed that the tumor was ALK-negative. Additionally, the PCR analysis determined that the tumor was negative for *ROS1* fusion and wild-type *EGFR*. Notably, we detected *TPR-ROS1* fusion by targeted NGS (Figure 2); this alteration has not been previously reported in lung cancer. Subsequent immunohistochemical analysis demonstrated diffuse and strong cytoplasmic positivity for ROS1 in the tumor cells. Thereafter, the patient began crizotinib treatment and remained stable exhibiting no symptoms.

In case 2, the patient had lung adenocarcinoma with multiple lymph node, pleura, and bone metastases. Our PCR and immunohistochemical analyses revealed that the tumor was negative for *EGFR* and *ROS1* and ALK, respectively (Figure 1). Subsequently, the NGS analysis detected an *EGFR-RAD51* fusion (Figure 2) and RICTOR deletions.

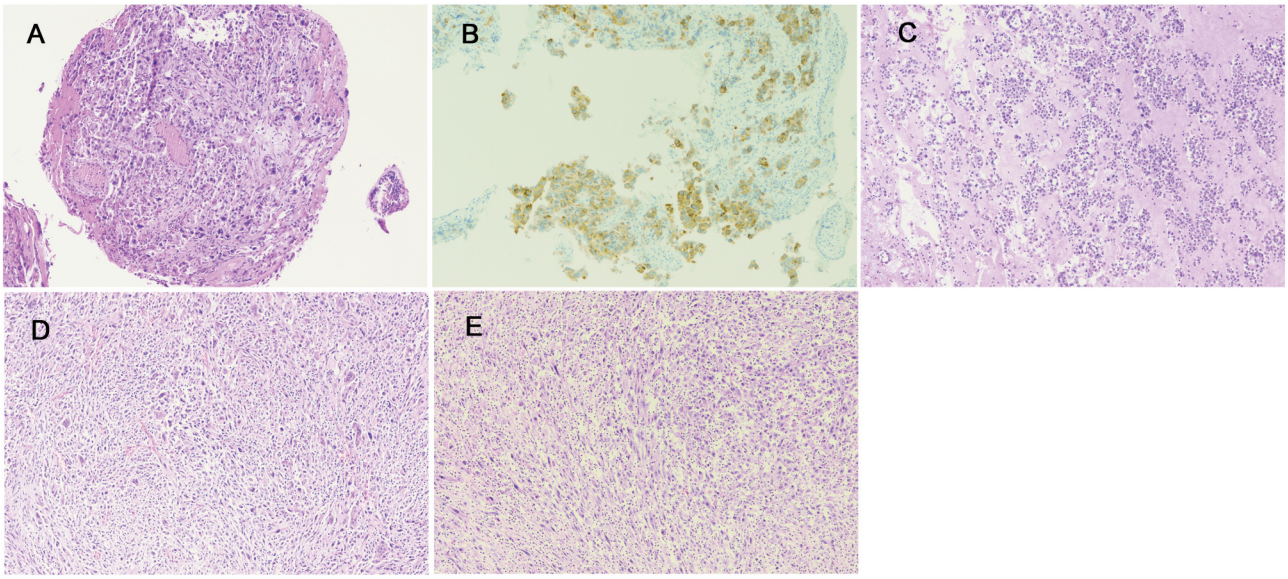

Figure 1. *Histopathological and immunohistochemical findings of cases 1-4. (A) and (B): Case 1. (A) Hematoxylin and eosin (H&E) staining and (B) immunohistochemical staining of ROS1. (C) H&E staining of case 2 sample. (D) H&E staining of case 3 sample. (E) H&E staining of case 4 sample. Original magnifications in (A)-(E): ×100.*

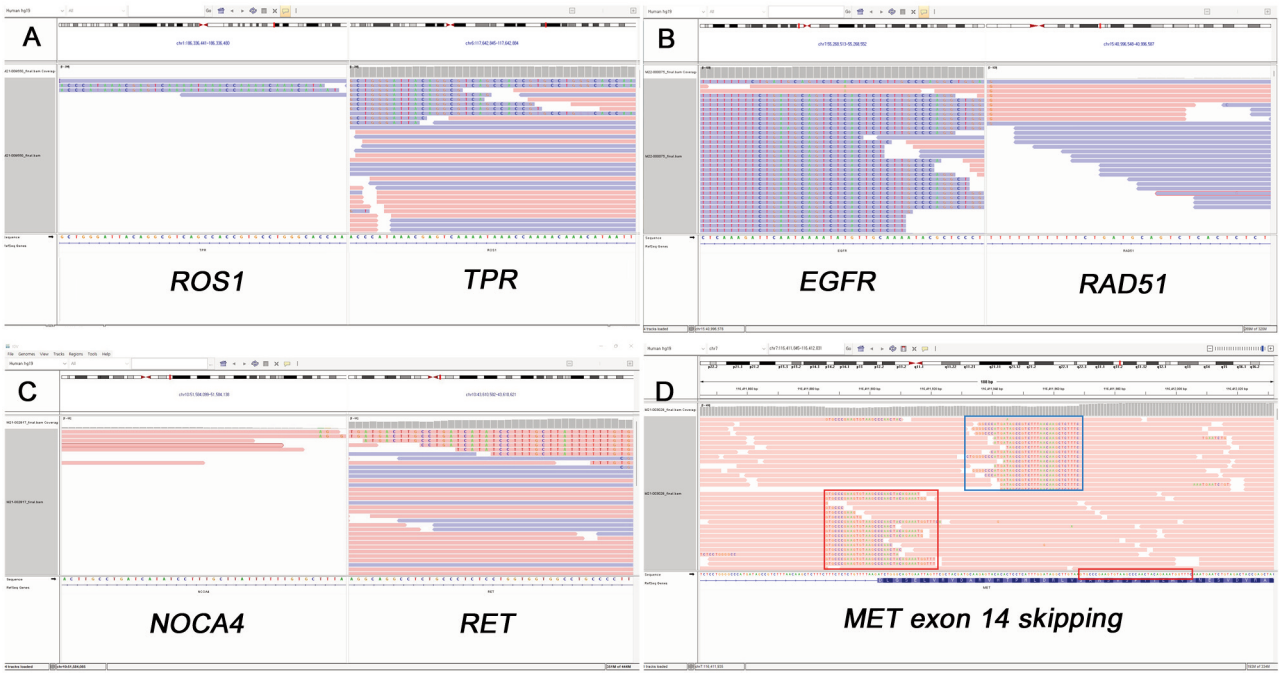

Figure 2. *Integrative Genomics Viewer snapshot of the (A) TPR-ROS1 fusion, (B) EGFR-RAD51 fusion, (C) NCOA4-RET fusion, and (D) MET exon 14 skipping.*

Case 3 was an anaplastic thyroid carcinoma with multiple lymph node and lung metastases. Total thyroidectomy was performed in this case. On histological examination we observed that the entire tumor had a sarcomatoid pattern (Figure 1). Remarkably, we detected a *NCOA4-RET* fusion and pathogenic alterations in *TP53* C238Y *and TERT* C228T

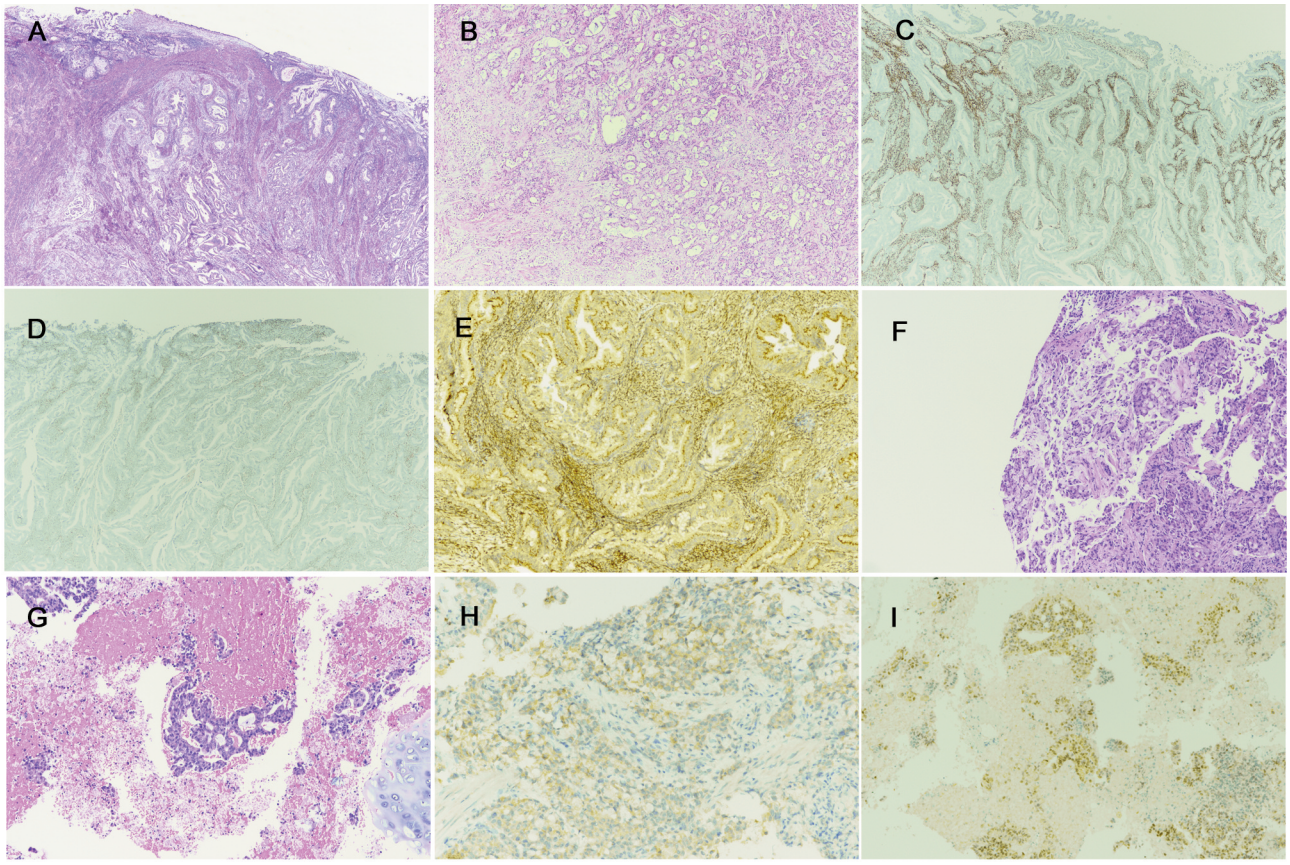

Figure 3. *Histopathological and immunohistochemical finding of cases 5-6. (A)-(E): Case 5. (A) Hematoxylin and eosin (H&E) staining of uterine and (B) pancreatic tumor samples. Immunohistochemical staining of (C) ER, (D), PR, and (E) SMAD4. (F)-(I): Case 6. (F) H&E staining of lung and (G) lymph node samples. Immunohistochemical staining of TTF-1 in the (H) lung and (I) lymph node samples. Original magnifications: (A), scan view; (B), ×40; (C)-(E), ×40; (F)-(I), ×100.*

by targeted NGS (Figure 2). Subsequently, the patient was treated with selpercatinib. Post one month of treatment, a follow-up chest computed tomography (CT) revealed a significant decrease in size of the metastasized tumor.

In case 4, the patient had a lung mass and multiple lymph node, peritoneum, bone, and small bowel metastases. Consequently, small bowel mass excision was performed. We diagnosed this patient with sarcomatoid carcinoma, as the tumor exhibited histologic characteristics of both carcinoma and sarcoma (Figure 1). Moreover, our immunohistochemical analysis revealed that the tumor was positive for TTF-1, cytokeratin (AE1/AE3), and vimentin. On the other hand, targeted NGS detected exon 14 skipping in *MET* (Figure 2) and a pathogenic mutation in *KRAS* Q61L, and an amplification of *KRAS*. Therefore, capatinib treatment was initiated for this patient. Of note, a follow-up chest CT post six months of treatment demonstrated a significant decrease in the size of the lung and metastasized masses.

Cases 5-6. NGS played a critical role in diagnosis. In case 5, the patient had a history of pancreatic cancer five years prior. A follow-up positron emission tomography-CT revealed a focal fluorodeoxyglucose uptake in the endometrium. The tumor was histologically diagnosed as an adenocarcinoma upon biopsy, and total abdominal hysterectomy was performed. Remarkably, the endometrial tumor was histologically indistinguishable from the metastatic pancreatic adenocarcinoma (Figure 3). Notably, our immunohistochemical analysis determined the endometrial tumor to be negative for ER, PR, PAX2, and PAX8; SMAD4 loss was also detected. We also performed targeted NGS of both the pancreatic and endometrial specimens to identify the origin of the endometrial tumor. In fact, the NGS analysis revealed that both the specimens had identical driver mutations: *KRAS, TP53, CDKN2A*, and *SMAD4*. Both the tumors also had identical mutational variants of uncertain significance. Interestingly, they also had identical amino acid changes in sequences of proteins encoded by all genes except *SMAD4*

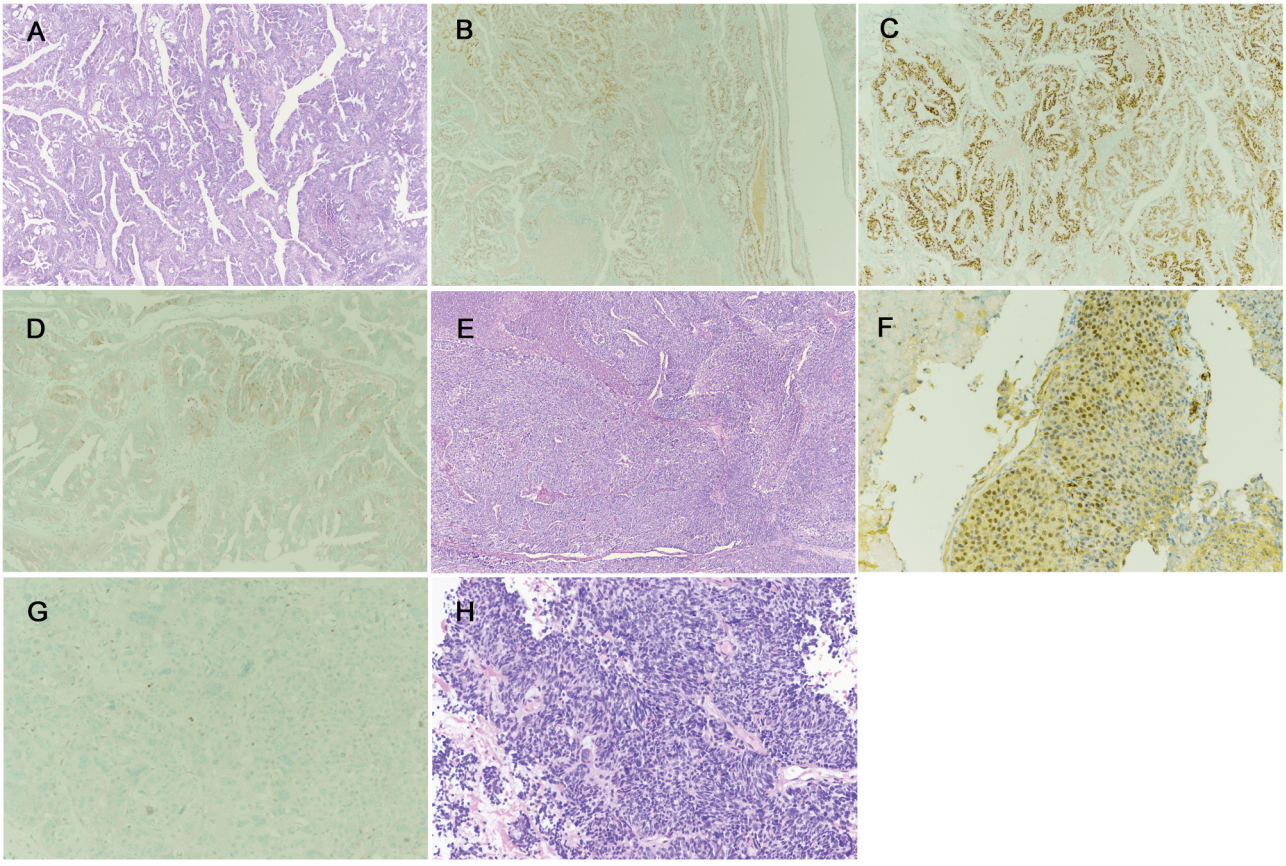

Figure 4. *Histopathological and immunohistochemical findings of cases 7-9. (A)-(D): Case 7. (A) Hematoxylin and eosin (H&E) staining and (B) immunohistochemical staining of ER, (C) PR, and (D) p53. (E)-(G): Case 8. (E) H&E staining and (F) Immunohistochemical staining of WT1 and (G) p53. (H) H&E staining of case 9 sample. Original magnifications: (A)-(C) and (E), ×40; (D), ×100; (F)-(H): ×200.*

(*SMAD4* p.H261Ifs*75 in the pancreatic specimen *vs*. *SMAD4* p.Tyr133* in the endometrial specimen).

The case 6 patient was diagnosed with lung cancer and presented with clinical symptoms of multiple lymph node, adrenal gland, chest wall, and abdominal metastases. Of note, lung and lymph node biopsies had significantly different histological and immunohistochemical characteristics. For instance, the lymph node biopsy sample had a typical lung adenocarcinoma histology and exhibited TTF-1 nuclear positivity in immunohistochemistry. In contrast, the lung biopsy sample was TTF-1 negative and demonstrated a solid growth pattern with a few signet-ring cells (Figure 3). Thus, we speculated that this case was of a double primary cancer. Indeed, targeted NGS determined that both the specimens shared all pathogenic genetic alterations (*KRAS* G12V, *STK11* D194Y, and *SMARCA4* S1409*) and exhibited identical amino acid changes.

Cases 7-9. Dilemma cases demonstrating significant discrepancies between NGS, histological, and immunohistochemical data. In case 7, the patient visited the hospital because of an incidentally detected 15.0 cm-sized left ovarian mass. Consequently, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and lymph node dissection were performed. Histological examination demonstrated that the tumor had back-to-back arrangements of small glands lined by an endometrioid-like epithelium. The tumor cells had nuclei that were round or oval in shape and occasionally were observed to undergo mitosis. Remarkably, the tumor was positive for ER, PR, and PAX8, and focally positive for WT1, as determined by immunohistochemistry. The immunohistochemical analysis also detected weak and focal cytoplasmic expression of p53 (Figure 4). Thus, an endometrioid adenocarcinoma diagnosis of the ovarian tumor was made in line with the histological and immunohistochemical findings. However, subsequent NGS analysis identified *BRCA1* W1815* and *TP53* T256Hfs*89 mutations that are typical for ovarian high-grade serous carcinoma. We also detected a pathogenic mutation in *ASC2* E1523Vfs*5.

Histological and immunohistochemical findings in case 8 were also consistent with ovarian high-grade serous carcinoma. The tumor demonstrated a solid, papillary, and glandular architecture with marked nuclear atypia. Moreover, the tumor cells underwent frequent mitosis. Immunohistochemical analysis of the tumor demonstrated WT1- and PAX8-positive nuclear expression, diffuse p16 expression, and complete negative p53 staining (mutant pattern; Figure 4). Furthermore, subsequent NGS analysis determined pathogenic mutations in the *BRCA2* D2242Ffs*2, *ARID1A* Q557*, and *PARP1* K15* genes. However, we did not detect genetic alterations in *TP53*.

Case 9 was diagnosed with small cell lung carcinoma and had consistent histological (Figure 4) and immunohistochemical findings (positive expression for chromogranin A, synaptophysin, and cytokeratin AE1/AE3 positive and a >90% Ki-67 labeling index). Interestingly, our NGS analysis detected no *RB1* loss, which is a characteristic of small cell lung carcinoma. Instead, we detected pathogenic alterations in *ERBB2* R896G, *TP53* R248L, *CREBBP* R1446L, *RUNX1* R162K, *MAP3K1* E1127*, and *FANCL* Q75*. We also identified deletions of *TP53,* and *PTEN*.

Discussion

In this study, we present real-world cases wherein NGS was beneficial in making an appropriate diagnosis and establishing an effective treatment plan for cancer patients.

First, targeted NGS identified targetable genetic alterations that were not detected by immunohistochemistry or PCR. For instance, we detected *TPR-ROS1* fusion in case 1, which has not been previously reported in lung adenocarcinoma. Of note, *ROS1* fusion occurs in 2-3% of invasive non-mucinous lung adenocarcinoma (32); such cases are treated with targeted tyrosine kinase inhibitors (TKIs) (33). *ROS1* frequently fusses with the following genes: cluster of differentiation 74 (*CD74*), ezrin (*EZR*), syndecan 4 (*SDC4*), and tropomyosin 3 (*TPM3*) (34). Indeed, the AmoyDx ROS1 Gene Fusions Detection Kit did not include a probe for translocated promoted region (*TPR*), thereby failing to detect the *TPR-ROS1* fusion. *TPR* encodes a large coiled coil protein that interacts with nuclear pore complexes (35); oncogenic fusion of *TPR* with several kinase genes has been reported in multiple types of neoplasms (36, 37). The breakpoint of the *TPR-ROS1* fusion detected in case 1 had an intact kinase domain but lacked other driver mutations, indicating an oncogenic nature. This speculation is further supported by the diffuse and strong ROS1-positive expression we observed upon immunohistochemistry. Furthermore, *EGFR-RAD51* fusion was detected in case 2. Although the majority of *EGFR*-activating mutations in lung adenocarcinomas are insertions and deletions (indels) or SNVs (38), *EGFR* fusion has been occasionally reported in previous studies. Although such cases of lung adenocarcinomas are limited in number, they have demonstrated a good response to *EGFR* TKIs (39-41). Since there is no commercially available kit specifically designed for the detection of *EGFR* fusion, NGS analysis is an essential alternative to identify such a rare fusion. In case 3, we detected *RET* fusion accompanied with *TP53* and TERT promoter mutations. Although *RET* fusion has rarely been reported in anaplastic thyroid carcinoma (42), the patient in case 3 exhibited a remarkable response to capatinib treatment during follow-up. Adenocarcinoma with *MET* exon 14 skipping occurs in a subset of lung adenocarcinomas. In fact, sarcomatoids with *MET* exon 14 skipping in lung cancer have been previously described (43-45), consistent with our observations in case 4. In conclusion, targeted NGS should be the first choice for multiplex testing to detect these rare but targetable genetic alterations.

Second, we investigated two cases wherein targeted NGS helped identify the origin of malignant tumors. Pancreatic adenocarcinoma is one of the deadliest cancers that metastasizes to many organs (46); nonetheless, metastasis to the uterus has rarely been reported in pancreatic adenocarcinoma (47). Interestingly, the endometrial mass in case 5 was histologically and immunohistochemically indistinguishable from the primary pancreatic adenocarcinoma. Since the primary pancreatic adenocarcinoma and endometrial mass shared most of the genetic alterations, we concluded that the endometrial mass was a metastatic pancreatic cancer. However, the two tumors had different amino acid changes in protein encoded by *SMAD4*, which is attributable to intratumor heterogeneity and branched clonal evolution (48-51).

Lung and lymph node biopsy samples in case 6 had different histological and immunohistochemical profiles. Nevertheless, we performed targeted NGS to confirm that both tumors shared identical genetic alterations and were of the same lineage. Although histological assessment and immunohistochemical evaluation are the cornerstone of pathological diagnosis, studies have reported inconsistent histological and immunohistochemical data between primary and metastatic tumors. For example, Bruehl *et al*. (2021) suggested that histologic assessment alone can mislead judgement regarding the possibility of a double primary cancer; instead, next-generation sequencing can be used to distinguish primary tumors from metastatic tumors (52).

Lastly, we assessed interesting cases that demonstrated substantial discrepancies between the histological, immunohistochemical, and NGS findings. It is challenging to distinguish ovarian high-grade serous carcinoma from endometrioid carcinoma, particularly poorly differentiated carcinoma, because of a morphological overlap (53). Furthermore, high-grade serous carcinoma has solid, pseudo-endometroid, and transitional cell patterns (54) designated as "SET" variant. While aberrant p53 staining pattern, diffuse WT1- and p16-positive staining, and negative ER and PR staining are immunohistochemical characteristics of high-grade

serous carcinoma, endometroid adenocarcinoma exhibits contrasting characteristics (53). Histologic findings in case 7 were characteristic of an endometrioid carcinoma demonstrating round, punched-out glandular spaces. While we did not detect any psammoma bodies, our immunohistochemical data revealed that the tumors were ER- and PR-positive and were only focally WT1-positive. Additionally, the tumor demonstrated weak and focal cytoplasmic p53 expression. On the other hand, histological and immunohistochemical evaluations in case 8 implicated it to be high-grade serous carcinoma. However, while the genomic profile of case 7 indicated at high-grade serous carcinoma with concurrent mutations of *BRCA1* and *TP53*, that of case 8 suggested endometrioid carcinoma possessing an *ARID1A* mutations and no *TP53* mutation. This might be of therapeutic significance because ovarian cancers with *BRCA* mutations or homologous recombination deficiency are treatable with *PARP* inhibitors (55). Taken together, ovarian cancer subtypes that are not high-grade serious carcinoma histology can also benefit from targeted NGS, although the number might be small. Furthermore, case 9 was a small-cell lung carcinoma. Bi-allelic inactivation of *RB1* and *TP53* is essential for small cell lung carcinoma (56). Our NGS analysis of case 9 identified pathogenic mutations in *TP53*, *ERBB2*, *CREBBP*, *RUNX1*, *MAP3K1*, and *FANCL*; however, *an RB1* mutation was not detected. Since inactivation of *RB1* often occurs through complex genomic rearrangements (57), it is possible that *RB1* loss was not detected by targeted NGS. Notably, we detected an *ERBB2* mutation in case 9. *ERBB2* mutations are frequently found in non-small cell lung cancer; in contrast, *ERBB2* is rarely mutated in small cell lung cancer (58). Indeed, we did not identify any pathogenic alterations in *ERBB2* upon screening the cBio Cancer Genomics Portal (http://cbioportal.org). Importantly, oncogenic mutation of *ERBB2* in non-small cell lung cancer is an indication for targeted therapy, such as ado-trastuzumab emtansine (59). However, there is a lack of evidence supporting targeted therapy of *ERBB2* mutations in small cell lung carcinoma.

This study had certain limitations. Firstly, owing to our use of a selected gene panel for targeted NGS, rare but targetable genetic alterations or mutations of diagnostic significance may have been missed. Secondly, only selected representative cases have been included in the study. Thus, this study does not provide the overall mutational landscape of tumors in real-world samples.

Despite these limitations, we demonstrated the usefulness of applying targeted NGS in clinical practice. Additionally, targetable genetic alterations that were not detected in other molecular methods were successfully detected by massive parallel sequencing. Targeted NGS was also proven to be efficient in confirming the origin of a malignant tumor. Therefore, NGS can be widely used for the management of cancer patients, as it can detect unexpected targetable alterations that go undetected during histological and immunohistochemical examinations.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

JYP conceived and designed the manuscript. JYJ and KMS drafted the manuscript. MSK, NJP analyzed previous articles on aggressive thyroid cancer. MSK, JYJ, NJP, and JYP reviewed and revised the manuscript carefully. All Authors have read and approved the final manuscript.

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References

1 Fouad YA and Aanei C: Revisiting the hallmarks of cancer. Am J Cancer Res *7(5)*: 1016-1036, 2017. PMID: 28560055.

2 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144(5)*: 646-674, 2011. PMID: 21376230. DOI: 10.1016/j.cell.2011.02.013

3 Chakravarty D and Solit DB: Clinical cancer genomic profiling. Nat Rev Genet *22(8)*: 483-501, 2021. PMID: 33762738. DOI: 10.1038/s41576-021-00338-8

4 Yatabe Y, Sunami K, Goto K, Nishio K, Aragane N, Ikeda S, Inoue A, Kinoshita I, Kimura H, Sakamoto T, Satouchi M, Shimizu J, Tsuta K, Toyooka S, Nishino K, Hatanaka Y, Matsumoto S, Mikubo M, Yokose T and Dosaka-Akita H: Multiplex gene-panel testing for lung cancer patients. Pathol Int *70(12)*: 921-931, 2020. PMID: 32956529. DOI: 10.1111/pin.13023

5 Pennell NA, Mutebi A, Zhou ZY, Ricculli ML, Tang W, Wang H, Guerin A, Arnhart T, Dalal A, Sasane M, Wu KY, Culver KW and Otterson GA: economic impact of next-generation sequencing versus single-gene testing to detect genomic alterations in metastatic non-small-cell lung cancer using a decision analytic model. JCO Precis Oncol *3*: 1-9, 2019. PMID: 35100695. DOI: 10.1200/PO.18.00356

6 Xue Y, Ankala A, Wilcox WR and Hegde MR: Solving the molecular diagnostic testing conundrum for Mendelian disorders in the era of next-generation sequencing: single-gene, gene panel, or exome/genome sequencing. Genet Med *17(6)*: 444-451, 2015. PMID: 25232854. DOI: 10.1038/gim.2014.122

7 Siroy AE, Boland GM, Milton DR, Roszik J, Frankian S, Malke J, Haydu L, Prieto VG, Tetzlaff M, Ivan D, Wang WL, Torres-Cabala C, Curry J, Roy-Chowdhuri S, Broaddus R, Rashid A, Stewart J, Gershenwald JE, Amaria RN, Patel SP, Papadopoulos

NE, Bedikian A, Hwu WJ, Hwu P, Diab A, Woodman SE, Aldape KD, Luthra R, Patel KP, Shaw KR, Mills GB, Mendelsohn J, Meric-Bernstam F, Kim KB, Routbort MJ, Lazar AJ and Davies MA: Beyond BRAF(V600): clinical mutation panel testing by next-generation sequencing in advanced melanoma. J Invest Dermatol *135(2)*: 508-515, 2015. PMID: 25148578. DOI: 10.1038/jid.2014.366

8 Goodwin S, McPherson JD and McCombie WR: Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet *17(6)*: 333-351, 2016. PMID: 27184599. DOI: 10.1038/nrg.2016.49

9 Metzker ML: Sequencing technologies - the next generation. Nat Rev Genet *11(1)*: 31-46, 2010. PMID: 19997069. DOI: 10.1038/nrg2626

10 Schwarze K, Buchanan J, Taylor JC and Wordsworth S: Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med *20(10)*: 1122-1130, 2018. PMID: 29446766. DOI: 10.1038/gim.2017.247

11 Jennings LJ, Arcila ME, Corless C, Kamel-Reid S, Lubin IM, Pfeifer J, Temple-Smolkin RL, Voelkerding KV and Nikiforova MN: Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn *19(3)*: 341-365, 2017. PMID: 28341590. DOI: 10.1016/j.jmoldx.2017.01.011

12 Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, Chandramohan R, Liu ZY, Won HH, Scott SN, Brannon AR, O'Reilly C, Sadowska J, Casanova J, Yannes A, Hechtman JF, Yao J, Song W, Ross DS, Oultache A, Dogan S, Borsu L, Hameed M, Nafa K, Arcila ME, Ladanyi M and Berger MF: Memorial sloan kettering-integrated mutation profiling of actionable cancer targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn *17(3)*: 251-264, 2015. PMID: 25801821. DOI: 10.1016/j.jmoldx.2014.12.006

13 Humphrey PA: Histopathology of prostate cancer. Cold Spring Harb Perspect Med *7(10)*: a030411, 2017. PMID: 28389514. DOI: 10.1101/cshperspect.a030411

14 Hanby AM: The pathology of breast cancer and the role of the histopathology laboratory. Clin Oncol (R Coll Radiol) *17(4)*: 234-239, 2005. PMID: 15997917. DOI: 10.1016/j.clon.2005.02.009

15 Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J, Richardson AL, Schnitt SJ, Schmitt FC, Tan PH, Tse GM, Badve S and Ellis IO: Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res *12(4)*: 207, 2010. PMID: 20804570. DOI: 10.1186/bcr2607

16 van den Bent MJ: Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. Acta Neuropathol *120(3)*: 297-304, 2010. PMID: 20644945. DOI: 10.1007/s00401-010-0725-7

17 Paech DC, Weston AR, Pavlakis N, Gill A, Rajan N, Barraclough H, Fitzgerald B and Van Kooten M: A systematic review of the interobserver variability for histology in the differentiation between squamous and nonsquamous non-small cell lung cancer. J Thorac Oncol *6(1)*: 55-63, 2011. PMID: 21107286. DOI: 10.1097/JTO.0b013e3181fc0878

18 Eefting D, Schrage YM, Geirnaerdt MJ, Le Cessie S, Taminiau AH, Bovée JV and Hogendoorn PC: Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. Am J Surg Pathol *33(1)*: 50-57, 2009. PMID: 18852676. DOI: 10.1097/PAS.0b013e31817eec2b

19 Longacre TA, Ennis M, Quenneville LA, Bane AL, Bleiweiss IJ, Carter BA, Catelano E, Hendrickson MR, Hibshoosh H, Layfield LJ, Memeo L, Wu H and O'malley FP: Interobserver agreement and reproducibility in classification of invasive breast carcinoma: an NCI breast cancer family registry study. Mod Pathol *19(2)*: 195-207, 2006. PMID: 16341153. DOI: 10.1038/modpathol.3800496

20 Hunter KD and Speight PM: The diagnostic usefulness of immunohistochemistry for odontogenic lesions. Head Neck Pathol *8(4)*: 392-399, 2014. PMID: 25409846. DOI: 10.1007/s12105-014-0582-0

21 Bae YS, Kim H, Noh SH and Kim H: Usefulness of immunohistochemistry for microsatellite instability screening in gastric cancer. Gut Liver *9(5)*: 629-635, 2015. PMID: 26343070. DOI: 10.5009/gnl15133

22 Moriya T, Kanomata N, Kozuka Y, Fukumoto M, Iwachido N, Hata S, Takahashi Y, Miura H, Ishida K and Watanabe M: Usefulness of immunohistochemistry for differential diagnosis between benign and malignant breast lesions. Breast Cancer *16(3)*: 173-178, 2009. PMID: 19475479. DOI: 10.1007/s12282-009-0127-7

23 Zhou M, Roma A and Magi-Galluzzi C: The usefulness of immunohistochemical markers in the differential diagnosis of renal neoplasms. Clin Lab Med *25(2)*: 247-257, 2005. PMID: 15848735. DOI: 10.1016/j.cll.2005.01.004

24 da Cunha Santos G, Shepherd FA and Tsao MS: EGFR mutations and lung cancer. Annu Rev Pathol *6*: 49-69, 2011. PMID: 20887192. DOI: 10.1146/annurev-pathol-011110-130206

25 Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar AK and Xing M: Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer *20(4)*: 603-610, 2013. PMID: 23766237. DOI: 10.1530/ERC-13-0210

26 Tsuta K, Kohno T, Yoshida A, Shimada Y, Asamura H, Furuta K and Kushima R: RET-rearranged non-small-cell lung carcinoma: a clinicopathological and molecular analysis. Br J Cancer *110(6)*: 1571-1578, 2014. PMID: 24504365. DOI: 10.1038/bjc.2014.36

27 Woehrer A, Sander P, Haberler C, Kern S, Maier H, Preusser M, Hartmann C, Kros JM, Hainfellner JA and Research Committee of the European Confederation of Neuropathological Societies: FISH-based detection of 1p 19q codeletion in oligodendroglial tumors: procedures and protocols for neuropathological practice – a publication under the auspices of the Research Committee of the European Confederation of Neuropathological Societies (Euro-CNS). Clin Neuropathol *30(2)*: 47-55, 2011. PMID: 21329613. DOI: 10.5414/npp30047

28 Selinger CI, Li BT, Pavlakis N, Links M, Gill AJ, Lee A, Clarke S, Tran TN, Lum T, Yip PY, Horvath L, Yu B, Kohonen-Corish MR, O'Toole SA and Cooper WA: Screening for ROS1 gene rearrangements in non-small-cell lung cancers using immunohistochemistry with FISH confirmation is an effective method to identify this rare target. Histopathology *70(3)*: 402-411, 2017. PMID: 27599111. DOI: 10.1111/his.13076

29 Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, Meng W, Dewal N, Milbury C, Clark T, Donahue A, Stover D, Kennedy M, Dacpano-Komansky J, Burns C, Vietz C, Alexander B, Hegde P and Dennis L: Clinical and analytical validation of

FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One *15(9)*: e0237802, 2020. PMID: 32976510. DOI: 10.1371/journal.pone.0237802

30 Pestinger V, Smith M, Sillo T, Findlay JM, Laes JF, Martin G, Middleton G, Taniere P and Beggs AD: Use of an integrated pan-cancer oncology enrichment next-generation sequencing assay to measure tumour mutational burden and detect clinically actionable variants. Mol Diagn Ther *24(3)*: 339-349, 2020. PMID: 32306292. DOI: 10.1007/s40291-020-00462-x

31 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL and ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med *17(5)*: 405-424, 2015. PMID: 25741868. DOI: 10.1038/gim.2015.30

32 Kohno T, Nakaoku T, Tsuta K, Tsuchihara K, Matsumoto S, Yoh K and Goto K: Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. Transl Lung Cancer Res *4(2)*: 156-164, 2015. PMID: 25870798. DOI: 10.3978/j.issn.2218-6751.2014.11.11

33 Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW and Iafrate AJ: Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med *371(21)*: 1963-1971, 2014. PMID: 25264305. DOI: 10.1056/NEJMoa1406766

34 He Y, Sheng W, Hu W, Lin J, Liu J, Yu B, Mao X, Zhang L, Huang J and Wang G: Different types of *ROS1* fusion partners yield comparable efficacy to crizotinib. Oncol Res *27(8)*: 901-910, 2019. PMID: 30940295. DOI: 10.3727/096504019X15509372008132

35 Nakano H, Funasaka T, Hashizume C and Wong RW: Nucleoporin translocated promoter region (Tpr) associates with dynein complex, preventing chromosome lagging formation during mitosis. J Biol Chem *285(14)*: 10841-10849, 2010. PMID: 20133940. DOI: 10.1074/jbc.M110.105890

36 Choi YL, Lira ME, Hong M, Kim RN, Choi SJ, Song JY, Pandy K, Mann DL, Stahl JA, Peckham HE, Zheng Z, Han J, Mao M and Kim J: A novel fusion of TPR and ALK in lung adenocarcinoma. J Thorac Oncol *9(4)*: 563-566, 2014. PMID: 24736082. DOI: 10.1097/JTO.0000000000000093

37 Al-Ibraheemi A, Folpe AL, Perez-Atayde AR, Perry K, Hofvander J, Arbajian E, Magnusson L, Nilsson J and Mertens F: Aberrant receptor tyrosine kinase signaling in lipofibromatosis: a clinicopathological and molecular genetic study of 20 cases. Mod Pathol *32(3)*: 423-434, 2019. PMID: 30310176. DOI: 10.1038/s41379-018-0150-3

38 Hsu WH, Yang JC, Mok TS and Loong HH: Overview of current systemic management of EGFR-mutant NSCLC. Ann Oncol *29(suppl 1)*: i3-i9, 2018. PMID: 29462253. DOI: 10.1093/annonc/mdx702

39 Konduri K, Gallant JN, Chae YK, Giles FJ, Gitlitz BJ, Gowen K, Ichihara E, Owonikoko TK, Peddareddigari V, Ramalingam SS, Reddy SK, Eaby-Sandy B, Vavalà T, Whiteley A, Chen H, Yan Y, Sheehan JH, Meiler J, Morosini D, Ross JS, Stephens PJ, Miller VA, Ali SM and Lovly CM: EGFR fusions as novel therapeutic targets in lung cancer. Cancer Discov *6(6)*: 601-611, 2016. PMID: 27102076. DOI: 10.1158/2159-8290.CD-16-0075

40 Raez LE, Pinto JA, Schrock AB and Ali SM: EGFR-RAD51 fusion: a targetable partnership originated from the tumor evolution? J Thorac Oncol *13(3)*: e33-e34, 2018. PMID: 29472054. DOI: 10.1016/j.jtho.2017.10.005

41 Zhu YC, Wang WX, Xu CW, Song ZB, Du KQ, Chen G, Lv TF and Song Y: EGFR-RAD51 fusion variant in lung adenocarcinoma and response to erlotinib: A case report. Lung Cancer *115*: 131-134, 2018. PMID: 29290255. DOI: 10.1016/j.lungcan.2017.12.001

42 Dias-Santagata D, Lennerz JK, Sadow PM, Frazier RP, Govinda Raju S, Henry D, Chung T, Kherani J, Rothenberg SM and Wirth LJ: Response to RET-specific therapy in *RET* fusion-positive anaplastic thyroid carcinoma. Thyroid *30(9)*: 1384-1389, 2020. PMID: 32292131. DOI: 10.1089/thy.2019.0477

43 Heist RS, Shim HS, Gingipally S, Mino-Kenudson M, Le L, Gainor JF, Zheng Z, Aryee M, Xia J, Jia P, Jin H, Zhao Z, Pao W, Engelman JA and Iafrate AJ: MET exon 14 skipping in non-small cell lung cancer. Oncologist *21(4)*: 481-486, 2016. PMID: 27022036. DOI: 10.1634/theoncologist.2015-0510

44 Reungwetwattana T, Liang Y, Zhu V and Ou SI: The race to target MET exon 14 skipping alterations in non-small cell lung cancer: The Why, the How, the Who, the Unknown, and the Inevitable. Lung Cancer *103*: 27-37, 2017. PMID: 28024693. DOI: 10.1016/j.lungcan.2016.11.011

45 Paik PK, Felip E, Veillon R, Sakai H, Cortot AB, Garassino MC, Mazieres J, Viteri S, Senellart H, Van Meerbeeck J, Raskin J, Reinmuth N, Conte P, Kowalski D, Cho BC, Patel JD, Horn L, Griesinger F, Han JY, Kim YC, Chang GC, Tsai CL, Yang JC, Chen YM, Smit EF, van der Wekken AJ, Kato T, Juraeva D, Stroh C, Bruns R, Straub J, Johne A, Scheele J, Heymach JV and Le X: Tepotinib in non-small-cell lung cancer with *MET* exon 14 skipping mutations. N Engl J Med *383(10)*: 931-943, 2020. PMID: 32469185. DOI: 10.1056/NEJMoa2004407

46 Kleeff J, Korc M, Apte M, La Vecchia C, Johnson CD, Biankin AV, Neale RE, Tempero M, Tuveson DA, Hruban RH and Neoptolemos JP: Pancreatic cancer. Nat Rev Dis Primers *2*: 16022, 2016. PMID: 27158978. DOI: 10.1038/nrdp.2016.22

47 Hartsough EM, Erickson BK, Chauhan A and Khalifa MA: Isolated metastatic pancreatic adenocarcinoma to the uterine cervix: A case report. Gynecol Oncol Rep *29*: 61-63, 2019. PMID: 31334322. DOI: 10.1016/j.gore.2019.07.003

48 Knijn N, Mekenkamp LJ, Klomp M, Vink-Börger ME, Tol J, Teerenstra S, Meijer JW, Tebar M, Riemersma S, van Krieken JH, Punt CJ and Nagtegaal ID: KRAS mutation analysis: a comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients. Br J Cancer *104(6)*: 1020-1026, 2011. PMID: 21364579. DOI: 10.1038/bjc.2011.26

49 Caswell DR and Swanton C: The role of tumour heterogeneity and clonal cooperativity in metastasis, immune evasion and clinical outcome. BMC Med *15(1)*: 133, 2017. PMID: 28716075. DOI: 10.1186/s12916-017-0900-y

50 Kaji T, Yamasaki O, Takata M, Otsuka M, Hamada T, Morizane S, Asagoe K, Yanai H, Hirai Y, Umemura H and Iwatsuki K: Comparative study on driver mutations in primary and metastatic melanomas at a single Japanese institute: A clue for intra- and inter-tumor heterogeneity. J Dermatol Sci *85(1)*: 51-57, 2017. PMID: 27771229. DOI: 10.1016/j.jdermsci.2016.10.006

51 Werner-Klein M, Scheitler S, Hoffmann M, Hodak I, Dietz K, Lehnert P, Naimer V, Polzer B, Treitschke S, Werno C, Markiewicz A, Weidele K, Czyz Z, Hohenleutner U, Hafner C, Haferkamp S, Berneburg M, Rümmele P, Ulmer A and Klein CA: Genetic

alterations driving metastatic colony formation are acquired outside of the primary tumour in melanoma. Nat Commun *9(1)*: 595, 2018. PMID: 29426936. DOI: 10.1038/s41467-017-02674-y

52 Bruehl FK, Doxtader EE, Cheng YW, Farkas DH, Farver C and Mukhopadhyay S: Does histological assessment accurately distinguish separate primary lung adenocarcinomas from intrapulmonary metastases? A study of paired resected lung nodules in 32 patients using a routine next-generation sequencing panel for driver mutations. J Clin Pathol, 2021. PMID: 33649140. DOI: 10.1136/jclinpath-2021-207421

53 Lim D, Murali R, Murray MP, Veras E, Park KJ and Soslow RA: Morphological and immunohistochemical reevaluation of tumors initially diagnosed as ovarian endometrioid carcinoma with emphasis on high-grade tumors. Am J Surg Pathol *40(3)*: 302-312, 2016. PMID: 26551621. DOI: 10.1097/PAS.0000000000000550

54 Howitt BE, Hanamornroongruang S, Lin DI, Conner JE, Schulte S, Horowitz N, Crum CP and Meserve EE: Evidence for a dualistic model of high-grade serous carcinoma: BRCA mutation status, histology, and tubal intraepithelial carcinoma. Am J Surg Pathol *39(3)*: 287-293, 2015. PMID: 25581732. DOI: 10.1097/PAS.0000000000000369

55 Ledermann JA: PARP inhibitors in ovarian cancer. Ann Oncol *27(Suppl 1)*: i40-i44, 2016. PMID: 27141070. DOI: 10.1093/annonc/mdw094

56 Meuwissen R, Linn SC, Linnoila RI, Zevenhoven J, Mooi WJ and Berns A: Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. Cancer Cell *4(3)*: 181-189, 2003. PMID: 14522252. DOI: 10.1016/s1535-6108(03)00220-4

57 George J, Lim JS, Jang SJ, Cun Y, Ozretić L, Kong G, Leenders F, Lu X, Fernández-Cuesta L, Bosco G, Müller C, Dahmen I, Jahchan NS, Park KS, Yang D, Karnezis AN, Vaka D, Torres A, Wang MS, Korbel JO, Menon R, Chun SM, Kim D, Wilkerson M, Hayes N, Engelmann D, Pützer B, Bos M, Michels S, Vlasic I, Seidel D, Pinther B, Schaub P, Becker C, Altmüller J, Yokota J, Kohno T, Iwakawa R, Tsuta K, Noguchi M, Muley T, Hoffmann H, Schnabel PA, Petersen I, Chen Y, Soltermann A, Tischler V, Choi CM, Kim YH, Massion PP, Zou Y, Jovanovic D, Kontic M, Wright GM, Russell PA, Solomon B, Koch I, Lindner M, Muscarella LA, la Torre A, Field JK, Jakopovic M, Knezevic J, Castaños-Vélez E, Roz L, Pastorino U, Brustugun OT, Lund-Iversen M, Thunnissen E, Köhler J, Schuler M, Botling J, Sandelin M, Sanchez-Cespedes M, Salvesen HB, Achter V, Lang U, Bogus M, Schneider PM, Zander T, Ansén S, Hallek M, Wolf J, Vingron M, Yatabe Y, Travis WD, Nürnberg P, Reinhardt C, Perner S, Heukamp L, Büttner R, Haas SA, Brambilla E, Peifer M, Sage J and Thomas RK: Comprehensive genomic profiles of small cell lung cancer. Nature *524(7563)*: 47-53, 2015. PMID: 26168399. DOI: 10.1038/nature14664

58 Chuang JC, Stehr H, Liang Y, Das M, Huang J, Diehn M, Wakelee HA and Neal JW: ERBB2-mutated metastatic non-small cell lung cancer: response and resistance to targeted therapies. J Thorac Oncol *12(5)*: 833-842, 2017. PMID: 28167203. DOI: 10.1016/j.jtho.2017.01.023

59 Peters S, Stahel R, Bubendorf L, Bonomi P, Villegas A, Kowalski DM, Baik CS, Isla D, Carpeno JC, Garrido P, Rittmeyer A, Tiseo M, Meyenberg C, de Haas S, Lam LH, Lu MW and Stinchcombe TE: Trastuzumab emtansine (T-DM1) in patients with previously treated HER2-overexpressing metastatic non-small cell lung cancer: efficacy, safety, and biomarkers. Clin Cancer Res *25(1)*: 64-72, 2019. PMID: 30206164. DOI: 10.1158/1078-0432.CCR-18-1590

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