Translational Oncology
www.transonc.com

Volume 12 Number 11 November 2019 pp. 1488–1495 1488

# Cancer Panel Assay for Precision Oncology Clinic: Results from a 1-Year Study

**Dohee Kwon*, Binnari Kim*,†, Hyeong Chan Shin*, Eun Ji Kim†, Sang Yun Ha*, Kee-Taek Jang*, Seung Tae Kim‡, Jeeyun Lee‡, Won Ki Kang‡, Joon Oh Park‡ and Kyoung-Mee Kim*,†**

*Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; †Center of Companion Diagnostics, Samsung Medical Center, Seoul, Republic of Korea; ‡Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

## Abstract

Next-generation sequencing (NGS)-based cancer panel tests are actively being applied in the clinic for precision oncology. Given the importance of NGS panel tests in the palliative clinical setting, it is critical to understand success rates, factors responsible for test failures, and the incidence of clinically meaningful genetic alterations. We performed NGS cancer panel test with tumors from the stomach (n = 234), colorectum (n = 196), and rare tumors (n = 105) from 535 recurrent or metastatic cancer patients for 1 year. Sequencing was successful in 483 (95.3%) archival tumor samples to find single nucleotide variant (SNV), copy number alteration (CNA), and fusion. NGS testing was unsuccessful in 52 (9.7%) specimens due to inadequate tissue (n = 28), low tumor volume (n = 19), and poor quality of nucleic acid (n = 5). According to the Tier system, variants were classified as Tier IA, 0.8%; IIC, 10.3%; IID, 2.0%; III, 66.7% for gastric: Tier IA, 3.6%; IIC, 11.6% for colorectal: Tier IA, 1.6%; IIC, 13.5%; IID, 0.5%; III, 70.8% for melanoma, and Tier IA, 9.1%; IIC, 1.8%; IID, 1.0%; III, 66.4% for GIST. In total, 30.8% of 483 sequenced cases harbored clinically meaningful variants. In Tier IA, *KRAS* and *ERBB2* were the most commonly altered genes. Interestingly, we identified *CD274 (PD-L1)* amplification, *PTPN11 (SHP2)* SNV, *TPM3-NTRK1* fusion, and *FGFR3-TACC3* fusion as a rare (<2%) alteration having therapeutic targets. In conclusion, although small biopsy samples constitute half of cases, informative NGS results were successfully reported in >90% of archival tissue samples, and 30.8% of them harbored clinically meaningful variants.

*Translational Oncology (2019) 12, 1488–1495*

## Introduction

Due to the increased efficiency of NGS, deep targeted sequencing panels with high depth and high exon coverage are rapidly being developed and applied in clinical practice and for clinical trials. NGS assays provide high accuracy, rapid turnaround time, and cost-effectiveness. [1,2] For an oncologist, the most important issue with an NGS cancer panel is whether the detected variants are useful for clinical management. To answer this question, a joint consensus recommended standards and guidelines in 2017. [3] These guidelines cover biomarkers for a specific tumor and a Tier system based on level of evidence. Areas of NGS application in clinical cancer care include

Address all correspondence to: Kyoung-Mee Kim, Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, #81, Irwon-ro, Gangnam-Gu, Seoul 06351, Korea. or Joon Oh Park, Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.
E-mails: oncopark@skku.edu, kkmkys@skku.edu
Received 20 June 2019; Revised 20 July 2019; Accepted 23 July 2019


1936-5233/19
https://doi.org/10.1016/j.tranon.2019.07.017

disease diagnosis, identification of therapeutic targets, and improvement of risk-stratification, which can guide treatment selection. [4]

The NGS cancer panel assays allow for rapid and reliable identification of the most commonly reported aberrations for precision oncology. Nearly all ongoing clinical trials of precision oncology can be performed on formalin-fixed paraffin-embedded (FFPE) tissue. [5] Differences in FFPE sample preparation, processing, and amount of DNA input can have substantial effects on the final outcome of NGS, [6] which can fail in some cases. For clinical application of an NGS-based cancer panel, there is considerable variability in clinical laboratories in terms of number and identities of genes tested, disease indication, and sample throughput. [7]

As development of a custom NGS test requires significant operational and bioinformatics infrastructure investment, some laboratories validate and use ready-made vendor solutions. [8,9] Many institutes without high infrastructure or samples with low quantity and/or poor quality of DNA use the oncomine comprehensive assay (OCA), a commercial platform consisting of 143 actionable genes that requires relatively small amounts of input DNA. However, the success rate of this NGS test in the palliative clinical setting and the overall incidence of clinically meaningful genetic alterations based on recommended guidelines are not reported in the pathology department. Moreover, the factors responsible for test failures in the real world have not been systematically evaluated. [4]

Herein, we analyzed the OCA cancer panel results from 535 gastrointestinal and rare cancers using FFPE tissue specimens used to discover molecular therapeutic targets in palliative cancer patients. We identified rare or novel genetic alterations linked to treatment and factors affecting NGS test failure.

## Materials and Methods

### *Patients and Tumor Samples*

A total of 535 solid tumor samples from gastrointestinal tracts (stomach, n = 234; colorectum, n = 196) and rare tumors (malignant melanoma (MM), n = 94; gastrointestinal stromal tumor (GIST), n = 11) were collected at Samsung Medical Center (SMC) from July 2017 to June 2018. All patients agreed to the collection and testing of genetic information (DNA/RNA) from their tumor samples.

### *Sample Preparation and DNA Extraction*

Following histological assessment with hematoxylin and eosin--stained sections by a pathologist to confirm tumor cell contents (tumor purity), the tumor areas of the FFPE sections were macro-dissected. The minimal tumor cellularity for NGS test was 10%. Paraffin blocks of FFPE samples were cut into 4-μm-thick sections, and 5 to 10 slides of unstained tissue were prepared. Then, the sample was deparaffinized using xylene and 100% ethanol. Genomic DNA (0.5−1.5 ng/μl) and RNA were extracted using a RecoverAll Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. DNA quantification was determined using a Qubit DNA HS assay kit (Thermo Fisher Scientific).

### *Library Preparation and Sequencing*

We used the Oncomine comprehensive assay v1 (Thermo Fisher Scientific), which examines 143 oncogenes and tumor suppressor genes detecting single nucleotide variant (SNV), copy number alteration (CNA), indels, and fusions. Targeted DNA/RNA amplification of each tumor sample was performed using the Ion AmpliSeq Library kit 2.0 (Thermo Fisher Scientific). For barcoded library preparation, the Ion Xpress Barcode Adapter 1−96 kit (Thermo Fisher Scientific) was substituted for the non-barcoded adapter mix in the Ion AmpliSeq Library Kit. The resulting amplicons were purified using Agencourt AMPure XP Reagent (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. The final library molecules were 50pM in concentration, which is appropriate for downstream template preparation. The libraries underwent quantification using the Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The final library molecules were 50pM in concentration, which is appropriate for downstream template preparation. The final libraries were transferred to the Ion Chef System (Thermo Fisher Scientific) for automated template preparation. Sequencing was performed on the Ion Torrent S5XL Machine platform (Thermo Fisher Scientific) with an Ion 540 Chip Kit (Thermo Fisher Scientific), according to the manufacturer's instructions.

### *Integrative Analysis and Reporting*

Automated analysis of sequencing raw data was performed by Ion Torrent software (Torrent Suite 5.10.0 with Ion Reporter 5.2) and Oncomine Knowledgebase Reporter (Thermo Fisher Scientific). All the genetic alterations were reported according to standards and guidelines for the interpretation and reporting of sequence variants in cancer: Tier I, variants of strong clinical significance such as FDA-approved, professional guideline or well-powered studies-appeared therapy; Tier II, variants of potential clinical significance such as FDA-approved treatment for different tumor types or investigational therapies; Tier III, variants of unknown clinical significance; and Tier IV, benign of likely benign variants. [3] Briefly, the criterion of variant allele frequency for SNVs/indels was ≥5%. An average CN ≥4 was interpreted as a gain (amplification) and <1 as a loss (deletion). For translocations, read counts ≥20 and total valid mapped reads ≥50,000 were interpreted as positive results. Most tumor samples were within the standards of sequencing results, such as mapped reads >5,000,000, on-target rate >90%, mean depth >1200, and uniformity >90%. Results with poor quality and suspected errors were filtered out based on <5% variant allele frequency, <100× coverage, and variants in the intron region. [10] Final analysis of each case was reviewed and reported by a professional pathologist.

### *Validation of assay*

To verify the workflow of the Ion S5XL system, we performed a verification test using commercially available control reference agents of Acrometrix oncology hotspot control (Thermo Fisher Scientific), 5 Fusion RNA multiplex positive/negative control (Horizon Diagnostics, Cambridge, United Kingdom), Structural Multiplex Reference Standard gDNA (Horizon Diagnostics), and Acrometrix frequency ladder (Thermo Fisher Scientific). NGS reactions were performed for 9 replicates to validate control testing, 32 replicates to demonstrate limit of detection testing, and 6 replicates to verify reproducibility between two operators.

### *Statistical Analysis*

Statistical analysis with the Mann−Whitney *U* test and visualization was performed using SPSS ver. 24.0 software (IBM Corp.,

Armonk, NY, USA). Two-sided *P* values <.05 were considered to statistically significant for all analyses.

## Results

### *Patient and Tumor Characteristics*

The median age of the patients was 58.36 years (range, 2−86), and 295 (55.1%) patients were male. The clinicopathological characteristics of patients and tumor tissue acquisition methods are described in Table 1. FFPE tissue samples consisted of 277 small (endoscopic) biopsies, 257 resections, and 1 fine-needle aspirate. The median age of the paraffin block was 1 month (range, 1 day to 105 months).

### *Analyses of NGS Test Failure Cases*

Out of 535 solid tumor samples that were used in the NGS cancer panel test, we were able to generate informative results in 483 cases (95.3%) comprising 211 gastric carcinoma (GC, 95%), 181 colorectal carcinoma (CRC, 96.3%), 82 MM (94.3%) and 9 GIST (90%). In 52 (9.7%) cases, the NGS test was not successful. The causes of failure are depicted in Figure 1 and include exhaustion of tissue due to other biomarker study for clinical trial enrollment (53.8%, 28/52), low (<10%) tumor volumes (36.5%, 19/52), and poor quality and quantity of nucleic acid (9.6%, 5/52). Most (>73%) cases with NGS test failure were biopsy samples.

The pie charts in Figure 1a illustrate the reasons for tissue insufficiency according to primary tumor site and sample type. The most frequent reason for test failure was tissue exhaustion (53.8%), followed by poor quality of DNA (9.6%) (Figure 1b). The success rates of resection specimens and biopsy specimens were 97.6% (248/254) and 92.9% (237/255), respectively. According to tumor type, the success rate was highest in CRC (181/188; 96.3%), followed by GC (211/222; 95%) and MM (82/87; 94.3%). When the NGS test was performed with unstained slides obtained from an outside hospital, the success rate was lower (87.1%) than that from in-house specimens (90.9%). For the failed cases, we obtained new samples in three cases and achieved final results with NGS.

**Table 1.** Clinicopathological characteristics of patients and tissue acquisition methods

| Variables | Category | N = 535 (%) |
|---|---|---|
| Age (y) | Continuous | median 59 (range 2−86) |
| Sex | Male | 295 (55.1) |
| | Female | 240 (44.9) |
| Source of tissue | Inside | 451 (84.3) |
| | Outside hospital | 84 (15.7) |
| Paraffin block age [1] | Continuous | median 8.76 mo (range 0−105) |
| Primary tumor | Gastric cancer | 234 |
| | Tubular adenocarcinoma | 206 (88) |
| | Signet ring cell carcinoma | 23 (9.8) |
| | Mucinous adenocarcinoma | 2 (0.9) |
| | Poorly differentiated carcinoma | 2 (0.9) |
| | Papillary adenocarcinoma | 1 (0.4) |
| | Colorectal cancer | 196 |
| | Adenocarcinoma | 185 (94.4) |
| | Mucinous adenocarcinoma | 9 (4.6) |
| | Signet ring cell carcinoma | 2 (1.0) |
| | Malignant melanoma | 94 |
| | Skin | 54 (57.4) |
| | Nasal cavity, larynx, and nasopharynx | 14 (14.9) |
| | Oral cavity | 5 (5.3) |
| | Anus | 6 (6.4) |
| | GI tract mucosa | 3 (3.2) |
| | Vagina | 3 (3.2) |
| | Others | 9 (9.6) |
| | GIST | 11 |
| | Small intestine | 7 (63.6) |
| | Stomach | 3 (27.3) |
| | Retroperitoneum | 1 (9.1) |
| Primary vs. metastasis | Primary | 410 (76.6) |
| | Metastasis | 123 (23.0) |
| | Unknown | 2 (0.4) |
| Specimen types | Biopsy | 277 (51.8) |
| | Resection | 257 (48.0) |
| | Cytology | 1 (0.2) |

Abbreviations: GI, gastrointestinal; GIST, gastrointestinal stromal tumor; NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma.

[1] This variable has missing values.

### *Sequencing Quality*

There was no statistically significant difference of mapped read, on target rate (%), mean depth, or uniformity according to cancer type, sample source (FFPE from SMC or unstained slide from outside hospital) (data not shown), or sample type (Supplementary Table 1). On the other hand, tumor purity was significantly higher in resection specimen compared to those of biopsy (60.59 ± 23.69% versus 55.40 ± 25.39%; $P = .028$).

### *Identified Genomic Alterations and Tier System*

A clinical report containing SNVs, CNAs, and gene rearrangements detected by NGS and with clinical implementation was generated for 483 cases. There were 7181 individual variants in the entire cohort. Those variants were classified according to the Tier system as follows (Supplementary Figure 1): Tier IA, 25/3112 (0.8%); IIC, 321/3112 (10.31%); IID, 63/3112 (2.02%); III, 2076/3112 (66.71%); IV, 627/3112 (20.15%) in GC; Tier IA, 99/2775 (3.57%); IIC, 321/2775 (11.57%); III, 1665/2775 (60%); IV, 690/2775 (24.86%) in CRC; Tier IA, 19/1184 (1.6%); IIC, 160/1184 (13.51%); IID, 6/1184 (0.51%); III, 838/1184 (70.78%); IV 161/1184 (13.6%) in MM. GIST harbored relatively higher numbers of Tier IA genetic alterations (10/110, 9.09%).

During clinical reporting, we weighted Tier I and II alterations because of their importance in molecularly guided therapy. In Tier I and II, 1029 alterations were found, comprising 630 (61.2%) SNVs, 53 indels, 343 CNAs, and 3 fusions. The most frequently altered gene (31%) in our report was *TP53,* and 89.7% (286/319) of all *TP53* variants were SNV, 9.7% (31/319) were indel, and 2 cases were CN deletion. *TP53* alterations were frequent in GC (54.2%) and CRC (42%). The second most frequently altered gene was *KRAS* (11.3%), including SNV in 82.8% (96/116) and amplifications in 17.2% (20/116). *KRAS* alterations occupied 19.9% of all Tier I and II variants in CRC.

### *Tier IA Alterations in 483 Cases*

Overall, 30.8% (149/483) of total cases harbored Tier IA genetic alterations (Figure 2). CRC was present in 65.1% of total cases with Tier IA alterations, and GC was present in 15.4%, MM in 12.8%, and GIST in 6.7% of cases. In Tier I, we observed alterations in five oncogenes (*KRAS,* 53%; *ERBB2,* 15.4%; *BRAF,* 14.8%; *KIT,* 12.1%, *NRAS,* 4.7%). The most frequent genetic alterations in Tier IA were *KRAS* (81.4%, 79/92) in CRC, *BRAF* (11.3%, 11/92), and *NRAS* (7.2%, 7/92) (Figure 3). In GC, *ERBB2* amplification was the most common Tier I genetic alteration, found in 16 cases (72.7%). GIST harbored *KIT* alterations in 88.9% of cases and occupied the largest proportion of tumors with Tier IA alteration. MM showed 12 *KIT* alterations (6 SNVs, 2 small indels, and 4 amplifications) and 11 *BRAF* SNVs.

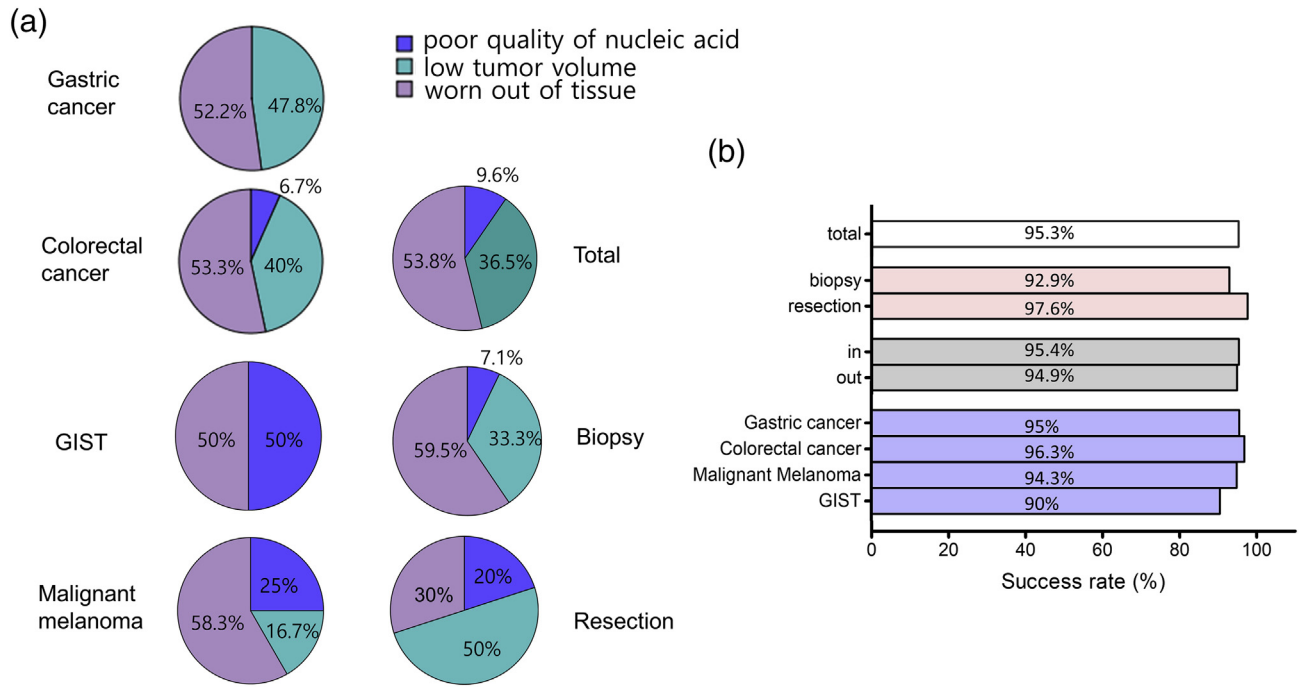


**Figure 1.** (A) Causes of failure according to tumor (left panels) and sample (right panels) type. (B) Sequencing success rate (%)-dependent variables.

### *Overview of Identified SNV*

The most prevalent SNV was found in *TP53,* and c.215C > G (p.Pro72Arg) was the most frequent SNV, found in 18.6% of total *TP53* SNVs and 8.3% of total SNVs of all genes (Figure 4). As the next prevalent, c.524G > A (p.Arg175His) occupied 6.5% of *TP53* SNVs. This SNV was found in *KRAS* and was enriched in c.35G > A (p.Gly12Asp) and c.38G > A (p.Gly13Asp). Then, *PIK3CA, PTEN, BRAF,* and *NRAS* mutations followed in frequency. In GC, *TP53* SNVs were the most frequent, followed by *KRAS* SNVs. The *ERBB2*

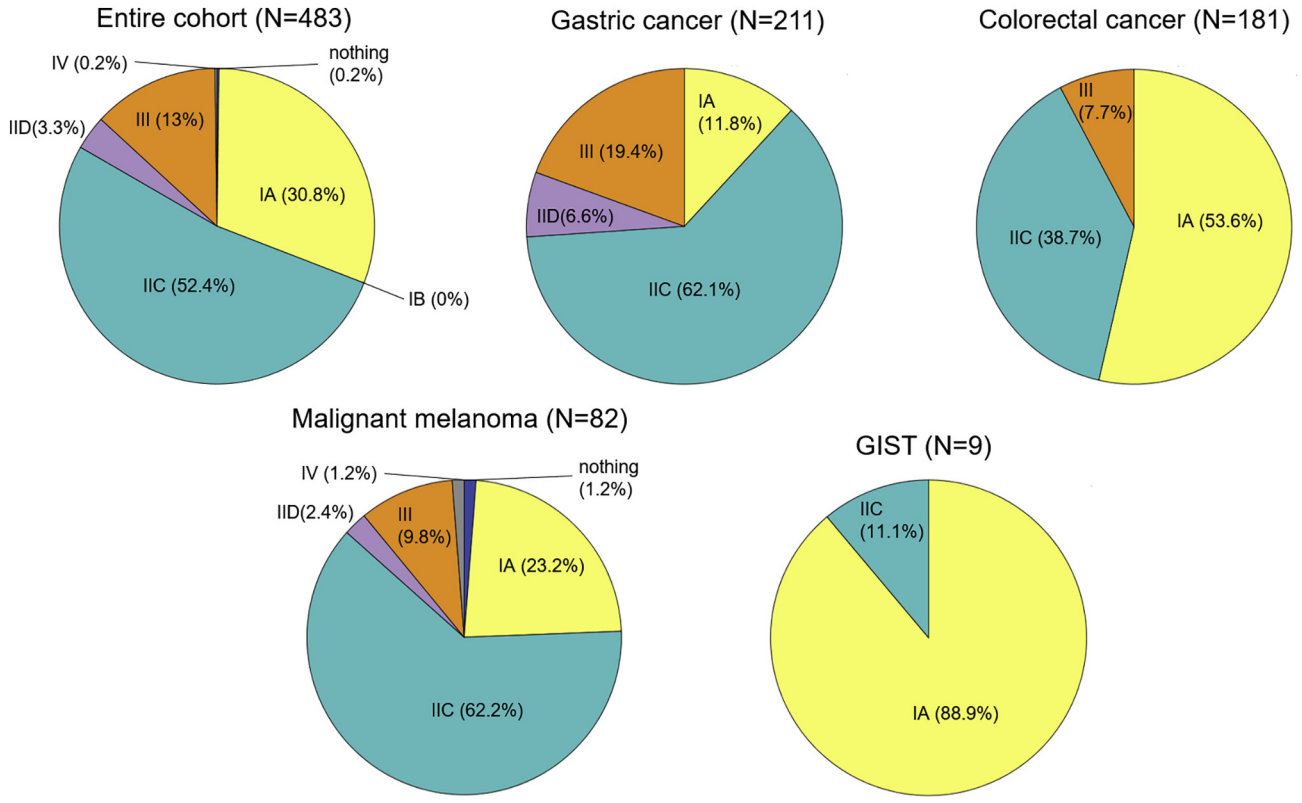


**Figure 2.** Pie charts showing the highest Tier distribution for all patients (upper left panel) and each tumor type.

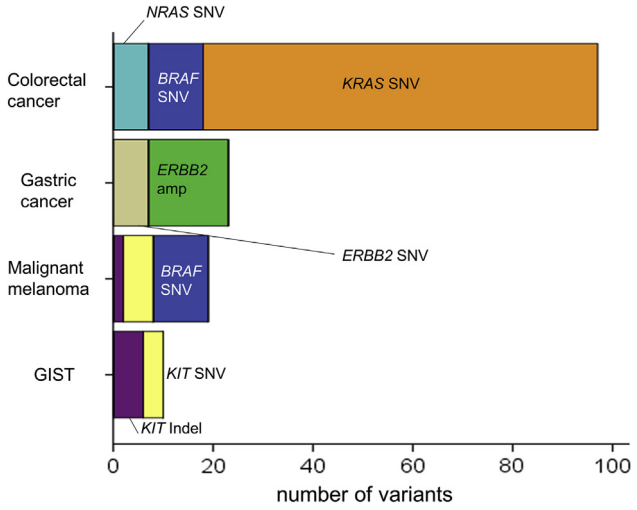


**Figure 3.** Bar graphs indicating distribution of Tier IA alterations.

2033G > A SNV was also common in GC, as were *KRAS* and *TP53* SNVs, followed by *BRAF* 1799 T > A (p.Val600Glu).

Unexpectedly, we found *PTPN11* SNV in 4 cases consisting 2 GC (Gln510His; 0.95%) and 1 each of CRC (Gln510His; 0.55%) and MM (Glu76Lys; 1.22%).

### *Overview of Identified Insertions and Deletions (indels)*

In Tier I and II variants, 49 indels were identified in the 8 genes *TP53, KIT, CDKN2A, ATM, NF1, NOTCH1, PTEN,* and *BRCA1*. Some of them were unusually large in size, and a 51 base pair deletion, p.Val560_Leu576del (c.1678_1728delGTTGAGGAGATAAATGGAAACAATTATGTTTAC ATAGACCCAACACAACTT), was found in GIST from the small intestine. This mutation was not called by Ion Reporter but has been detected by Sanger sequencing. Careful examinations of Integrative Genomics Viewer (IGV) confirmed this deletion mutation (Supplementary Figure 2). However, indels <50 base pairs (bp) were successfully called and reported by Ion Reporter including *KIT* p.Val560_Pro573delinsAlaGluGluIle AsnGlyAsnAsnTyrValTyrIleAspGln (c. 1679_1718delinsCT GAGGAGATAAATGGAAACAATT ATGTTTACATAGACCA) from MM and *TP53* p.Asn131fs (c.390_426delCAACAAGATGTTTTGCCAACT G GCCAAGACCTGCCCT) from GC. All 46 indels classified as Tier I or II alteration and with <30 bp are listed in Supplementary Table 2. Among 49 indels in Tier I or II, 10 were novel, and their IGV are depicted in Supplementary Figure 3.

### *Overview of identified copy number alterations (CNAs)*

CNAs were observed in 347 cases consisting of 17 Tier IA alterations (4.9%), and *ERBB2* was the most frequently observed CNA especially in GC. *ERBB2* amplification was also found in CRC as Tier IIC. The median CN of 22 *ERBB2*-amplified tumors was 37.7 (range; 5.3−640.2). The next frequent CNA was *CCND1* (Tier IIC) found in 28 GC, 15 MM, and 3 CRC (median CN, 12.8; range, 4.35−152.5). *MYC* amplification was found in 10 GC and 6 each of CRC and MM (median CN, 8; range, 4.34−101.1). *FGFR2* amplification was identified in 15 GC and 5 CRC (median CN, 7.9; range, 3.97−295.3). *EGFR* amplification was observed in 5 GC, 4 CRC, and 4 MM (median CN, 12.3; range, 5.6−123). *MET* amplification was found in 7 GC, 2 MM, and 1 CRC (median CN, 8.8; range, 7.1−177.7). Unexpectedly, we identified *CD274* (PD-L1) amplifications in 2 GC and 1 MM. In those cases, immunohistochemistry of PD-L1 with 22C3 pharmDx (Agilent Pathology Solutions) revealed a few tumor infiltrating lymphocytes stained with PD-L1 in 2 GCs and PD-L1 expression in tumor cells in MM cases, although the numbers of tumor cells were small because the tissue specimens were mostly depleted. In addition, *KRAS, FGFR1, ATM,* and *CCNE1* CNAs were identified and classified as Tier II (Figure 5).

### *Overview of Identified Fusions*

Fusion detection was performed with RNA components through 3′/5′ expression imbalance. [11] Out of 483 cases, *TPM3-NTRK1* fusion was found in 2 CRC samples (1.1%). In MM of the nasal cavity, we identified a *FGFR3-TACC3* fusion that had previously been reported. [12]

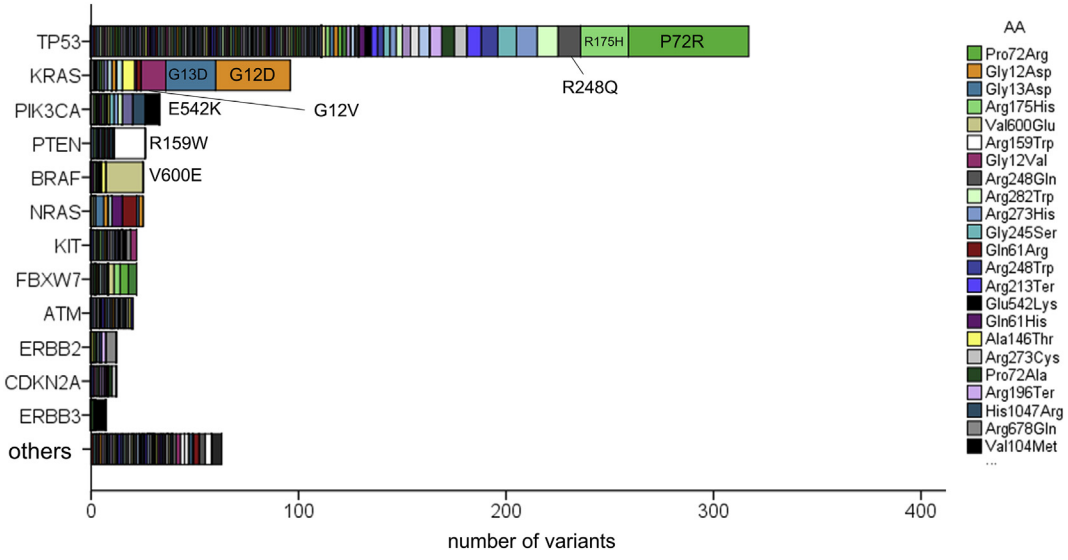


**Figure 4.** Bar graphs illustrating amino acid changes of Tier I and II gene alterations according to frequency.

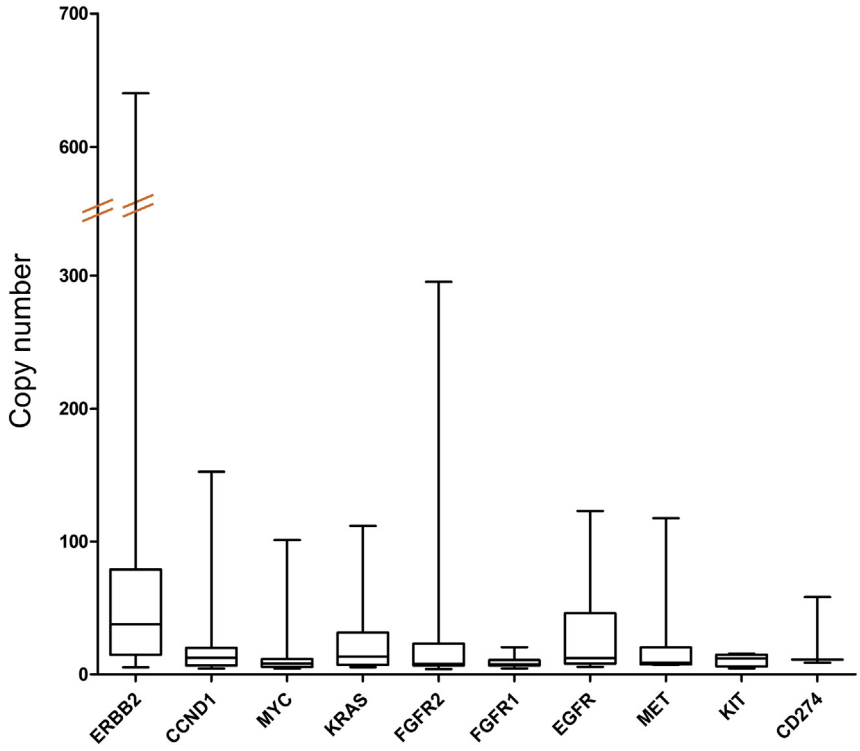


**Figure 5.** Each box plot represents amplified copy numbers in selected genes.

## Discussion

Given the importance of NGS cancer panel tests in the palliative clinical setting, it is critical to understand the success rate of NGS test, factors responsible for test failures, and the overall incidences of clinically significant genetic alterations based on recommended guidelines reported for real-world pathology departments. In this study, we analyzed OCA cancer panel results in 535 gastrointestinal and rare cancers using FFPE tissue specimens from recurrent or metastatic cancer patients. We analyzed factors affecting NGS test failure and identified common and rare genetic alterations linked to treatment.

The overall success rate of our patient cohort was 95.3%, and the success rate of resection was higher than that with biopsy. Prior study with a large number of patients with a 467-gene oncology panel showed sequencing success in 92.3% of cases, with a higher success rate in the resection specimens. [13] An MSK-IMPACT study reported successful sequencing in 91% of cases using either archival or new (fresh) tumor samples. [14] A study from the MD Anderson Cancer Center reported significantly different success rate across resection, biopsy, and cytology samples: 97% in resection, 80% in biopsy, and 50% in FNA samples. [15] When we focused on biopsy samples in a clinically palliative setting, the proportion of biopsy samples was high in the present study compared to previous studies [13,14] suggesting that the higher success rate observed in the present study was caused by coverage of a smaller number of genes and lower input DNA for this amplicon-based sequencing method.

Al-Kateb et al. reported that age and type of tissue specimen and DNA degradation cause library failure. [4] In our study, we experienced 5 cases of failed library construction because of poor DNA quality, in which 3 were from MM with dense melanin pigment depositions, 1 was from mucinous colonic carcinoma with excessive mucin, and 1 was from GIST with extensive necrosis after neoadjuvant chemotherapy. So, necrotic or mucin-rich areas should be avoided to acquire better DNA quality if possible. [16]

In the present study, the incidence of Tier IA variants is similar to previous studies [13,14] using a cancer panel with larger numbers of genes compared to ours. When we expand clinically relevant genomic alterations to Tier IA to IIC variants as previously described, 83.4% of our patients could be enrolled in genotype-matched clinical trials, an incidence similar to that of previous studies. [17,18] In addition to diverse distributions of primary tumor, different definitions and broad concepts were applied to the Tier system for classifying genetic alterations guiding molecular therapy. In the present study, we applied the Tier system recommended by the joint consensus of Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists in 2017. [3] In an MSK-IMPACT study, the most common genetic alterations used for patient enrollment to matched therapies were *PIK3CA* mutations (25%), *ERBB2* amplifications (14%), and *PTEN* alterations (9%). [19] We classified *ERBB2* amplification as Tier IA and *PIK3CA* and *PTEN* alterations as Tier IIC. This Tier system allows clinicians to concentrate on two groups; however, clinical judgment by the treating physician is still necessary. [20]

For Tiers I and II, the frequently altered genes in CRC were *KRAS*, *TP53,* and *BRAF*. Recent studies evaluating the genomic landscapes of metastatic CRC revealed that *APC, TP53, KRAS,* and *PIK3CA* are commonly mutated. [21,22] In a previous study, the highest actionability level (level-1) based on OncoKB classification showed *KRAS* and *NRAS* as resistance markers for anti-EGFR antibody therapy. In the present study, we classified *KRAS, NRAS,* and *BRAF* alterations as Tier IA by a recommended guideline. For GC, *TP53* SNV and indel occupied 50% of total somatic alterations, and *ERBB2*

alterations represented 8.6% of total variants. [17] Another GC NGS panel data reported *ERBB2* amplification in 14.9% of cases. [23] In MM, a previous study showed *BRAF* alterations in over 50% and *NRAS* in 15% of total alterations [13]; however, *KIT* mutation was observed in 5% of the cases. [24] In this study, *NRAS* was the most frequent in 82 MM cases, followed by *BRAF* and *KIT*. Although detailed codon changes in major genes (e.g., *BRAF, KIT,* and *NRAS*) are similar to those in previous studies, the differences in incidence may be caused by ethnic differences and the fact that almost half of cases were mucosal melanoma cases.

Unexpectedly, we found a rare *PTPN11* SNV in 4 cases each of GC, CRC, and MM, ant their incidence is similar to that of COSMIC. *PTPN11* mutation induces production of oncoprotein SHP2 and activates the RAS/RAF/MAPK pathway. [25] *PTPN11(SHP2)* inhibitors are being developed and are currently in clinical trials (NCT03114319).

Indels are abundant in the human genome, and their functionally is important. [26] For detection of indel, errors have been reported in the Ion Torrent platform, [22] although the torrent suite has been improved with a version upgrade. [27–29] We found a unique case in a GIST patient with a 51-bp deletion mutation of *KIT* confirmed by Sanger sequencing but missed by NGS. Given that accurate mapping of indels larger than 7 nucleotides is a known limitation of the GATK genotyping pipeline, [30] caution is needed for medium-size indel detection with this platform.

CNA is a crucial somatic variation of cancer and is important for enrollment in clinical trials. [31,32] In this study, all CNA results in genes with strong clinical significance were validated with immunohistochemistry, and all cases with *ERBB2* amplifications were matched with strong HER2 overexpression. As previously reported, CN estimation and tumor purity are linearly correlated with amplicon-based NGS. [32] Meticulous evaluation of tumor purity measured during the pathologic review process may have contributed to our accurate CNA results.

Interestingly, we found three cases with *CD274* amplifications found in two EBV-negative and microsatellite stable (MSS) GCs and one MM. Immunohistochemistry of PD-L1 with 22C3 pharmDx (Agilent Pathology Solutions) revealed a few tumor infiltrating lymphocytes stained with PD-L1 in two GCs, and PD-L1 expression in tumor cells was observed in MM cases, although the numbers of tumor cells were small because the tissue specimens were mostly depleted. Those patients with *CD274* amplifications would be candidates for anti-PD-1/PD-L1 therapy. Recent studies reported that *STK11* alteration is associated with resistance to immunotherapy. [33,34] We observed *STK11* alterations in 3 CRCs, 2 GCs, and 1 MM case. Genetic alterations related to immunotherapy would be useful in those palliative setting patients.

This study had some limitations. First, we did not match to normal blood to filter out germline variants. Second, the clinical application of NGS results for precision oncology was not covered because we wanted to concentrate on analyzing the success rate of NGS and the Tier system and avoid any overlap with a clinical study.

In conclusion, although small biopsy samples constituted half of our cases, informative NGS results were successfully reported in >90% of archival tissue samples, and 30.8% of those harbored clinically meaningful variants for guideline-recommended target therapy. In addition to variants of strong clinical significance, we identified many variants with potential clinical significance and rare alterations with therapeutic targets.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2019.07.017.

## Acknowledgements

This research was supported by a grant from the National Research Foundation of Korea funded by the Ministry of Science and ICT (2017R1D1A1B03032449 and NRF-2017R1A2B4012436).

## References

[1] Horak P, Frohling S and Glimm H (2016). Integrating next-generation sequencing into clinical oncology: strategies, promises and pitfalls. *ESMO Open* **1**:e000094.

[2] Paasinen-Sohns A, Koelzer VH and Frank A, et al (2017). Single-center experience with a targeted next generation sequencing assay for assessment of relevant somatic alterations in solid tumors. *Neoplasia* **19**, 196–206.

[3] Li MM, Datto M and Duncavage EJ, et al (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology. *and College of American Pathologists J Mol Diagn* **19**, 4–23.

[4] Al-Kateb H, Nguyen TT, Steger-May K and Pfeifer JD (2015). Identification of major factors associated with failed clinical molecular oncology testing performed by next generation sequencing (NGS). *Mol Oncol* **9**, 1737–1743.

[5] Wagle N, Berger MF and Davis MJ, et al (2012). High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov* **2**, 82–93.

[6] Lee C, Bae JS and Ryu GH, et al (2017). A method to evaluate the quality of clinical gene-panel sequencing data for single-nucleotide variant detection. *J Mol Diagn* **19**, 651–658.

[7] Cheng DT, Mitchell TN and Zehir A, et al (2015). 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**, 251–264.

[8] Luthra R, Patel KP and Routbort MJ, et al (2017). A targeted high-throughput next-generation sequencing panel for clinical screening of mutations, gene amplifications, and fusions in solid tumors. *J Mol Diagn* **19**, 255–264.

[9] D'Haene N, Fontanges Q and De Neve N, et al (2018). Clinical application of targeted next-generation sequencing for colorectal cancer patients: a multicentric Belgian experience. *Oncotarget* **9**, 20761–20768.

[10] Kim S, Lee J and Hong ME, et al (2014). High-throughput sequencing and copy number variation detection using formalin fixed embedded tissue in metastatic gastric cancer. *PLoS One* **9**:e111693.

[11] Hovelson DH, McDaniel AS and Cani AK, et al (2015). Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia* **17**, 385–399.

[12] Jiyun Lee JL, Hong Sang Duk, Jang Kee-Taek and Jin Lee Su (2018). FGFR3-TACC3: A novel gene fusion in malignant melanoma. *Precision and Future Medicine* **2**, 71–75.

[13] Sireci AN, Aggarwal VS and Turk AT, et al (2017). Clinical genomic profiling of a diverse array of oncology specimens at a large academic cancer center: Identification of Targetable Variants and Experience with Reimbursement. *J Mol Diagn* **19**, 277–287.

[14] Zehir A, Benayed R and Shah RH, et al (2017). Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* **23**, 703–713.

[15] Goswami RS, Luthra R and Singh RR, et al (2016). Identification of factors affecting the success of next-generation sequencing testing in solid tumors. *Am J Clin Pathol* **145**, 222–237.

[16] Kim J, Park WY and Kim NKD, et al (2017). Good laboratory standards for clinical next-generation sequencing cancer panel tests. *J Pathol Transl Med* **51**, 191–204.

[17] Ali SM, Sanford EM and Klempner SJ, et al (2015). Prospective comprehensive genomic profiling of advanced gastric carcinoma cases reveals frequent clinically relevant genomic alterations and new routes for targeted therapies. *Oncologist* **20**, 499–507.

[18] Rangachari D, VanderLaan PA and Le X, et al (2015). Experience with targeted next generation sequencing for the care of lung cancer: insights into

promises and limitations of genomic oncology in day-to-day practice. *Cancer Treat Commun* **4**, 174–181.
[19] Schram AM, Reales D and Galle J, et al (2017). Oncologist use and perception of large panel next-generation tumor sequencing. *Ann Oncol* **28**, 2298–2304.
[20] Kurnit KC, Dumbrava EEI and Litzenburger B, et al (2018). Precision oncology decision support: current approaches and strategies for the future. *Clin Cancer Res* **24**, 2719–2731.
[21] Yaeger R, Chatila WK and Lipsyc MD, et al (2018). Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell* **33**: 125–136.e123.
[22] Del Vecchio F, Mastroiaco V and Di Marco A, et al (2017). Next-generation sequencing: recent applications to the analysis of colorectal cancer. *J Transl Med* **15**, 246.
[23] Kuboki Y, Yamashita S and Niwa T, et al (2016). Comprehensive analyses using next-generation sequencing and immunohistochemistry enable precise treatment in advanced gastric cancer. *Ann Oncol* **27**, 127–133.
[24] de Unamuno Bustos B, Murria Estal R and Perez Simo G, et al (2017). Towards personalized medicine in melanoma: implementation of a clinical next-generation sequencing panel. *Sci Rep* **7**, 495.
[25] Ruess DA, Heynen GJ and Ciecielski KJ, et al (2018). Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med* **24**, 954–960.
[26] Mullaney JM, Mills RE, Pittard WS and Devine SE (2010). Small insertions and deletions (INDELs) in human genomes. *Hum Mol Genet* **19**, R131–R136.
[27] Yeo ZX, Wong JC, Rozen SG and Lee AS (2014). Evaluation and optimisation of indel detection workflows for ion torrent sequencing of the BRCA1 and BRCA2 genes. *BMC Genomics* **15**, 516.
[28] Loman NJ, Misra RV and Dallman TJ, et al (2012). Performance comparison of benchtop high-throughput sequencing platforms. *Nat Biotechnol* **30**, 434–439.
[29] Shin S, Kim Y and Chul Oh S, et al (2017). Validation and optimization of the Ion Torrent S5 XL sequencer and Oncomine workflow for BRCA1 and BRCA2 genetic testing. *Oncotarget* **8**, 34858–34866.
[30] Yohe S, Hauge A and Bunjer K, et al (2015). Clinical validation of targeted next-generation sequencing for inherited disorders. *Arch Pathol Lab Med* **139**, 204–210.
[31] Shlien A and Malkin D (2009). Copy number variations and cancer. *Genome Med* **1**, 62.
[32] Grasso C, Butler T and Rhodes K, et al (2015). Assessing copy number alterations in targeted, amplicon-based next-generation sequencing data. *J Mol Diagn* **17**, 53–63.
[33] Skoulidis F, Goldberg ME and Greenawalt DM, et al (2018). STK11/LKB1 mutations and PD-1 inhibitor resistance in KRAS-mutant lung adenocarcinoma. *Cancer Discov* **8**, 822–835.
[34] Hellmann MD, Nathanson T and Rizvi H, et al (2018). Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. *Cancer Cell* **33**:843–852.e844.