scientific reports

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Clinical implementation of next-generation sequencing testing and genomically-matched therapy: a real-world data in a tertiary hospital

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Next-generation sequencing (NGS) cancer profiling has gained traction in routine clinical practice in South Korea. Here, we evaluated the use of NGS testing and genomically-matched therapies for patients with advanced solid tumors in a real-world clinical practice. We analyzed results from NGS cancer panel tests (SNUBH pan-cancer version 2) ordered from June 2019 to June 2020. Genomically-matched treatment was determined based on the novel information obtained from NGS testing, while results from conventional molecular tests were excluded. A total of 990 patients were included in the analysis (median age: 62, Stage IV: 82.5%). Using the Association for Molecular Pathology genetic variant classification system, we found that 257 (26.0%) patients harbored tier I variants, and 859 (86.8%) patients carried tier II variants. Among the tier I cases, the most frequently altered genes we detected were *KRAS* (106 patients, 10.7%), followed by *EGFR* (27 patients, 2.7%) and *BRAF* (17 patients, 1.7%). Of patients with tier I variants, 13.7% received NGS-based therapy as follows: Thyroid cancer (2/7, 28.6%), skin cancer (2/8, 25.0%), gynecologic cancer (7/65, 10.8%), and lung cancer (12/112, 10.7%). Of 32 patients with measurable lesions who received NGS-based therapy, 12 (37.5%) achieved a partial response, and 11 (34.4%) achieved stable disease. The median treatment duration was 6.4 months (95% CI, 4.4–8.4), and the median OS was not reached. In conclusion, NGS tumor profiling was successfully implemented in real-world clinical practice. This enabled the use of molecular profiling-guided therapy which improved survival outcome of selected patients.

The last decade has seen a significant shift in how patients with cancer are diagnosed and managed through the clinical application of next-generation sequencing (NGS) technology. Large-scale tumor molecular profiling programs using NGS have fostered the growth of precision cancer medicine[1–3]. NGS-based molecular pathology has become an essential tool in not only diagnosing and predicting the prognosis of tumor, but also in driving

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therapeutic decision-making. Indeed, several clinical trials have employed deep sequencing to randomize cancer patients to new genomically-matched treatments[1]. Further, the number of druggable tumor-specific molecular alterations has grown substantially, with a significant survival benefit obtained from biomarker-matched therapies in several cancer types[4–6].

The Korean National Health Insurance Service now includes NGS testing in its insurance coverage, and NGS tests for target genes are currently being implemented in clinical practice in South Korea[7,8]. Although NGS testing has become more affordable, bringing these tests into routine clinical use has proven to be challenging due to several reasons. First of all, significant financial investment is required to establish and maintain the bioinformatics infrastructure that enables genomic testing and research[9]. Further, bioinformatics specialists and server engineers are needed to run the sophisticated software and manage the scientific computing required for NGS[2]. In addition, rigorous quality control mechanisms and reasonable turn-around times are essential to implement NGS testing in daily clinical practice[10,11].

One of the main goals of NGS-based oncology is to identify genomically-matched therapies based on NGS results that can directly benefit patients. However, interpreting NGS test results and identifying actionable genetic alterations remains challenging, making it difficult to administer genomically-matched therapies. Restrictions on the off-label use of matched drugs out of the context of clinical trials further complicate this implementation[12]. Moreover, there are substantial differences among the various tier systems, depending on the group that proposed the system and the criteria of each tier. This gap between research and the clinical application of NGS could make the clinical application of NGS more difficult[13–16].

To address the challenges in implementing NGS-based oncology profiling in routine clinical practice, we assessed the clinical utility of this technique in a tertiary hospital in South Korea. Here we present the use of NGS tests for patients with advanced solid tumors and investigated the actual frequency and effect of genomically-matched therapies.

Methods

Patients

Patients and tumor specimens

We analyzed the results of NGS test (SNUBH Pan-Cancer v2.0) conducted from June, 2019 to June, 2020 at Seoul National University Bundang Hospital (SNUBH). All solid tumors were included. Cases with proper NGS results were included. Hematologic malignancy was excluded. Cases with sequencing failure were excluded. All NGS tests were ordered at the discretion of the attending physician. NGS tests were performed on stored formalin-fixed paraffin-embedded (FFPE) tumor specimens.

Sample preparation

For manual microdissection, representative tumor areas with sufficient tumor cellularity were chosen. To extract the genomic DNA, a QIAamp® DNA FFPE Tissue kit (Qiagen, Hilden, Germany) was used. The DNA concentration was quantified with the Qubit dsDNA HS Assay kit (Invitrogen; Thermo Fisher Scientific, Inc. SA) on the Qubit 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.SA). In addition, DNA purity was measured using NanoDrop Spectrophotometer (Invitrogen; Thermo Fisher Scientific). At least 20 ng of DNA with A260/A280 ratio between 1.7 and 2.2 was used for library generation. The hybrid capture method was used for DNA library preparation and target enrichment, according to Illumina's standard protocol using an Agilent SureSelectXT Target Enrichment Kit (Agilent Technologies, Santa Clara, CA, USA). Finally, average library size and quantity are calculated using an Agilent 2100 Bioanalyzer system (Agilent Technologies) using an Agilent High Sensitivity DNA Kit (Agilent Technologies). Cutoff for size and concentration of the library were 250–400 bp and 2nμ, respectively. Less than 80% of ×100 coverage was considered as failure of sequencing and the average mean depth for the cohort was 677.8×.

NGS panel information and data analysis

Tumor tissue specimens were sequenced using the SNUBH Pan-Cancer v2.0 Panel, a targeted sequencing platform in SNUBH. The panel targets 544 genes (Supplementary Table 4) and microsatellite instability (MSI) status as well as tumor mutational burden (TMB) were reported. The SNUBH pan-cancer version 2 panel is based on the Axen master cancer panel, which is provided as a service by Macrogen, Korea.

Samples were sequenced on the NextSeq 550Dx (Illumina, San Diego, CA, USA) for SNUBH Pan-Cancer v2.0 panel. Reads were aligned to the human reference genome hg19. Mutect2 was used to detect single nucleotide variants (SNVs) and small insertion/deletions (INDELs), and SnpEff was used to annotate the identified variants. Only SNVs/INDELs with variant allele frequency (VAF) greater than or equal to 2% were selected. CNVkit was used to identify copy number variation (CNV) and an average CN ≥ 5 was regarded as a gain (amplification). Gene fusions were identified using LUMPY, and read counts ≥ 3 were interpreted as positive results for structure variation detection.

MSI phenotype was detected using mSINGs and TMB was calculated as the number of eligible variants within the panel size (1.44 megabase). Eligible variants were missense mutations with the following criteria: (1) Variants reported in the population database > 1% (East Asian, gnomAD) were excluded; (2) Pathogenic, likely pathogenic mutations reported in ClinVar were excluded; (3) Variants with allele frequency less than 2% were excluded; and (4) Variants below depth 200 were also excluded.

Reporting system

All genetic alterations were reported and classified into tiers according to standardized guidelines for the interpretation and reporting of sequence variants in cancer provided by the Association for Molecular Pathology: (1) Tier I, variants of strong clinical significance such as FDA-approved, professional guidelines, or well-powered

research-based therapy; (2) Tier II, variants of potential clinical significance such as FDA-approved treatment for different tumor types or investigational therapies; (3) Tier III, variants of unknown clinical significance; and (4) Tier IV, benign or likely benign variants[15].

NGS-based therapy

NGS-based therapy was defined as the genomically-matched treatment selected based on novel information obtained from NGS tests. Therapies identified using conventional molecular tests—such as targeted therapies against *HER2* overexpression that were identified using immunohistochemistry (IHC) or silver in situ hybridization (SISH)—were excluded. Targeted therapies for known *EGFR* mutations in non-small cell lung cancer identified by polymerase chain reaction, Sanger sequencing, and pyrosequencing were also excluded.

HER2 IHC and silver in situ SISH

IHC for *HER2* (ready to use; clone 4B5; Ventana Medical Systems, Tucson, USA) was performed using BenchMark XT autostainer (Ventana Medical Systems) according to the manufacturer's protocol. *HER2* SISH analysis was performed with INFORM *HER2* DNA and Chromosome 17 probes (Ventana Medical Systems), using UltraView SISH Detection Kit (Ventana Medical Systems). In breast cancer and non-gastrointestinal tract cancer cases, *HER2* status was determined according to the 2018 ASCO/CAP guidelines[17]. CAP/ASCP/ASCO guidelines for gastroesophageal adenocarcinoma were used in gastrointestinal tract, hepatobiliary and pancreatic cancer cases[18].

MSI PCR analysis

MSI status was assessed by fragmentation analysis, using a DNA autosequencer (ABI 3730 Genetic Analyzer, Applied Biosystems). Allele profiles of five markers (BAT-26, BAT-25, D5S346, D17S250, and D2S123) in tumor cells were compared with those of matched normal cells. MSI status was determined according to the Revised Bethesda Guidelines[19].

Statistical analysis

Categorical variables were summarized using frequencies and percentages, whereas the continuous variables were summarized using descriptive statistics such as the median and range. Survival analysis was performed using Kaplan–Meier curves. P-values less than 0.05 were considered statistically significant. All statistical analysis and mutational mapping in this study were performed with SPSS for Windows (SPSS Inc. Chicago, IL, USA) and the open software R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients

A total of 1014 NGS tests were ordered by attending physicians during the course of the study. Of these, 23 tests did not yield proper results, and therefore were cancelled due to the following reasons; insufficient tissue specimen (7 cases), failure to extract DNA (10 cases), failure of library preparation (4 cases), poor sequencing quality (1 case), decalcification of the tissue specimen (1 case). And 1 case was cancelled upon the request from clinic. This led to the failure rate of 2.4% (24/1014). Finally, 990 patients with NGS results were included in this analysis. The median age was 62 years (range: 2–92 years), and 50.9% of the patients were male. 82.5% of the patients had stage IV cancer. The most common cancer type was colorectal cancer (22.3%), followed by biliary-pancreatic cancer (18.1%), lung cancer (11.3%), stomach cancer (9.2%), breast cancer (7.8%), and brain cancer (7.7%) (Table 1).

NGS test

The time to result for NGS testing was 30 days (range: 14–82). NGS testing was most frequently performed during first-line treatment (53.3%), followed by the postoperative period (18.7%), second-line treatment (15.9%), and ≥ third-line treatment (12.1%). In total, 60.5% of the study samples used for NGS analysis were obtained from primary tissues, followed by hepatic metastatic lesions (14.1%). All NGS tests were performed on stored FFPE tumor specimens, with a median age of 0.8 months (range: 0–170). Of the study samples, 49.0% were obtained by biopsy. The tests were performed using specimens obtained within Seoul National University Bundang Hospital (90.1%) or specimens referred from other hospitals (9.9%).

Results of NGS testing

257 (26.0%) patients harbored tier I variants, while 859 (86.8%) carried tier II variants. Among the tier I SNV/INDEL variants, alterations were found most frequently in *KRAS* (10.7%), followed by *EGFR* (2.7%), *BRAF* (1.7%), *IDH1*(1.6%), *KIT* (1.4%), *BRCA1/2* (1.3%, 1.3%), and *NRAS* (1.2%) (Fig. 1A). For the tier II SNV/INDEL variants, alterations were identified in *TP53* (50.3%), *APC* (19.2%), *KRAS* (12.9%), *PIK3CA* (10.4%), *SMAD4* (5.1%), *CDKN2A* (3.9%), and *PTEN* (3.9%) (Fig. 1B). For tier I CN amplifications, alterations were found in *ERBB2* (1.2%), while for tier II variants, alterations were identified in *FGFR1* (4.3%), *CCNE1*(4.1%), *MYC* (3.7%), *EGFR* (3.1%), *ERBB2* (2.7%), *KRAS* (2.6%), *MDM2* (2.4%), *PIK3CA* (1.1%), *MET* (1.0%), *KIT* (0.8%), and *BRAF* (0.5%) (Fig. 1C,D). A total of 11 cases had tier I fusions, while 9 cases showed tier II fusions (Fig. 1E,F).

Regarding MSI status, 36 (3.6%) cases were detected as MSI-H and 953 (96.3%) were identified as MSS/MSI-L. MSI status was not evaluable in one case (0.1%) due to quality control failure of the specimen. MSI-H was detected in 13 (36.1%) colorectal cancers, 11 (30.6%) gastric cancers, 6 (16.7%) biliary-pancreatic cancers, 1 (2.8%) small bowel cancer, 1 (2.8%) breast cancer, 1 (2.8%) ovarian cancer, 1 (2.8%) lung cancer, 1 (2.8%) sarcoma, and 1 (2.8%) cancer of unknown origin.

Variables		N = 990	%
Age	Median (range)	62 (2–92)	
Sex	Male	504	50.9
	Female	486	49.1
Stage*	≤ III	97	9.8
	IV	817	82.5
Tumor type	Colorectal cancer	221	22.3
	Lung cancer	112	11.3
	Biliary tract cancer	104	10.5
	Stomach cancer	91	9.2
	Breast cancer	77	7.8
	Brain tumor	76	7.7
	Pancreatic	75	7.6
	Gynecologic cancer	65	6.6
	Genitourinary cancer	37	3.7
	Sarcoma	29	2.9
	Hepatocellular carcinoma	16	1.6
	Gastrointestinal stromal tumor	15	1.5
	Metastasis of unknown origin	12	1.2
	Neuroendocrine tumor	13	1.3
	Head and neck cancer	4	0.4
	Skin Cancer	8	0.8
	Thyroid cancer	7	0.7
	Small bowel cancer	9	0.9
	Others	19	1.9
NGS result- turnaround time	Median (range), days	30 (14–82)	
NGS testing—time	Post-operation	185	18.7
	During first-line	528	53.3
	During second-line	157	15.9
	During ≥ third-line	120	12.1
NGS testing—tumor site	Primary	599	60.5
	Metastatic lesions	391	39.5
	Liver	140	14.1
	Lymph node	82	8.3
	Lung	40	4.0
	Others	129	13.0
Paraffin block age	Median (range), months	0.8 (0–170)	
Specimen types	Biopsy	485	49.0
	Resection	505	51.0
	Cytology	0	0.0
Source of tissue	Inside	892	90.1
	Outside	98	9.9

Table 1. Baseline characteristics of patients and NGS testing. *Brain tumor (76) was excluded.

The median TMB was 9.2/Mb (range: 0–221.9). When analyzed according to MSI status, the median TMB was 10.634 (range: 0–53.2) in the MSI-H cases and 9.216 (range: 0.7–221.9) in the MSS/MSI-L cases. The difference between MSI subgroups was not statistically significant. The highest TMB (221.9/Mb) was detected in a colorectal cancer case with a POLE mutation.

Concordance between NGS CN alteration and *HER2* IHC

Among the 990 patients who underwent successful NGS testing, *HER2* IHC was performed in 424 cases. The intensity of *HER2* IHC was classified as negative in 208 (49.1%) cases, 1+ in 125 (29.5%) cases, 2+ in 74 (17.4%) cases, and 3+ in 17 (4.0%) cases (Supplementary Table 1). There was few discrepancy between NGS testing and *HER2* IHC in rare cases. *HER2* CN gain, determined through NGS, was detected in 3 (0.9%) out of 333 specimens that had a *HER2* intensity of 0 or 1+ (Supplementary Table 1). *HER2* SISH analysis confirmed the absence of amplification in two samples that had *HER2* intensity 0. One sample with a *HER2* intensity 1+ exhibited amplification in SISH (Supplementary Fig. 1A,B). Among the samples with a *HER2* intensity of 3+, *HER2* CN gain was not detected in 3 (17.6%) out of 17 cases (Supplementary Table 1). Further analysis of the

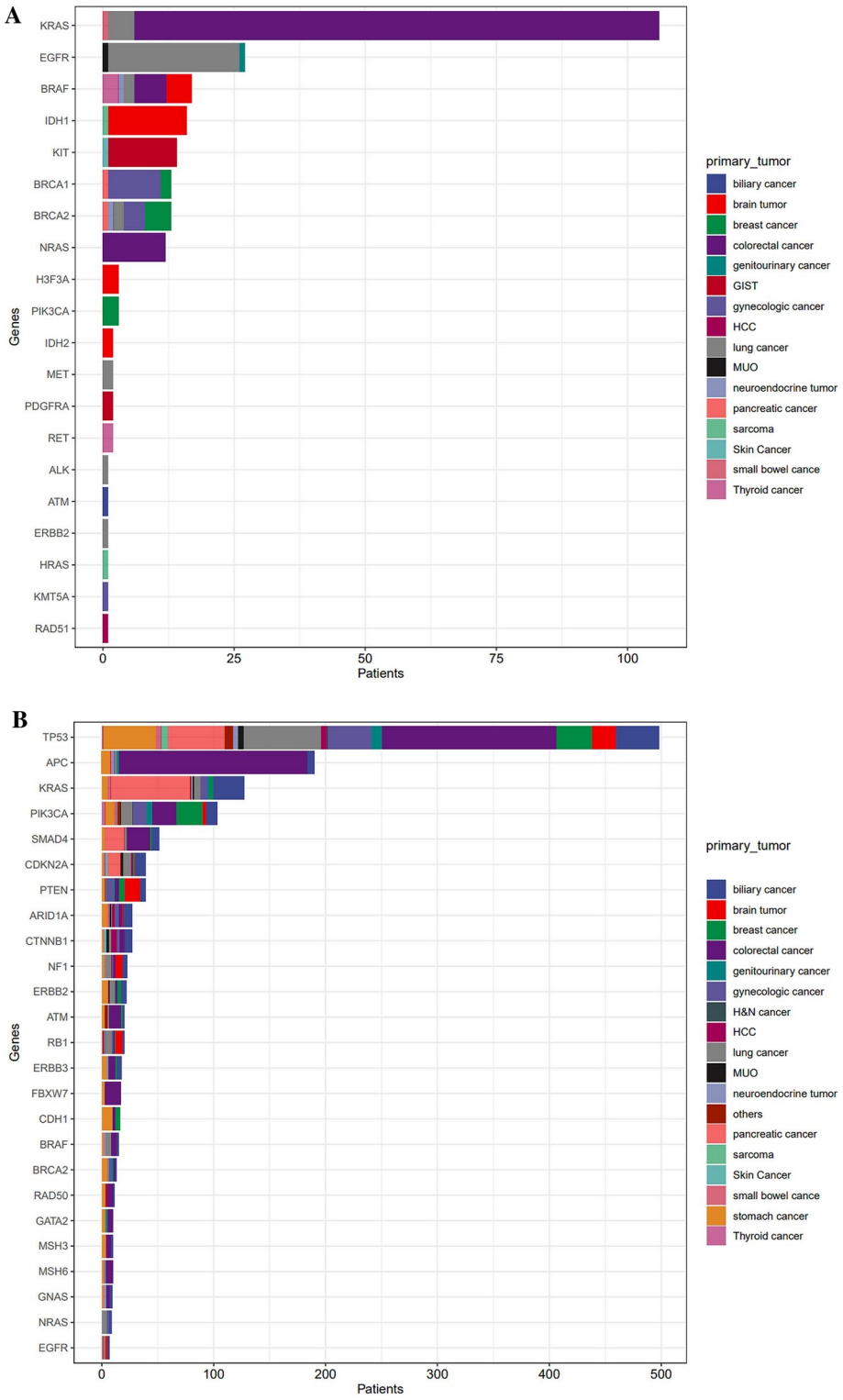

Fig. 1. Genetic alterations identified using NGS profiling. (**A**) Tier I SNV/INDEL variants, (**B**) Tier II SNV/INDEL variants, (**C**) Tier I CN amplification, (**D**) Tier II CN amplification, (**E**) Tier I SV variants, (**F**) Tier I SV variants.

HER2 IHC in these samples revealed that the staining pattern was heterogeneous, and intensity 3+ staining was only observed in a minor component of the tumor area dissected for NGS testing (Supplementary Fig. 1C). Among the samples with a *HER2* intensity of 2+, a total of 58 cases were also tested for SISH analysis, revealing a total of 14 (24.1%) discrepant cases (Supplementary Table 2). In samples with positive *HER2* SISH results (amplification and polysomy), the median *HER2* CN determined via SISH was significantly lower when the

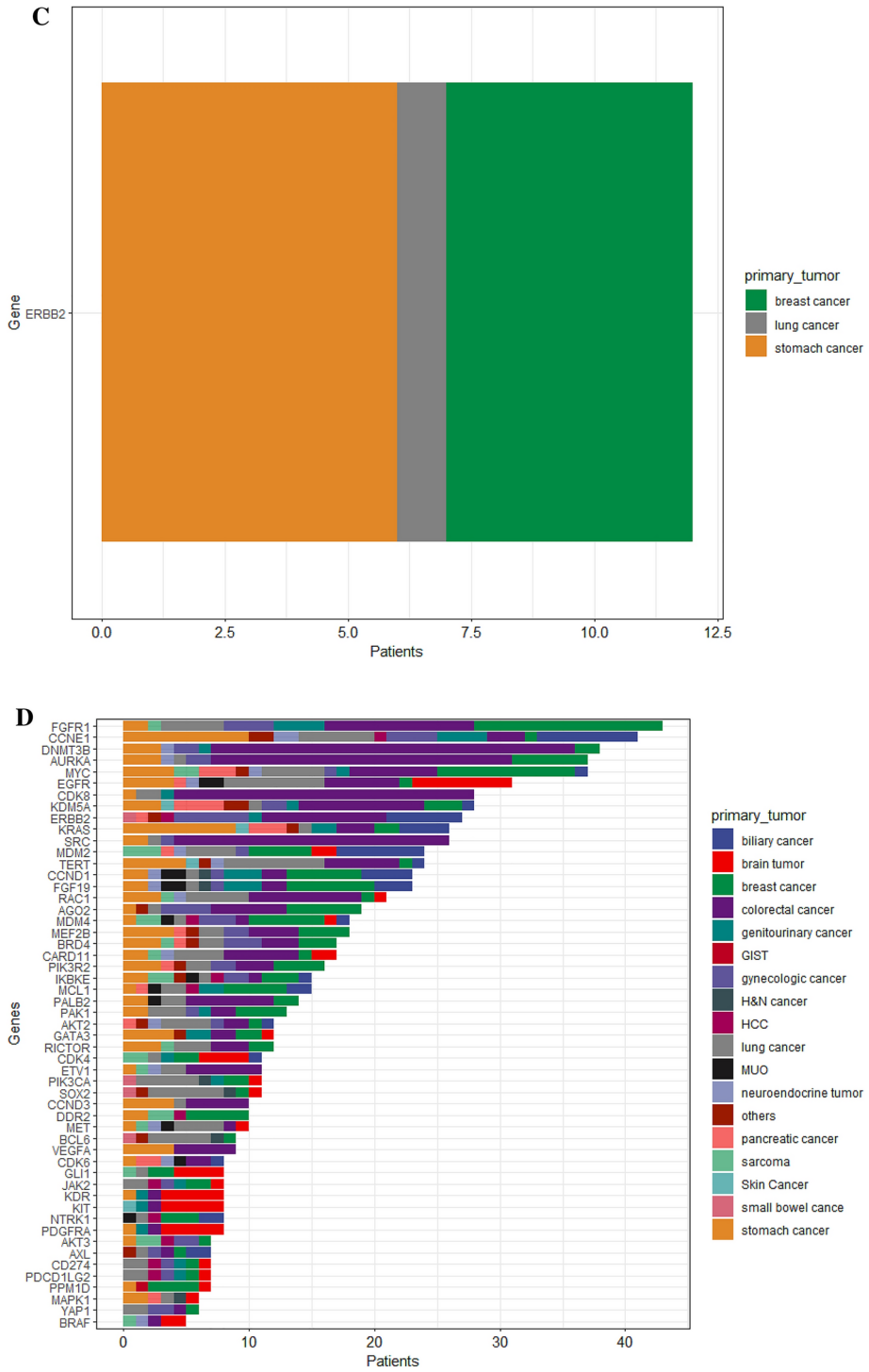

Figure 1. (continued)

NGS results were reported as negative for CN alteration [5.4 (range: 3.5–8.1)] than when the NGS results were reported as positive [10.7 (range: 7.3–29.2)] ($p < 0.001$).

Concordance between NGS and MSI PCR

MSI PCR analysis was also performed for 326 of the total 990 patient samples (Supplementary Table 3). The concordance rate between MSI PCR and NGS was 99.1%. There were a total of 3 (0.9%) cases showing discrepant results. Positive and negative predictive values of NGS against MSI PCR were 100.0% (16/16) and 99.0% (308/311) for MSI PCR.

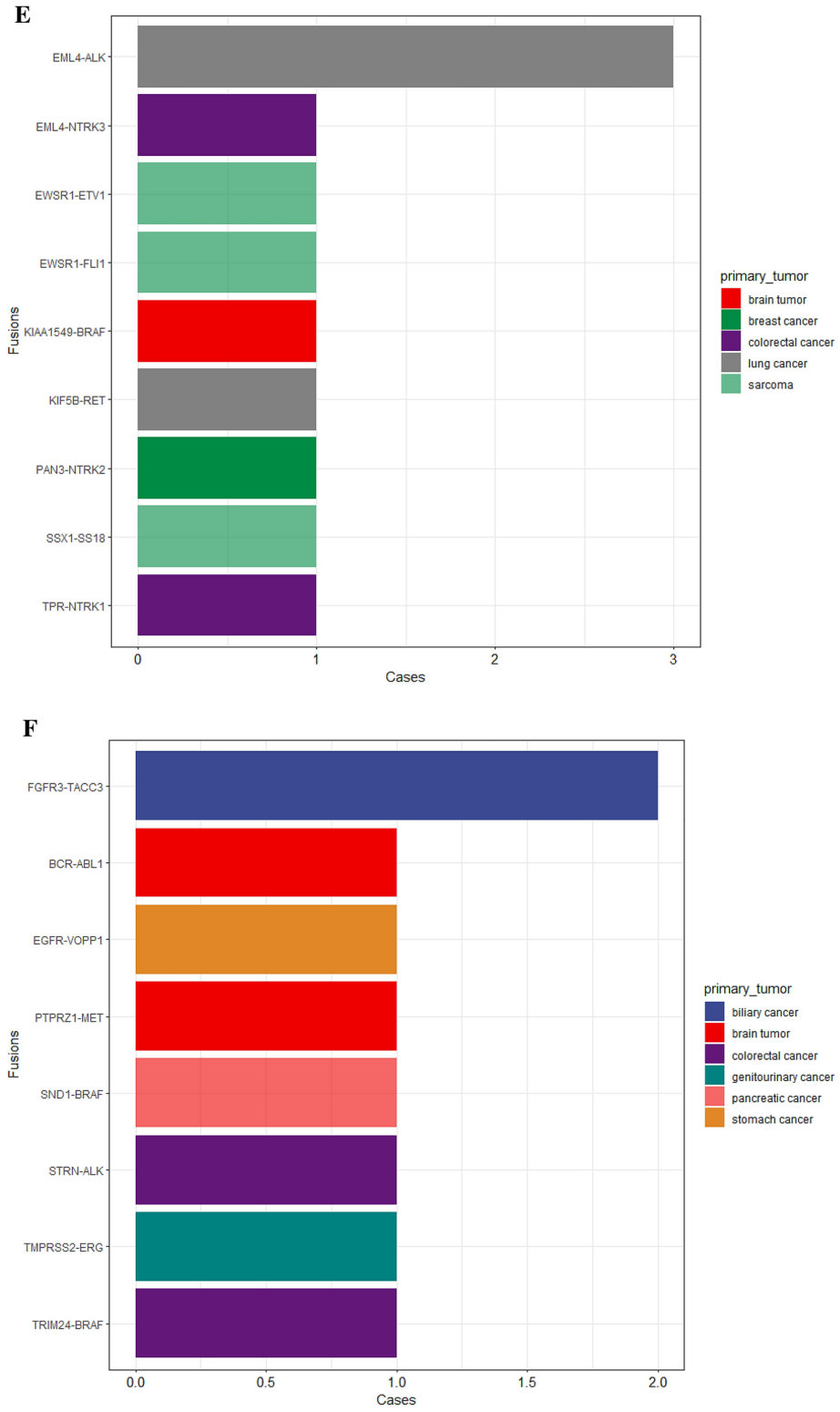

Figure 1. (continued)

Application of NGS-based therapy

Of the patients tested using NGS, 37 (3.7%) received NGS-based therapy. For patients with tier I genomic alterations, 13.7% received NGS-based therapy. The median time between NGS testing and NGS-based therapy was 4.3 months (range: 0.8–11.7). Of the patients who received NGS-based therapy, the median age was 63 years (range: 36–85) (Table 2), and 40.5% were male. The predominant cancer types found in patients who received NGS-based therapy included lung cancer (32.4%), gynecologic cancer (18.9%), hepato-biliary-pancreatic cancer

Variables		N = 37	%
Age	Median (range), years	63 (36–85)	
Sex	Male	15	40.5
	Female	22	59.5
Tumor types	Lung cancer	12	32.4
	Gynecologic cancer	7	18.9
	HCC/Pancreas/Biliary tract cancer	5	13.5
	Colorectal cancer	3	8.1
	Breast cancer	2	5.4
	Skin cancer	2	5.4
	Thyroid cancer	2	5.4
	Stomach cancer	1	2.7
	Metastasis of unknown origin	1	2.7
	Neuroendocrine tumor	1	2.7
	Adrenal cortical carcinoma	1	2.7
ESCAT*	IA	18	48.6
	IB	5	13.5
	IIIA	3	8.1
	IIIB	5	13.5
	IVA	6	16.2
Drug source	Approved drug	25	67.6
	Clinical trial	9	24.3
	Compassionate use/Expanded access program	3	8.1
Tumor response⁺	Partial response	12	37.5
	Stable disease	11	34.4
	Progressive disease	9	28.1

Table 2. The characteristics and outcomes of NGS-based therapy. *ESMO Scale for Clinical Actionability of Molecular Targets. ⁺Disease of 5 patients were non-evaluable.

(13.5%), and colorectal cancer (8.1%). For each cancer type, adrenal cortical carcinoma was the most common cancer type for which NGS-based therapy was applied (1/3, 33.3%), followed by thyroid cancer (2/7, 28.6%), skin cancer (2/8, 25.0%), gynecologic cancer (7/56, 10.8%), and so on. The matched NGS-based drugs were obtained from daily practice with approved drug (67.6%), clinical trials (24.3%), and compassionate use/expanded access programs (8.1%) (Table 2). All genetic alterations with matched NGS-based therapy were classified as tier I or tier II (Fig. 2). Alterations were detected in *EGFR* (9 cases), *BRCA1* (6), *BRAF* (4), *BRCA2* (4), *ATM* (2), *EML4-ALK* fusion (2), *KIT* (2), and *MET* (2). When classifying genetic alterations for NGS-based therapy according to ESCAT guidelines (ESMO Scale for Clinical Actionability of Molecular Targets), IA and IB alterations were common (48.5% and 13.5%, respectively). IIIB and IVA alterations were also identified (13.5% and 16.2%, respectively, Table 2).

Compared to standard of care, NGS-based therapy resulted in a higher response rate of 37.5%. Of 32 patients with measurable lesions, 12 (37.5%) achieved a partial response, and 11 (34.4%) achieved stable disease. The median follow-up duration was 6.7 months (95% CI, 4.3–9.1), and the median treatment duration was 6.4 months (95% CI, 4.4–8.4). The median overall survival following NGS therapy was not reached during the course of this study. Detailed information for all NGS-based therapies is described in Table 3.

NGS-matched therapy case study

A 53-year-old male patient was diagnosed with metastatic adenocarcinoma, with a primary mass in the descending colon involving the parietal peritoneum and multiple liver metastases. NGS was performed at the time of diagnosis, and we identified an *ALK:STRN* fusion and mutations in *TP53* and *RNF43*, which were classified as tier II. Mutations in *BRAF* and *RAS* were not detected, and the MSI status was MSS/MSI-L. His tumor also showed strong *ALK* expression in IHC. After 17 cycles of bevacizumab combined with a FOLFOX regimen, he was treated with oral brigatinib 180 mg qd as second-line treatment, provided through a compassionate use/expanded access program from the drug company. CT scans revealed a partial response for the liver metastases based on RECIST 1.1, and the tumor markers showed a decrease in serum CEA and CA 19-9. However, the disease progression was confirmed after 11.5 months of brigatinib administration. Brigatinib was changed to lorlatinib, provided through a compassionate use/expanded access program from the drug company. Serial CT scans revealed stable disease, but the disease rapidly progressed after 3 months. *ALK* IHC on a biopsy sample taken following the switch to lorlatinib revealed the sample to be *ALK*-negative. The patient participated in a clinical trial using immunotherapy and then received additional chemotherapy, including bevacizumab with FOLFIRI, but it had no effect (Supplementary Figs. 2 and 3).

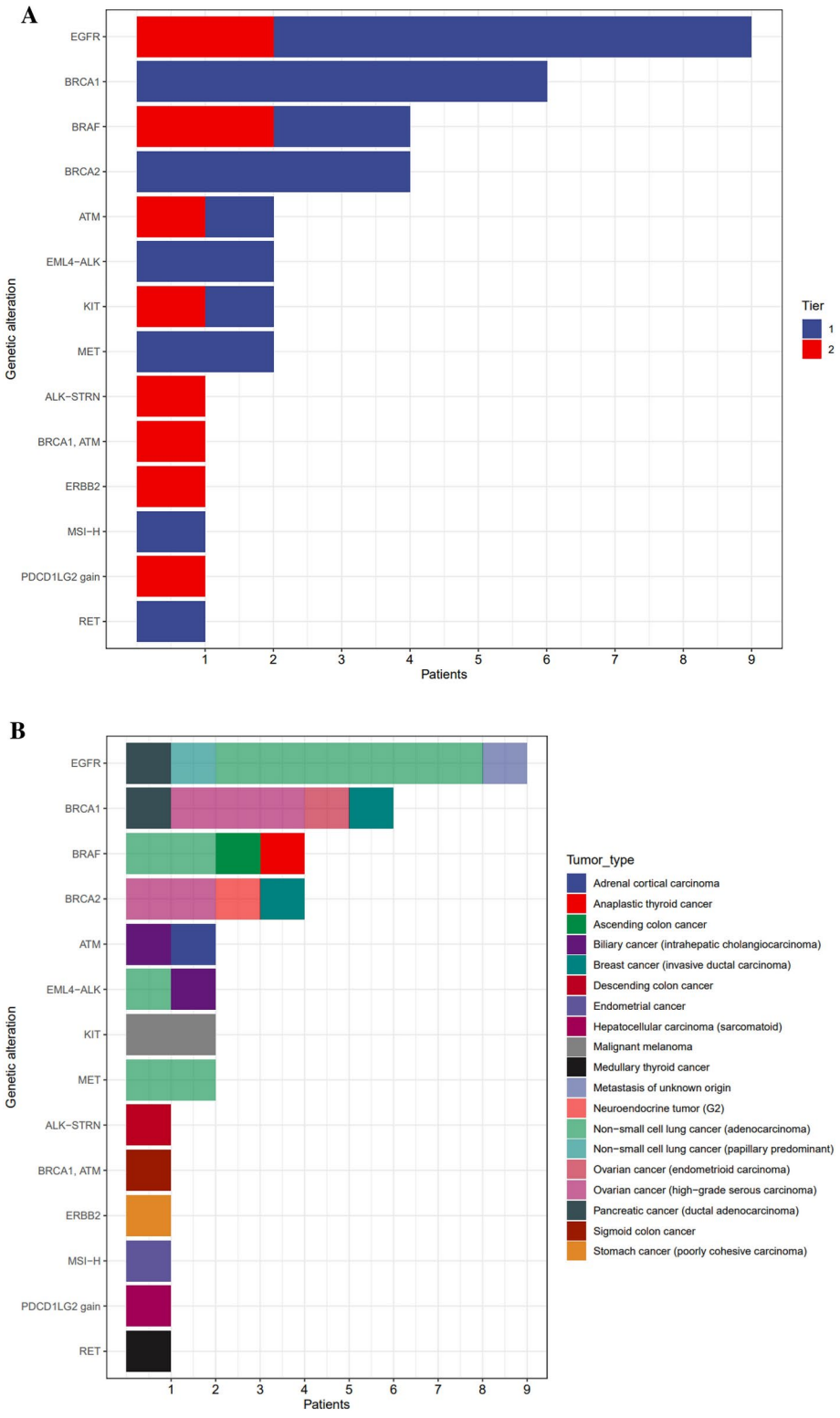

Fig. 2. Genetic alterations that were candidates for NGS-based therapy. (**A**) Genetic alterations grouped by tier, (**B**) Genetic alterations grouped by tumor type.

Discussion

In this study, we sought to demonstrate the real-world utility of NGS testing for detecting pathogenic alterations in patients with solid tumors and the subsequent application of matched therapeutics. NGS tumor profiling and NGS-matched therapy have the potential to revolutionize the diagnosis and treatment of cancer[4,5,13,14]. The shift to precision medicine afforded by this technology is already benefiting patients in daily clinical practice.

Genetic alteration	Tumor type	Therapy	Drug source	Best response	Treatment duration*	Overall survival from NGS-based therapy*	Time between NGS testing and NGS-based therapy
EGFR exon18 c.2127_2129delAAC(E709_T710delinsD)	Non-small cell lung cancer (adenocarcinoma)	Afatinib	Approved drug	SD	15.0+	15.0+	1.4
ERBB2 p.Arg678Gln	Stomach cancer (poorly cohesive carcinoma)	Neratinib	Compassionate use/Expanded access program from drug company	PD	1.9	2.8+	5.7
PDCD1LG2 gain	Hepatocellular carcinoma (sarcomatoid)	Pembrolizumab	Approved drug	PR	14.8+	14.8+	1.6
BRCA1 p.Gln905*, p.Glu1630Lys ATM p.Gln2277* PALB2 p.Gln343*, p.Arg753*	Sigmoid colon cancer	JPI547 (PARP/TNKS inhibitor)	Clinical trial	PD	1.3	10.9+	5.7
EML4-ALK translocation	Non-small cell lung cancer (adenocarcinoma)*	Alectinib	Approved drug	PR	14.2+	14.2+	2.1
RET M918T	Medullary thyroid cancer	Vandetanib	Approved drug	SD	6.8	11.1	2.1
ATM p.Asp2708Asn, FLCN p.His429fs, RAD50 p.Lys722fs, TP53 p.Arg175His	Adrenal cortical carcinoma	JPI547 (PARP/TNKS inhibitor)	Clinical trial	PD	1.3	8.6+	8.0
Braf p.Val600Glu	Anaplastic thyroid cancer	Dabrafenib+Trametinib	Approved drug	PD	0.9	1.1+	11.7
EGFR exon 21 L833V/H835L mutation	Non-small cell lung cancer (adenocarcinoma)	Erlotinib	Approved drug	PR	7.4	9.0+	6.5
MET exon14 skipping mutation (c.3028+2T>C)	Non-small cell lung cancer (adenocarcinoma)	Crizotinib	Approved drug	PR	5.9	14.7+	0.8
BRCA1 p.Val1833fs	Breast cancer (invasive ductal carcinoma)	Olaparib	Approved drug	SD	3.1	7.7+	6.6
EGFR p.L858R	Non-small cell lung cancer (adenocarcinoma)	Erlotinib	Approved drug	SD	8.6+	8.6+	5.5
Kit p.K642E	Malignant melanoma	Imatinib	Approved drug	PR	4.5	4.5+	2.0
BRCA1 p.L1780P	Ovarian cancer (high-grade serous carcinoma)	Olaparib	Approved drug	NE	6.5+	6.5+	6.5
BRCA2 p.N1824fs	Neuroendocrine tumor (G2)	Pembrolizumab + Olaparib	Clinical trial	SD	4.1	9.1+	3.5
ATM, p.R1875*, p.Y2437*	Biliary cancer (intrahepatic cholangiocarcinoma)	Pembrolizumab + Olaparib	Clinical trial	SD	5.2	13.9	7.6
MET c.3028G>C (MET exon 14 skipping)	Non-small cell lung cancer (adenocarcinoma)	Tepotinib	Clinical trial	SD	11.2	11.2+	2.3
BRAF p.G466V	Ascending colon cancer	HM95573 (RAF inhibitor) + Cobimetinib	Clinical trial	PD	1.8	4.0+	11.1
BRCA 1 c.3412G>T	Pancreatic cancer (ductal adenocarcinoma)	Olaparib	Compassionate use/Expanded access program from drug company	SD	8.1+	8.3+	6.3
BRAF mutation pT599 duplication	Non-small cell lung cancer (adenocarcinoma)	Dabrafenib + Trametinib	Approved drug	PR	1.7	8.3+	2.8
EGFR E21 L861R	Non-small cell lung cancer (papillary predominant)	Erlotinib	Approved drug	PD	1.8	3.5+	7.8
EGFR p.E746_A, 750del	Pancreatic cancer (ductal adenocarcinoma)	Erlotinib + Gemcitabine	Approved drug	PD	1.5	6.7+	4.4
EML4/ALK translocation	Non-small cell lung cancer (adenocarcinoma)	Alectinib	Approved drug	PR	7.7+	7.7+	1.3
KIT p.D820G, p.N822K	Malignant melanoma	Imatinib	Approved drug	PD	1.7	8.9+	2.2
EGFR exon 20 S768I, V769L	Non-small cell lung cancer (adenocarcinoma)	Erlotinib	Approved drug	PD	2.3	6.5+	1.5
EGFR p.E746_A750del	Metastasis of unknown origin	Erlotinib	Approved drug	NE	F/U loss without tumor response evaluation	0.9+	1.1
High TMB (MSI-H)	Endometrial cancer	Pembrolizumab	Approved drug	PR	5.1+	5.1+	5.0
BRCA1 p.E572fs	Ovarian cancer (high-grade serous carcinoma)	Rucaparib/ Placebo + Nivolumab/Placebo	Clinical trial	NE	5.6+	5.6+	4.6
EGFR p. G724S	Non-small cell lung cancer (adenocarcinoma)	Afatinib	Approved drug	PR	4.7+	4.7+	4.2
Continued							

Genetic alteration	Tumor type	Therapy	Drug source	Best response	Treatment duration*	Overall survival from NGS-based therapy*	Time between NGS testing and NGS-based therapy
BRCA1 p.E1210fs	Ovarian cancer (high-grade serous carcinoma)	Olaparib	Approved drug	NE	4.7+	5.0+	4.7
ALK-STRN fusion	Descending colon cancer	Brigatinib	Compassionate use/Expanded access program from drug company	PR	1.4+	1.4+	9.6
BRCA2 p.R2494* (germline)	Ovarian cancer (high-grade serous carcinoma)	Durvalumab + Olaparib	Clinical trial	PR	6.4	18.7+	Known germline mutation
EGFR p.E746_A750del	Non-small cell lung cancer (adenocarcinoma)	Lazertinib vs. Gefitinib	Clinical trial	PR	5.6+	5.6+	1.3
BRCA2 c.5576_5579delTTAA (p.I1859fs)	Breast cancer (invasive ductal carcinoma)	Olaparib	Approved drug	SD	5.4+	5.4+	1.4
BRAF p.V600E	Non-small cell lung cancer (adenocarcinoma)	Dabrafenib + Trametinib	Approved drug	SD	0.4+	0.4+	4.1
BRCA1 p.T1677fs	Ovarian cancer (endometrioid carcinoma)	Olaparib	Approved drug	NE	1.9+	1.9+	4.3
BRCA2 c.7007+1G>C	Ovarian cancer (high-grade serous carcinoma)	Olaparib	Approved drug	SD	0.9+	0.9+	1.2

Table 3. Detailed information for NGS-based therapies. + indicates ongoing or alive. *Initially suspected as intrahepatic cholangiocarcinoma.

Previous clinical trials have shown considerable efficacy of the NGS profiling approach. However, NGS testing is still in its infancy, and not all patients who undergo NGS testing will receive matched therapy. In the NCI-MATCH trial, an actionable alteration was found in 37.6% of cases, and 17.8% were assigned to clinical trials[6]. In the K-MASTER program, 10.9% of patients were enrolled in clinical trials[7].

Previous studies for implementation of NGS focused on the potential and early clinical applications of NGS technology, primarily concentrating on specific tumor types and the utility of tissue-based NGS in a controlled clinical trial setting[1,2,6,7]. While these studies demonstrated the potential of NGS technology, they lacked evaluations of its practical applicability in routine clinical practice. In contrast, our study utilized a large dataset collected from a tertiary hospital in South Korea to assess how NGS-based therapy is being applied in real-world clinical settings. This study provides critical insights into the clinical utility of NGS technology within the Korean healthcare system, offering a distinct contribution by practically validating its implementation and effectiveness in routine practice. This represents a significant differentiation from previous studies.

In our study, lung cancer (32.4%) was the most predominant cancer type that was treated with NGS-based therapy. However, the relatively low number of lung cancer patients receiving NGS-based therapy is interesting, given the high mutation rates typically associated with lung cancer. This finding could be attributed to the fact that lung cancer treatment has well-established protocols involving EGFR, ALK, and ROS1 mutations, and patients with these mutations are often treated with targeted therapies as part of standard care, which may not have been captured in our NGS-based therapy category if they were identified through conventional molecular tests. For each cancer type, adrenal cortical carcinoma was the most common cancer type for which NGS-based therapy was applied (1/3, 33.3%), followed by thyroid cancer (2/7, 28.6%), skin cancer (2/8, 25.0%), gynecologic cancer (7/56, 10.8%), and so on. The unexpected distribution of tumor types that received NGS-based therapy in our study could be attributed to various factors, including tumor biology, clinical practice guidelines, access to therapies, referral patterns, and specific characteristics of our patient cohort. Understanding these factors helps to interpret our findings and highlights the complexity of implementing NGS-based therapies across different tumor types.

Our study assessed the success of NGS testing out of the context of a clinical trial, focusing on patients in a tertiary hospital in South Korea. Of the cohort, 257 (26.0%) patients harbored tier I variants, and 859 (86.8%) patients carried tier II variants. Detection of these variants could lead to NGS-based therapy. However, we found that only 3.7% of patients who underwent NGS testing in our tertiary hospital received NGS-based therapy. Although therapies based on molecular alterations identified via conventional molecular tests were excluded in this study, such as *HER2*-directed therapy or targeted therapy for known *EGFR* mutations, the proportion of patients who received NGS-based therapy was significantly lower than the 10.9% to 17.8% reported in previous clinical trials[6,7]. In the NCI-MATCH trial, 37.6% of patients had actionable mutations, and 17.8% were assigned to clinical trials. This higher rate of NGS-based therapy in a clinical trial setting can be attributed to the structured framework and availability of targeted therapies within the trial. Similarly, the K-MASTER program reported that 10.9% of patients were enrolled in clinical trials based on NGS findings, facilitated by the integration with clinical trials. In contrast, the primary reason for the low rate of NGS-based therapy (3.7%) in our study was the lack of accessibility to matched drugs outside clinical trials. In routine clinical practice, the availability of approved targeted therapies is limited compared to the range of actionable mutations identified by NGS. Regulatory hurdles and financial constraints often limit the use of off-label targeted therapies in routine practice. Additionally, not all patients met the stringent criteria for available clinical trials and further limiting access to NGS-based therapies. To gain evidence for NGS-based therapy and expand its use in daily clinical practice, more clinical trials—designed as "basket" studies where eligibility is based on alterations identified through NGS testing—must be undertaken. To access molecular-guided therapies, flexible use of off-label treatment should also be increased.

NGS profiling is also an important tool in the clinic due to its diagnostic and prognostic value. Identifying specific genetic alterations has proven essential for the diagnosis and subgroup classification of several cancers, including brain cancer, sarcoma, and kidney cancer[13,14,16]. Some genetic alterations, such as the *BRAF* mutation in colorectal cancer, can strongly predict prognosis. Other alterations, such as expended *RAS* mutations in colorectal cancer, can predict response to anti-*EGFR* therapy. Our study detected the *PDGFRA D842V* mutation using NGS in a gastrointestinal stromal tumor, a well-known genetic alteration predicting resistance to imatinib therapy[20]. After this mutation was identified, adjuvant imatinib was stopped in the patient.

The reporting of NGS test results remains controversial due to the complexity of NGS data and the competing priorities of bioinformaticians, pathologists, and physicians[14–16]. Several reporting systems have been developed, including ESCAT, KSCAT, and ONcoKB, which use their own criteria to classify genetic alterations[13,14,21]. In our institute, we've adopted the AMP tier system, which classifies genetic variants based on clinical significance—Tier I: Variants of strong significance; Tier II: Variants of potential clinical significance; Tier III: Variants of unknown clinical significance; and Tier IV: Benign or likely benign variants[15]. Clinical significance in the AMP tier system includes all therapeutic, prognostic, and diagnostic aspects. However, the ESCAT and KCAT systems specifically focus on the clinical actionability of molecular targets, which can directly guide the use of NGS-based therapy[13,14]. Of the specimens reported as AMP tier I in our study, only 34% would be classified as ESCAT I (IA: 30%, IB: 4%), while 47% of cases would be classified as ESCAT IVA (Supplementary Fig. 4). Therefore, standardization of the NGS reporting system by reaching a consensus between clinicians and pathologists would greatly benefit future studies.

In our study, the cohort of patients included heterogeneous cancer types. The prevalence of genetic variants for each cancer type was comparable to that of public data[22]. Although stored FFPE specimens were used, the overall quality failure rate for NGS was very low (2.4%). The turnaround time of NGS testing from order to diagnosis was 30 days, which was acceptable for daily practice. Thus, the implementation of clinical NGS testing was successful in our institute.

To validate the accuracy of our NGS results, we compared the NGS results with results from *HER2* IHC and SISH, and MSI PCR test. The concordance rate for *HER2* status was 95.3% (404/424), which was high and comparable to previous studies[23–25]. Although there were a few false positive or false negative cases, the NGS test detected *HER2* amplification in one intrahepatic cholangiocarcinoma case that exhibited a *HER2* IHC intensity of 1+. Additional SISH results confirmed the gene amplification. It would not have been possible to detect this potentially targetable alteration if NGS testing had not been performed. In particularly, NGS test results of CN for *HER2* IHC 2+ could have some limitations to identify the candidate for *HER2*-directed therapy due to higher threshold compared with SISH testing. In cases of low CN of *HER2*, SISH test would be needed. The concordance rate for MSI status was 99.1% (323/326), which was also higher than or similar to previous studies[26,27]. Our NGS results also showed high concordance rates with conventional molecular tests, such as pyrosequencing for *KRAS* or *NRAS*, or PANAMutyper™ for *EGFR* (data not shown). Taken together, these data indicate that the results of our custom NGS panel are reliable. Although all NGS testing was performed using stored FFPE tumor specimens, the results were reliable regardless of the clinical situation and tissue status. The clinical meaning of genetic variants from NGS was sometimes ambiguous. Therefore, we hold a monthly molecular tumor board meeting, which includes bioinformaticians, pathologists, and physicians, to interpret and share the NGS results that they are willing to discuss in detail.

Our study has some limitations. First, NGS testing in our institute was only performed using tumor tissue, which hindered the precise filtering of germline variants. Second, because our NGS panel only included DNA samples, the accuracy of detecting fusion variants might be lower than other panels that include both DNA and RNA. Our institute recently adopted RNA-based testing to detect more genetic variants, including fusions, as the number of druggable fusions has increased.

In summary, NGS profiling was successfully implemented in daily clinical practice in our tertiary hospital for patients with solid tumors. We found that NGS tests provided valuable additional information compared with conventional molecular testing, leading to molecular profiling-guided therapy that benefited selected patients.

Data availability

All data have been deposited and hosted on our portal at SNUBH. Data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: 28 December 2023; Accepted: 30 December 2024
Published online: 16 January 2025

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Author contributions

JHK and J-HC designed the study. JWK, HYN, SL, J-WK, KJS, SHK, YJK, K-WL, JSL, JK, J-HH, KH, C-YK, YBK, SA, KSL, HK, HSL, SYP, GC, JHK and J-HK were involved in data collection. JWK, HYN, and SL were involved in data analysis and interpretation. JWK, HYN, and SL drafted the manuscript, with input and approval from J-WK, KJS, SHK, YJK, K-WL, JSL, JK, J-HH, KH, C-YK, YBK, SA, KSL, HK, HSL, SYP, GC, JHK and J-HK.

Competing interests

The authors declare no competing interests.

Ethical approval

The Institutional Review Board of SNUBH approved this study (IRB no. B-2010-645-106) and waived the requirement for written informed consent from the participants because of the retrospective nature of this study. This study was conducted in accordance with the principles of the Declaration of Helsinki, and all study procedures were conducted following the relevant guidelines and regulations.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-024-84909-9.

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