Clinical Benefit and Agenda for Clinical Sequencing Using Cancer Panel Testing: A Retrospective Clinical Study

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Research article

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Abstract

Background: Clinical sequencing using a multiplex gene panel has recently been applied worldwide for patients with refractory solid tumors, but the significance of clinical sequencing using multiplex gene panel testing remains uncertain. Here we sought to clarify the feasibility and utility of clinical sequencing in the treatment of refractory tumors at our hospital

Methods: A total of 28 patients with advanced solid tumors treated at our hospital between 2018 and 2020 were enrolled in the clinical sequencing. Among them, we identified 26 patients whose tissue samples were of suitable quality for clinical sequencing, and we analyzed the genomic profiles of these tumors.

Results: Pathogenic alterations were detected in 20 (77%) of the 26 patients. The most common mutation was *TP53* (6/26, 35%), followed by *KRAS* (5/26, 19%), and the highest frequency of gene amplification was *ERBB2* (5/26, 19%). Five of the 26 patients were identified as candidates for novel molecular-targeted therapy based on their actionable gene alterations, but none of the cases had an advantage for novel targeted therapy following the genetic tests; the reasons were clinical deterioration, refusal for off-label use, and complexity of clinical trial access.

Conclusions: Our current results suggested that clinical sequencing might be useful for the detection of pathogenic alterations and the management of additional cancer treatment. Both improved optimal timing of clinical sequencing and a consensus about its off-label use might help patients receive greater benefit from clinical sequencing.

Trial registration: This study was performed as a part of clinical study approved by Ethical Committee of Osaka City University Graduate School of Medicine (Permission number: 4199, 3925)

Background

Several studies have defined the genomic landscape of cancer with the use of next-generation sequencing (NGS) technology, and they have detected some candidate driver-gene alterations of cancer that allow the tumor cells to survive and spread (1-4). Clinically, multiplex gene panel testing (which uses NGS to target a limited number of cancer-associated genes) has been the most practical genome profiling method worldwide (5-8). NGS is often used for patients with advanced refractory cancer who fail to respond to standard therapy, and it is linked to a new alternative known as 'clinical sequencing.'

FoundationOne® CDx and MSK-IMPACT, which are comprehensive genome profiling tests for all solid tumors, have been approved by the U.S.FDA and used for the clinical sequencing of over 10,000 patients (9, 10). In Japan, a total of 230 patients with advanced solid tumors underwent clinical sequencing using the NCC Oncopanel as part of the TOP-GEAR project at the National Cancer Center Hospital (UMIN000011141), and the genetic tests revealed that 13% of the patients were candidates for novel targeted therapy (11). In this context, the use of the NCC Oncopanel as well as the FoundationOne® CDx

has been covered under Japan's national health insurance since June 2019. Thus, there have been few evaluations of the feasibility and utility of clinical sequencing in cancer treatment in Japan (11, 12). At our hospital, clinical sequencing has been introduced for cancer patients as an application of precision oncology. Here we summarize the results of 28 patients who underwent panel testing as clinical sequencing. We also provide the results of our assessment of the feasibility of clinical sequencing using panel testing.

Methods

Patients

A total of 28 patients with advanced solid tumor, including primary unknown site, treated at our hospital between 2018 and 2020, were enrolled in clinical sequencings using multiplex gene panel testing. All protocols were conducted after obtaining written informed consent from all patients in accordance with the approved procedures at our hospital. Specimens obtained by surgery, core, and fine needle biopsy were available. Among these 28 patients, we identified 26 patients with formalin- fixed paraffin-embedded (FFPE) tumor tissues samples which were available for sequencing with NGS, whereas the remaining 2 patients were excluded from clinical sequencing due to lack of quality FFPE samples. Therefore, for the 26 patients included in clinical sequencing, the following eligibility was confirmed before initiation of clinical sequencing: (1) refractory to standard treatment for solid tumor or no evidence of standard therapy for primary unknown cancer; (2) evidence of good performance status; (3) evidence of over 16 years old.

Multiplex gene panel testing based on NGS

For current clinical sequencing, four types of multiplex gene panel testing were performed in this study, as follows; (1) OncoGuide™ NCC oncopanel (Sysmex, Kobe Japan) targeted the coding exons of 114 cancer associated genes including 12 fusion genes was performed after implement for coverage under national health insurance. To detect somatic mutation, peripheral blood was used as a reference of germline mutation in this sequencing. (2) FoundationOne® CDx (Foundation Medicine, Inc., Cambridge, MA), which detected substitutions, insertion-deletions and copy-number alterations in 309 genes and select 36 gene arrangements, was approved by Japanese regulators, as well as NCC oncopanel. (3) Oncomine™ Target Test (Thermo Fisher Scientific, Waltham, MA), which interrogated prominent mutational hotspots only in 46 genes except for *TP53* and examined 12 gene rearrangements, was underwent as a part of Japan medical care sponsored by Osaka University hospital. Over 20% of tumor celluarity in FFPE sample was available for this panel testing. (4) ION Ampliseq™ hotspot panel v2 (Thermo Fisher Scientific, Waltham, MA), which research somatic mutations across the hotspot regions of 50 cancer associated genes, was performed as a part of prospective clinical trial sponsored by Kindai hospital. Oncomine™ Target Test and ION Ampliseq™ hotspot panel v2 were performed as clinical study approved by Ethical Committee of Osaka City University Graduate School of Medicine (Permission number: 4199, 3925), whereas FoundationOne® CDx and NCC oncopanel were clinically available. **Supplemental Table S1**, **S2, S3 and**

S4 lists details of genes in these panels, including actionable genes caused by variant, copy number alteration and rearrangement. Among a total of 324 genes targeted in these panels, 24 genes were confirmed in the intersection of all four panels (**Figure 1A**), whereas 70 actionable genes for FDA-approved targeted therapies, including genome-matched and related target, was observed in these panels (**Figure 1B**).

Tumor mutational Burden

Tumor mutational burden (TMB), which is related to response immune checkpoint therapy, was also analyzed in NCC oncopanel and FoundationOne® CDx (11, 13). TMB was classified as 2 categories, High-TMB and Low-TMB. High-TMB was defined as over 10 mutations/Megabase, otherwise Low-TMB was defined as under 10 mutations/Mb, as previously reported.

Reporting and curation of Genetic alteration

Sequencing was performed using NGS system according to each platform. After sequencing with NGS, bioinformatics analysis was performed to call genetic alterations using each NGS data analysis pipeline, as previously described (11, 14, 15). Finally, important filtered variants, which were annotated using biological database, were discussed at the molecular tumor board, called the “expert panel”. Each expert panel was composed of several specialists in specific disciplines, including oncology, pathology, bioinformatics and genetics. They notably curate the explanations on genetic alteration and provide the patients with treatment option according to knowledgebase for clinical interpretation of cancer variants, such as OncoKB (https://www.oncokb.org/) and CIVic (https://civicdb.org/home).After expert panel discussion , the curated report based on this council was returned to clinician.

Statistical analysis

Survival rates were estimated using the Kaplan-Meier method, and the differences in survival according to the cancer type in detail were analyzed by log-rank test. The presence of a statistically significant difference was denoted by $p<0.05$. All analyses were performed using GraphPad Prism 8.3.0 (GraphPad Software, La Jolla CA).

Results

The clinicopathological features of patients with refractory tumor

Of the 26 patients, four were clinically examined with the NCC oncopanel, one with the FoundationOne® CDx, 16 with the ION Ampliseq™ Cancer Hotspot Panel v2, and nine with the Oncomine™ Target Test (n=9); two patients with pancreatobiliary cancer were examined with both the ION panel and the Oncomine test (**Table 1,Fig. 2A**). **Table 1** summarizes the clinical characteristics of the 26 patients. Their median age was 69 years. Among the 26 patients, we identified 18 patients with gastrointestinal (GI) cancer (n=9) or pancreatobiliary (n=9) cancer; The remaining eight patients had different tumor types,

including ovarian cancer (n=2), primary unknown site (n=2), and lung cancer (n=2). The rate of DNA extraction from the formalin-fixed paraffin-embedded (FFPE) primary lesion samples was 54% (14/26), and an FFPE sample archived within 3 years from the biopsy was obtained in 20 cases. A successful NGS assay was achieved for all of the patients in this clinical sequencing.

Genomic profile of patients using multiplex gene panel

Figure 2A provides a list of all of the mutated genes identified with the use of cBioportal (16, 17). Pathogenic genetic alterations were detected in 20 (77%) of the 26 patients; the panel test showed no mutation in the other six patients. The most common mutation was *TP53* (35%), followed by *KRAS* (19%). The highest frequency of gene amplification was *ERBB2* (19%) in the clinical sequencing, whereas *MET*, *RB1*, and *CDK4* amplification was observed in one patient. For the two patients who underwent both the ION panel and the Oncomine test, no difference was observed in cancer treatment according to results of the panel testing. There were no patients with a high tumor mutational burden (TMB) in this clinical sequencing, and a germline mutation which contributes to hereditary disease was identified in 1/25 (4%) patients. Rearrangement was not observed in any of these patients with solid tumors.

Influence on cancer therapy according to result of panel test

The panel results revealed actionable mutations that might be a therapeutic target in eight of the 20 patients with pathogenic genetic alterations (**Table 2**). Three of the eight patients with gastric cancer harboring *ERBB2* amplification had already received targeted therapy as a first-line treatment for gastric cancer, and the other five patients were identified as candidates for novel molecular-targeted therapy based on their actionable gene alterations. One of these five patients had cholangiocarcinoma that harbored *ERBB2* missense mutation (V777L) (**Fig. 2B**), which was the therapeutic target for patients with different types of tumors as reported (18, 19). However, treatment with *ERBB2* inhibitors, including neratinib, was approved for solid tumors with *ERBB2* amplification but not for solid tumors with *ERBB2* variant by the Osaka City University Hospital Certified Review Board (certification no. CRB5180003). Another two of these five patients did not receive genotype-matched therapy because of their poor performance status when they were considered for enrollment in a clinical trial that evaluated the investigational drug efficacy in patients with solid tumors harboring *ERBB2* amplification. The remaining two patients were not followed up because of their transfer to another hospital after testing. Consequently, none of the patients was a candidate for novel targeted therapy after the genetic tests in this study.

Short survival

The median period between the date of consent for undergoing the NGS and the date when the NGS results were received was 43 days. Five patients died before their results were returned to the clinician (**Table 1, Fig. 3A**). **Figure 3B** illustrates the survival rate of the 21 patients with advanced solid tumors (the five patients who died are excluded). The median period of survival after the expert panel discussion was approx. 4 months. Most of the patients with pancreato-biliary cancer died within 1 year after the expert

panel discussion, whereas most of patients with GI cancer were alive >1 year later despite being refractory to standard therapy (**Fig. 3C**) (log rank test; $p<0.01$).

Discussion

We performed practical clinical sequencing using four types of multiplex gene panel testing by NGS. Differences were observed among the four panels in the number of actionable genes and the number of cancer-associated genes (Fig. 1). It has been demonstrated that the rate of patients who received a subsequent therapy based on actionable gene alteration was not significantly different among several types of panel testing (7, 10, 11). These findings might suggest that the actionable genes to be detected are limited to major cancer-associated genes, such as *ERBB2* and *EGFR*. However, the number of candidates for actionable genes has been increasing, as has the number of molecular-targeted therapeutics. In fact, a number of novel molecular-targeted therapeutics were approved by the FDA during the current clinical sequencing (https://www.fda.gov/Drugs). It could thus provide a great impact on future cancer treatment to evaluate as many cancer-associated genes as possible, using larger-panel testing, *i.e.,* whole exome sequencing or whole genome sequencing.

In the present clinical sequencing, 20 of the 26 patients had pathogenic genetic alterations, and eight of those 20 patients (40%) had an actionable gene for a therapeutic target, which was concordant with the results of earlier studies (ranging from 37% to 70%) (7, 10-12). However, none of the present patients underwent a novel targeted therapy after their clinical sequencing. There are two important points to be noted about the present results. First, the most common reason for not undergoing targeted therapy was clinical deterioration during the turnaround time from consent to genetic results (approx. 6 weeks in this patient series). Several reports demonstrated that 6% to 27% of patients fail to receive genome-matched therapy after panel testing because of declining performance status (7, 8). Second, there were two patients in this study who were unable to enroll in a genome-matched trial due to the complexity of gaining entry to a clinical trial even in Japan, and clinical deterioration occurred during the delay. To address these problems, Chantrill et al. suggested that establishing a dedicated multidisciplinary team (including a molecular pathologist responsible for extracting high-quality samples from specimens) is necessary to generate a quick turnaround time (6). Regarding the complexity of gaining access to a clinical trial, the utility of a virtual clinical trial — which is designed as a remote trial to evaluate the clinical data of patients via the Internet without hospital visits — has been proposed (20, 21). This online method might allow clinical patients to reduce their activities for a clinical trial, including the trial entry and travel to a hospital. A combination of these tools might help make clinical sequencing more widely available and accessible for patients hoping to undergo treatment based on genomic profiling.

Another unexpected finding of the present study is that the off-label use of an *ERBB2* inhibitor was not approved for patients with cholangiocarcinoma harboring an *ERBB2* pathogenic variant. Notably, a molecular-targeted drug approved for cancer with a specific gene alteration might be active or inactive in patients with different types of tumors. However, patients in Japan could receive subsequent therapy from another clinical sequencing (12). This observation might suggest the lack of consensus about off-

label use. It is well known that the guidelines for off-label uses of drugs are not harmonized across the world (22). It may thus be difficult to assess comparisons of off-label drug uses in studies conducted in different countries or regions, but the practice of off-label use should nevertheless be regulated based on greater security and robust clinical guidance.

In the present study, 19% of the patients died during the turnaround time, and seven patients died within 4 months of the testing in this clinical sequencing. A low frequency of actionable genes and poor prognoses were observed in the patients with pancreato-biliary cancer, whereas the patients with GI cancer could be treated with an alternative molecular-targeted drug (*e.g.*, ramucirumab and nivolumab) for standard therapy. These findings will contribute to the identification of the appropriate timing and clinical stage at which clinical sequencing should be performed according to tumor type. Considering that some patients with refractory solid tumor (such as rare tumors and pancreatobiliary cancer) experience impressive responses to targeted therapy following genetic tests (11, 12), we recommend that clinical sequencing for those patients be performed at an earlier stage (*i.e.,* at the time of tumor diagnosis or prior to standard chemotherapy).

Since TMB data was obtained from only five patients, it is difficult to analyze the significance of the TMB for this clinical sequencing. In general, the TMB is calculated from the results of whole exome sequencing, but several studies demonstrated the accuracy of TMB data from panel testing as well as from whole exome sequencing (10, 11, 13). TMB data from panel testing might be more widely available and useful for cancer treatment in the future.

Conclusion

The result of the current study indicate that clinical sequencing might be useful for the detection of pathogenic alterations and management of cancer treatment. Specially, the presence of actionable gene mutation might be associated with improved outcomes in patients with solid tumor refractory to standard therapy. However, none of the present patients received a novel targeted therapy based on this clinical sequencing, and thus further explorations of the optimal timing of clinical sequencing and a consensus about off-label use could help cancer patients benefit from clinical sequencing.

Abbreviations

FFPE; formalin- fixed paraffin- embedded

GI; gastrointestinal

NGS; Next Generation Sequencing

TMB; Tumor Mutation Burden

Declarations

Ethics statement

This study was approved by Osaka City University Hospital Certified Review Board (Permission number: CRB5180003) and carried out according to the guidelines of the committee. Written informed consent was obtained from all individual participants included in the study. And this study has been conducted according to the principles of the declaration of Helsinki.

Consent for publication

Consent for publication was obtained from all individual participants included in the study.

*A*vailability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

There are not any financial or other interests with regard to the submitted manuscript that might be construed as a conflict of interest.

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Authors` contributions

SN contributed to study conception and design, acquisition of data, analysis and interpretation of data, drafting manuscript. MY contributed to study conception and design, acquisition of data, analysis and interpretation of data, drafting manuscript. Drafting and revisiting manuscript, and interpretation of the results were performed by AS, SK, ST, KK, MY, TS, HK, TK, MK, KK, HN, SL, KM, TT, SK, MO.

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Tables

Table 1: **Patient demographics in clinical sequencing**			
Variables		Number of patients	(%)
Age	Mean [range] years old	69 [60-75]†	
Sex			
	Female	9	(35)
	Male	17	(65)
Type of Panel testing			
	OncoGuide™ NCC Oncopanel	4	(15)
	FoundationOne® CDx	1	(4)
	Oncomine™ Target System	9	(35)
	ION Ampliseq™ hotspot panel v2	16	(62)
Tumor type			
	Bile duct	4	(15)
	Bone	1	(4)
	Colorectal	1	(4)
	Esophagus	1	(4)
	Lung	2	(8)
	Ovary	2	(8)
	Pancreas	5	(19)
	Primary unknown	2	(8)
	Stomach	7	(27)
	Urinary Tract	1	(4)
Specimen lesion			
	Primary lesion	14	(54)
	Metastasis lesion	8	(31)
	Unknown	4	(15)
Time from extraction of specimen to sequencing			

	3 years <	6	(23)
	≤3 years	20	(77)
Time from consent to result	Mean [range] Days	43[32-49] †	
† Values are median [interquartile range]			

Table 2: 8 candidates for molecular-targeted therapy based on their actionable gene alterations

Age	Sex	Tumor type	Gene	Genetic Alteration	Drug	Received target Therapy
79	F	Stomach	*ERBB2*	Amplification	Approved Drug	YES (standard therapy)
84	F	Stomach	*ERBB2*	Amplification	Approved Drug	YES (standard therapy)
75	M	Stomach	*ERBB2*	Amplification	Approved Drug	YES (standard therapy)
67	F	Bile Duct	*ERBB2*	Missense (V777L)	Off-label Use	No (refusal of off-label use)
68	F	Ovary	*ERBB2*	Amplification	Investigational Drug	No (poor performance status, PS2*)
72	M	Bile Duct	*ERBB2*	Amplification	Investigational Drug	No (poor performance status, PS2*)
51	M	Lung	*CDK4*	Amplification	Investigational Drug	Unknown
61	F	Primary Unknown Site	*PIK3CA*	Missense (E777K)	Investigational Drug	Unknown

* PS means the scale of performance status developed by the Eastern Cooperative Oncology Group.

Figures

Fig.1

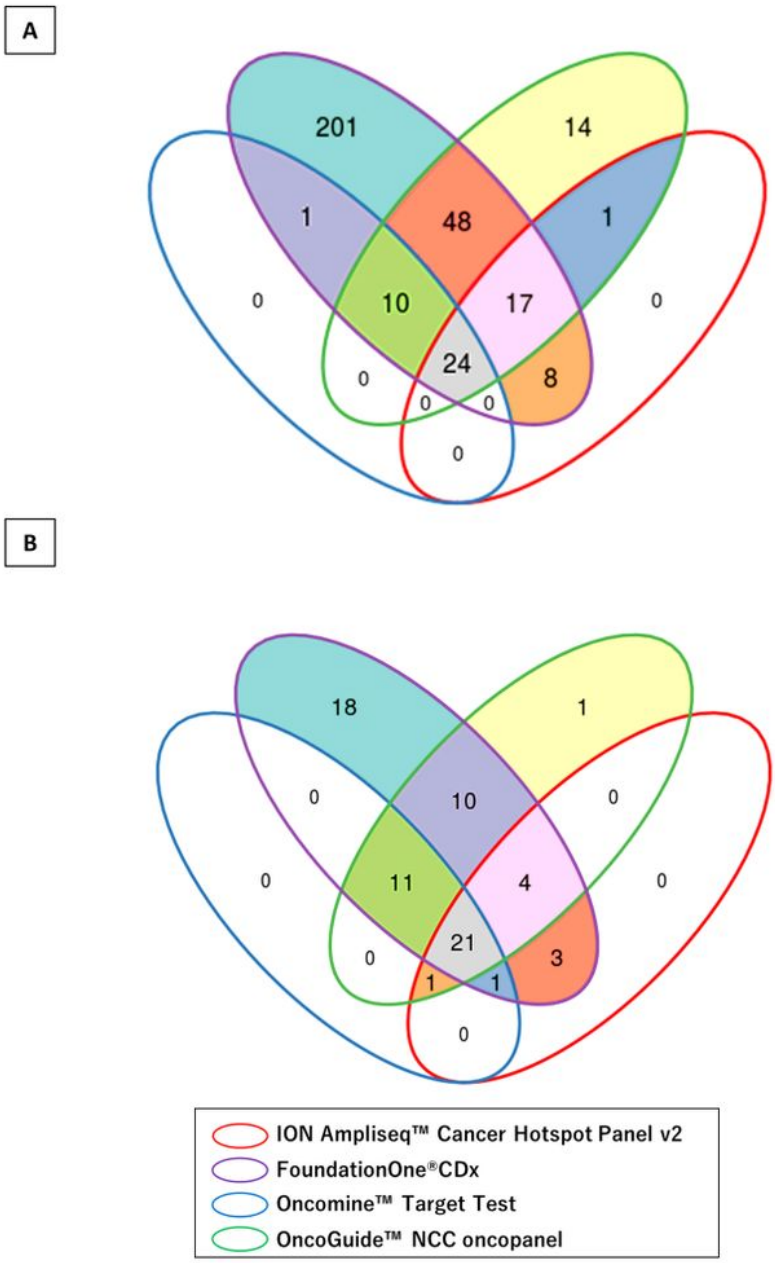

Figure 1

Venn diagram of gene lists overlap by four types of panel testing. A, The Venn diagram of the distribution of 324 genes targeted for all exons or hotspot regions in four panels. B, The Venn diagram of the distribution of druggable genes listed in four panels.

Fig.2

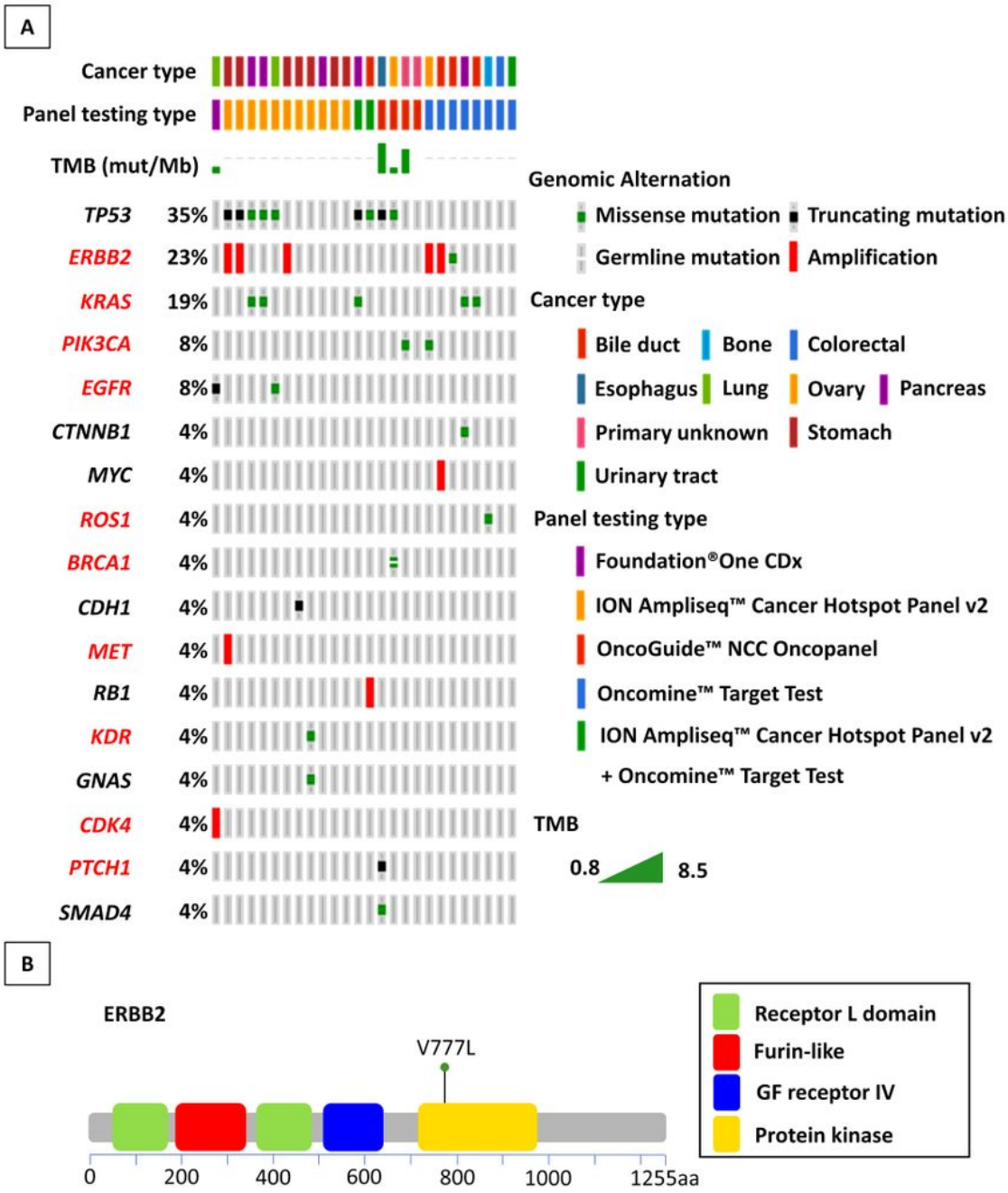

Figure 2

A, Pathogenic gene alterations in the 26 patients enrolled in clinical sequencing. A total of 17 mutated genes that harbored a point mutation or copy number variation were detected among the 26 patients. Genome signatures such as the TMB are also described in the list. Bold red gene symbols indicate druggable genes for FDA-approved targeted therapies. B, Mutations Map on a liner protein of ERBB2 and

its domains (lollipop plots). Missense mutation, a mutation in codon 777 (Valine → Leucine), was located on the protein kinase position in the patient with cholangiocarcinoma.

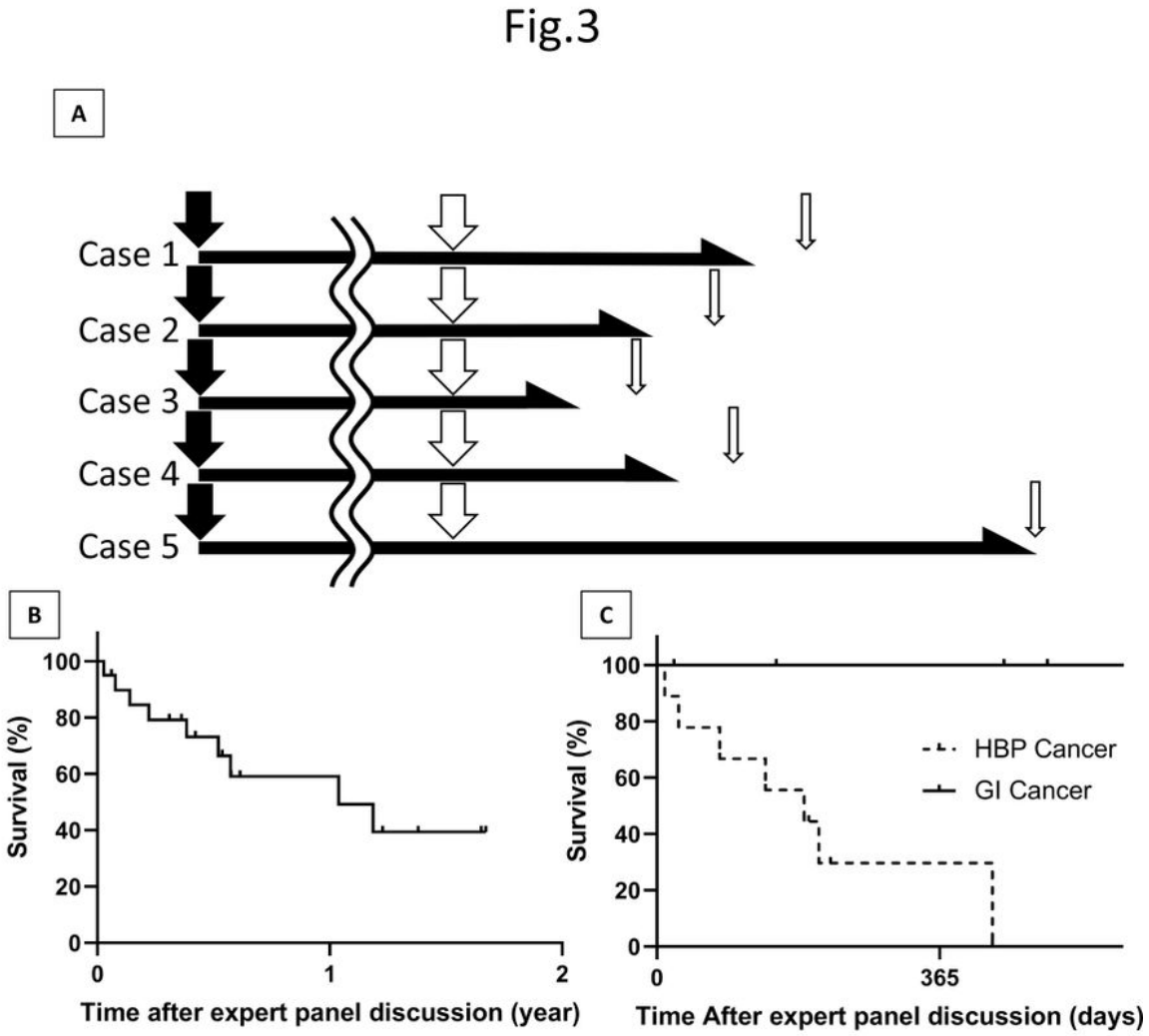

Figure 3

A, Time series from surgery to the result of the panel testing. Thick black arrow: The date of surgery. Thin black arrow: The individual survival date. Thick white arrow: The date of consent for testing. Thin white arrow: The date the result is returned to the clinician. B, Survival curve of post-panel testing cases in 21

patients. Most of the patients died within 1 year after the panel testing. C, Survival curves of the patients with GI cancer and those with pancreatobiliary cancer. The 1-year survival rates of the patients with GI cancer and pancreatobiliary cancer were 100% and 30%, respectively ($p<0.01$). PB means pancreatobiliary.

Supplementary Files

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