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Feasibility and utility of a panel testing for 114 cancer-associated genes in a clinical setting: A hospital-based study

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Next-generation sequencing (NGS) of tumor tissue (ie, clinical sequencing) can guide clinical management by providing information about actionable gene aberrations that have diagnostic and therapeutic significance. Here, we undertook a hospital-based prospective study (TOP-GEAR project, 2nd stage) to investigate the feasibility and utility of NGS-based analysis of 114 cancer-associated genes (the NCC Oncopanel test). We examined 230 cases (comprising more than 30 tumor types) of advanced solid tumors, all of which were matched with nontumor samples. Gene profiling data

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were obtained for 187 cases (81.3%), 111 (59.4%) of which harbored actionable gene aberrations according to the Clinical Practice Guidelines for Next Generation Sequencing in Cancer Diagnosis and Treatment (Edition 1.0) issued by 3 major Japanese cancer-related societies. Twenty-five (13.3%) cases have since received molecular-targeted therapy according to their gene aberrations. These results indicate the utility of tumor-profiling multiplex gene panel testing in a clinical setting in Japan. This study is registered with UMIN Clinical Trials Registry (UMIN 000011141).

KEYWORDS
actionable gene aberration, clinical sequencing, gene panel test, insurance reimbursement, NCC Oncopanel

1 | INTRODUCTION

In a clinical setting, massively parallel next-generation sequencing (NGS) has enabled simultaneous examination of more than 100 genes to detect "actionable" mutations that help oncologists with respect to diagnosis and selection of potential treatment regimens involving molecular-targeted drugs.[1,2] Such systems are referred to as "tumor-profiling multiplex gene panel tests" or more simply "gene panel tests." Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories in the USA have implemented a variety of NGS-based gene panel tests. For example, scientists at the Memorial Sloan Kettering Cancer Center implemented the MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) test to examine 348 genes and reported that 37% of 10 000 investigated patients harbored at least 1 actionable mutation and that 11% of the first 5009 patients who received an MSK-IMPACT test were subsequently enrolled in genomically matched clinical trials.[3] Foundation Medicine (Cambridge, MA, USA) developed the FoundationOne CDx test to examine 324 genes and the tumor mutational burden (TMB),[4] which is an emerging biomarker of sensitivity to immune checkpoint blockade therapy.[5,6] These 2 tests have now been approved by the FDA, further facilitating cancer genome medicine in the USA by promoting insurance reimbursement.[7]

Gene panel tests have not yet been implemented in routine oncological practice in Japan; ie, they have not been reimbursed by the national insurance system run by the Japanese Ministry of Health, Labor, and Welfare (MHLW).[8] However, several academic institutions have examined the feasibility and utility of gene panel tests,[1,2] and 3 major Japanese cancer-related societies (the Japanese Society of Medical Oncology, the Japanese Society of Clinical Oncology, and the Japanese Cancer Association) have issued consensus clinical practice guidance for NGS-based cancer tests (the Consensus Clinical Practice Guidelines for Next Generation Sequencing in Cancer Diagnosis and Treatment [Edition 1.0];[9] http://www.jsmo.or.jp/about/kanko.html#guideline) . Therefore, it is likely that implementation of gene panel tests in Japan will happen soon.

We have been undertaking a prospective hospital-based cohort study to investigate the feasibility and utility of NGS-based analysis of 114 cancer-associated genes using the National Cancer Center (NCC) Oncopanel test (Table 1). Many different cases of advanced solid tumors were analyzed at a quality-assured laboratory at the NCC Hospital (NCCH; Tokyo, Japan). Detected gene aberrations and their annotations were reported to the treating physicians. This study formed the second stage of the TOP-GEAR project (Trial of Onco-Panel for Gene-profiling to Estimate both Adverse events and Response during cancer treatment; UMIN 000011141). This follows the first[10] stage in which tumor samples were analyzed at the NCC Research Institute.

Here, we summarize the results of the first 230 cases analyzed during the second stage of TOP-GEAR. The results indicate the feasibility and utility of the gene panel test in a clinical oncology setting. From April 2018, the NCC Oncopanel test is being tested by 50 Core and Liaison Hospitals for Cancer Genomic Medicine in Japan (within the Advanced Medical Care B system) to validate its feasibility and utility.[11]

2 | PATIENTS AND METHODS

2.1 | Patient population

Patients aged 16 years or older, diagnosed histopathologically with a solid tumor, and who would finish or had finished standard chemotherapy were enrolled in the TOP-GEAR study (n = 248). Next, the availability of archival formalin-fixed paraffin-embedded (FFPE) tumor tissues with tumor cell content 10% or higher was checked for each case (pathologists estimated tumor cell content by counting the nuclei of tumor and nontumor cells within each tissue); appropriate cases were analyzed in the study to address the feasibility and utility of the NCC Oncopanel test (n = 230). The study was approved by the NCC Institutional Review Board, and all patients provided written informed consent for the use of genomic and clinical data for research purposes. When consent was obtained, patients were also asked whether they will be reported for the results of somatic and germline gene alteration, respectively, from treating physicians. Among the 230 analyzed cases, 228 (99.1%) and 219 cases (95.2%) gave consent to receive results of somatic and germline tests, respectively; therefore, results were returned to patients accordingly.

TABLE 1 Genes examined by the NCC Oncopanel test (n = 114)

Mutations and copy number alterations for all exons					Fusions
ABL1	*CRKL*	*IDH2*	*NF1*	*RAC2*	*ALK*
ACTN4	*CREBBP*	*IGF1R*	*NFE2L2/Nrf2*	*RAD51C*	*AKT2*
AKT1	*CTNNB1*	*IGF2*	*NOTCH1*	*RAF1/CRAF*	*BRAF*
AKT2	*CUL3*	*IL7R*	*NOTCH2*	*RB1*	*ERBB4*
AKT3	*DDR2*	*JAK1*	*NOTCH3*	*RET*	*FGFR2*
ALK	*EGFR*	*JAK2*	*NRAS*	*RHOA*	*FGFR3*
APC	*ENO1*	*JAK3*	*NRG1*	*ROS1*	*NRG1*
ARAF	*EP300*	*KDM6A/UTX*	*NTRK1*	*SETBP1*	*NTRK1*
ARID1A	*ERBB2/HER2*	*KEAP1*	*NTRK2*	*SETD2*	*NTRK2*
ARID2	*ERBB3*	*KIT*	*NTRK3*	*SMAD4*	*PDGFRA*
ATM	*ERBB4*	*KRAS*	*NT5C2*	*SMARCA4/BRG1*	*RET*
AXIN1	*ESR1/ER*	*MAP2K1/MEK1*	*PALB2*	*SMARCB1*	*ROS1*
AXL	*EZH2*	*MAP2K2/MEK2*	*PBRM1*	*SMO*	
BAP1	*FBXW7*	*MAP2K4*	*PDGFRA*	*STAT3*	
BARD1	*FGFR1*	*MAP3K1*	*PDGFRB*	*STK11/LKB1*	
BCL2L11/BIM	*FGFR2*	*MAP3K4*	*PIK3CA*	*TP53*	
BRAF	*FGFR3*	*MDM2*	*PIK3R1*	*TSC1*	
BRCA1	*FGFR4*	*MDM4*	*PIK3R2*	*VHL*	
BRCA2	*FLT3*	*MET*	*POLD1*		
CCND1	*GNA11*	*MLH1*	*POLE*		
CD274/PD-L1	*GNAQ*	*MTOR*	*PRKCI*		
CDK4	*GNAS*	*MSH2*	*PTCH1*		
CDKN2A	*HRAS*	*MYC*	*PTEN*		
CHEK2	*IDH1*	*MYCN*	*RAC1*		

2.2 | Next-generation sequencing-based multiplex gene assay (NCC Oncopanel test)

The NCC Oncopanel test is a hybridization capture-based NGS assay designed to examine mutations, amplifications, and homozygous deletions of the entire coding region of 114 genes of clinical or preclinical relevance, along with rearrangements of 12 oncogenes included in the panel (Table 1). For the analysis, 5 10-μm sections or 10 4-5-μm sections were prepared from FFPE tumor tissues. Peripheral blood (5 mL) collected from the same patients was used as a control to allow discrimination of somatic and germline mutations. Genomic DNA was extracted from tumor tissues and peripheral blood cells using a QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany) and a Maxwell RSC Blood DNA kit (Promega, Fitchburg, WI, USA), respectively. The extracted DNA was quantified using a Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Quantitative PCR analysis of the *RPPH1* (*RNase P*) locus was carried out, and the ratio of PCR-amplifiable DNA to total double-stranded DNA was used to indicate DNA quality. When this value (*Q*-value) was greater than or equal to 0.1, the DNA was sent for sequencing.[10] The *Q*-value reflects the fraction of PCR-active DNA molecules in each sample. Our previous clinical sequencing study[10] verified empirically that, in the cases with high-quality DNA, it is possible to reduce the amount of input DNA to 50 ng. However, in cases with poor-quality DNA, use of large amounts of DNA (more than 800 ng) did not efficiently improve the results; this was likely due to saturation of the DNA capture-based system. Therefore, the threshold for DNA quantity was set according to DNA quality: the threshold was 50 ng or more for samples with a *Q*-value less than or equal to 0.8; 100 ng or more for samples with a *Q*-value greater than or equal to 0.4 and less than 0.8; 200 ng or more for samples with a *Q*-value greater than or equal to 0.2 and less than 0.4; and 400 ng or more for samples with a *Q*-value greater than or equal to 0.1 and less than 0.2. Sequencing libraries were prepared from 50-800 ng DNA (depending on the *Q*-value) using the SureSelect XT reagent (Agilent Technologies, Santa Clara, CA, USA) and a KAPA Hyper Prep kit (KAPA Biosystems, Wilmington, MA, USA) and then analyzed on the Illumina MiSeq

or NextSeq platform (Illumina, San Diego, CA, USA) with 150 bp paired-end reads.

2.3 | Bioinformatics analysis

Mapping of NGS reads to the human reference genome was carried out using the Burrows-Wheeler Aligner[12] and the Burrows-Wheeler Aligner-Smith-Waterman algorithm[13] after removal of adapter sequences using a Cutadapt program.[14] Thresholds for mean read depth of coverage for gene aberration calls were set according to tumor cell content, as defined by pathological examination; the threshold was 200 for samples with more than 50% cellularity, 250 for samples with 20%-50% cellularity, and 500 for samples with less than 20% cellularity. For samples with a mean read depth of coverage above these thresholds, somatic mutations (single nucleotide variants and short insertions and deletions (indels)), gene amplifications, homozygous deletions, and gene fusions were detected using the cisCall program (version 7.1.5).[15] Mutations with 5% or more variant allele frequencies and amplifications with more than 4-fold copy number increases were defined as positive. Genes with less than 0.5-fold copy number decreases were considered as homozygous deletion candidates and judged by manual inspection using the Integrative Genomics Viewer (IGV).[16] Data from the refGene (20150219), ensGene (20140406), and COSMIC (version 71)[17] databases were used to annotate each gene aberration. The level of cross-individual contamination in tumor tissues was estimated by the ContEst program,[18] as well as by manual inspection of single nucleotide polymorphism sites using IGV.[16] Tumor samples thought to show cross-individual contamination were removed from the study.

Germline mutations in 13 genes responsible for hereditary cancers (*APC*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *PTEN*, *RB1*, *RET*, *SMAD4*, *STK11*, *TP53*, *TSC1*, and *VHL*), for which the American College of Medical Genetics and Genomics (ACMG) recommends reporting of incidental or secondary findings,[19] were detected by the GATK program[20] (version 3) using NGS data obtained from peripheral blood DNA. Single nucleotide polymorphisms were removed if they showed a threshold of 0.01 or more allele frequency in any of the following databases: 1000 Genomes (1 kgp, 201204) (http://www.1000genomes.org); the NHLBI GO Exome Sequencing Project (ESP6500) (http://evs.gs.washington.edu/EVS/); the Human Genetic Variation Database (HGVD, 20131010) (http://www.genome.med.kyoto-u.ac.jp/SnpDB); and the Integrative Japanese Genome Variation Database (iJGVD, 20151218) (https://ijgvd.megabank.tohoku.ac.jp/).[21] All somatic and germline aberrations judged to be positive were validated by manual inspection on IGV.[16]

2.4 | Definition of actionable

Actionable gene aberrations for drug selection were those predicted to confer sensitivity/resistance to either an approved targeted agent or an experimental targeted agent currently in clinical trials. Evidence levels were added to each gene aberration according to Clinical Practice Guidance for Next Generation Sequencing in Cancer Diagnosis and Treatment (issued by the Japanese Society of Medical Oncology, Japan Society of Clinical Oncology, and the Japanese Cancer Association).[9] The guidance cites the following levels of evidence for each gene aberration: level 1A, a Pharmaceuticals and Medical Devices Agency (PMDA)-approved biomarker for the tumor type; 1B, an FDA-approved biomarker for the tumor type (not approved by the PMDA) or a biomarker verified by a prospective molecularly driven clinical trial; 2A, a biomarker identified by subgroup analysis in a prospective clinical trial; 2B, an approved biomarker for a different tumor type or a biomarker with evidence supporting its clinical utility; 3A, a biomarker with evidence of proof-of-concept in at least one case report; 3B, a biomarker with evidence obtained from in vitro/in vivo experiments; and 4, other gene mutations in cancer. In the present study, gene aberrations with evidence levels 1A-3A were judged as actionable for drug selection. Evidence levels 1A-3A correspond to evidence levels A-C listed in guidance documents published by the Association for Molecular Pathology, ACMG, the American Society of Clinical Oncology, and the College of American Pathologists.[22]

In addition, actionable aberrations for diagnosis and prognosis were also considered according to Japanese guidelines. As for germline mutations in the above-mentioned 13 genes, truncating mutations and mutations deposited as "pathogenic" in the ClinVar database[23] (20150629) (https://www.ncbi.nlm.nih.gov/clinvar/) were judged as deleterious and, therefore, significant.

2.5 | Tumor mutational burden

Tumor mutational burden was defined as the number of somatic, coding, base substitutions, and indel mutations per megabase of genome examined (ie, the total number of mutations divided by 1.38 Mb [the genome size of target regions covered by the NCC Oncopanel assay]). All base substitutions and indels in the coding region of targeted genes, including synonymous alterations, were counted. DNAs extracted from tumor and nontumor tissues of lung, breast, and ovarian cancers (n = 20), whose TMBs were measured previously by whole exome sequencing,[24-26] were subjected to NCC Oncopanel analysis to verify their utility for estimating TMB.

2.6 | Molecular tumor board (expert panel)

Actionable gene aberrations and possible treatments were discussed at the molecular tumor board meeting by a multidisciplinary team at NCCH, called the "expert panel," which met twice per month. The board included medical oncologists, pediatric oncologists, pathologists, genome researchers, bioinformaticians, and genetic counselors. Board members discussed genetically informed treatment options and other issues such as authorization of pathological diagnoses and interpretation of somatic/germline variants. The report

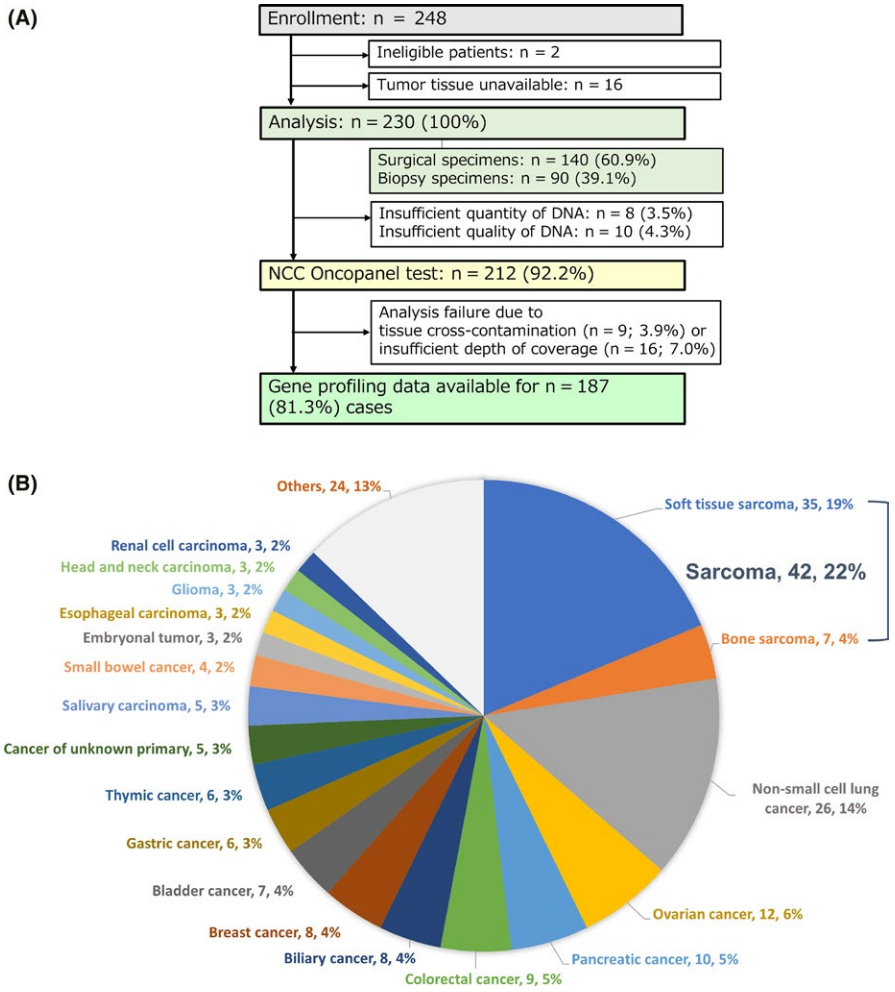

FIGURE 1 Feasibility of the NCC Oncopanel test for 114 cancer-associated genes in a cohort of Japanese patients with solid tumors who would complete or had completed standard chemotherapy. A, Success rate. Among the 230 cases analyzed, 18 were excluded due to insufficient quantity or quality of DNAs. Then 212 cases were subjected next-generation sequencing analysis and gene profiling data were obtained for 187 cases (success rate, 81.3%). B, Tumor types of the 187 cases for which gene profiling data were available

was returned to the treating physicians, who explained the details to their patients.

3 | RESULTS

3.1 | Feasibility of testing

Between May 2016 and May 2017, 248 patients were enrolled in TOP-GEAR and the availability of appropriate tumor tissues was checked (Figure 1A, Table S1). Eighteen cases were excluded due to lack of sufficient tumor tissue sample (n = 16) or diagnosis of a benign tumor upon pathological re-review (n = 2). Thus, 230 cases were analyzed to test the feasibility and utility of the NCC Oncopanel test. The 230 cases comprised 140 surgical (60.9%) and 90 (39.1%) biopsy specimens (Figure 1A). Eighteen of these were removed due to low DNA yield (n = 8) or quality (n = 10), measured according to the criteria described above[10] (Figure 1A). Therefore, 212 (92.2%) of the 230 cases were subjected to NGS analysis. After analysis, 9 (3.9%) cases were judged as having tissue cross-contamination. ContEst program analysis revealed that 2 of these had >5% tissue cross-contamination; the remaining 7 cases were inferred by IGV inspection (Figure S1). In addition, the mean read depth in another 16 (7.0%) cases was below the set thresholds. Thus, gene profiling data were obtained for 187 (88.2%) of 212 patients (Table S1), making the success rate 81.3%. In these samples, medians for the mean read depth and allele frequencies of detected mutations were 626 and 27.2%, respectively (Figure S2). The average turnaround time, defined as the interval between the date of sample arrival and the date of the molecular tumor board meeting, was 37 days (median, 32 days; range, 9-84 days).

The 187 cases comprised more than 30 types of tumor. The major tumor types are shown in Figure 1B and listed in Table S2. Sarcoma was the most common tumor type, accounting for 22.5% (n = 42) of cases, followed by non-small-cell lung cancer (n = 26, 13.9%), ovarian cancer (n = 12, 6.4%), and pancreatic cancer (n = 10, 5.3%). Notably, 97 cases (51.9%) were rare cancers (defined as those with an incidence rate of fewer than 6 per 100 000 persons per year) (Table S2).

3.2 | Percentage of cases harboring actionable gene aberrations

At least 1 genetic aberration was detected in 156 of the 187 cases for which gene profiling data were obtained (83.4%) (Figure 2A, detailed data in Table S3). Frequently altered genes were *TP53* (40.1%, 75/187), *KRAS* (15.5%, 29/187), *PIK3CA* (11.8%, 22/187), and *APC* (5.3%, 10/187). Notably, *EGFR* mutations were detected in 6 lung cancer cases that received companion diagnostics for *EGFR* mutations and 3 of them were judged to be negative. All of these *EGFR* mutations detected by the NCC Oncopanel test were rare variants not detected by existing companion diagnostics. The NCC Oncopanel test also detected an Asian-specific polymorphism in *BCL2L11/BIM*, which is thought to be associated with resistance of lung cancer to epidermal growth factor receptor tyrosine kinase inhibitors.[27] The deletion allele conferring resistance was observed in 24 (12.8%) cases, which is consistent with the percentage in the Asian population.[28]

According to evidence levels 1A-3A, 109 cases (58.2%) harbored at least 1 actionable gene aberration (Figure 2A). The 156 cases were ranked according to the strongest (maximum) evidence as follows: 14 (7.4%) cases harbored level 1A aberrations; 9 (4.8%) harbored level 1B aberrations; 9 (4.8%) harbored level 2A aberrations; 33 (17.6%) harbored level 2B aberrations; and 44 (23.5%) harbored level 3A aberrations. The other 47 cases harbored level 3B aberrations (n = 25; 13.3%) or level 4 aberrations (n = 22; 11.8%).

Next, we examined the percentage of cases with each tumor type (Figure 2B). When the 187 cases were categorized as carcinoma

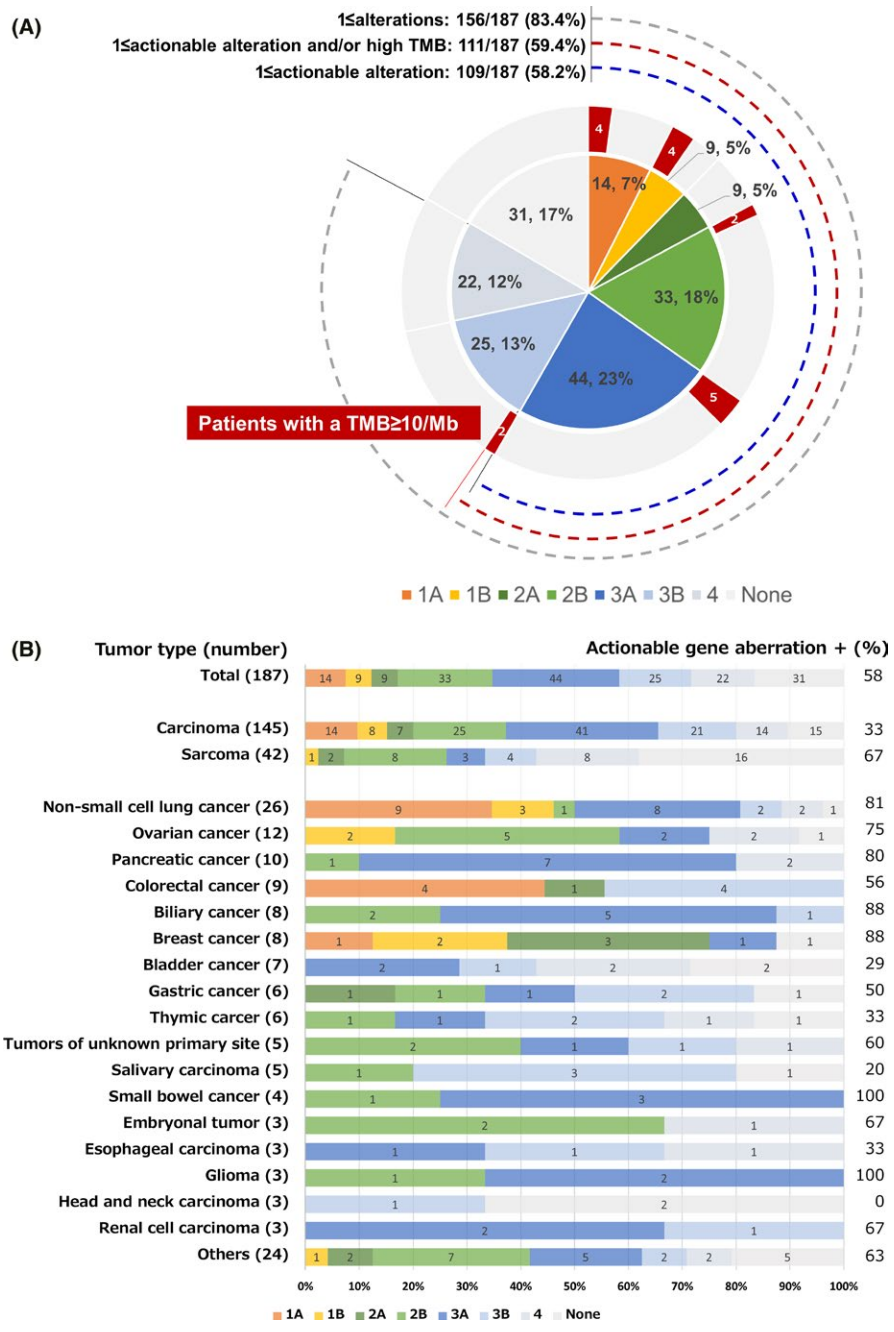

FIGURE 2 Utility of the NCC Oncopanel test in a cohort of Japanese patients with solid tumors who would complete or had completed standard chemotherapy. A, Gene aberration detected in 187 cases. Cases are categorized according to maximum evidence for drug selection. The percentage of cases with actionable gene aberrations was calculated taking (or not) into account a high tumor mutational burden (TMB; defined as ≥10 mutations/Mb). B, Percentage of cases with actionable gene aberrations according to tumor type. The number of cases is presented on the graph according to maximum evidence for drug selection

or sarcoma, we found that the percentage of carcinoma cases with actionable gene aberrations was greater than that of sarcoma cases (95/145, 65.5% vs 14/42, 33.3%, respectively). The difference was statistically significant ($P = 2.0 \times 10^{-4}$; χ^2 test). In agreement with previous genome-wide sequencing studies,[29-31] we frequently identified actionable aberrations (>80%) in cases of non-small-cell lung cancer, biliary cancer, and breast cancer.

3.3 | Fraction of cases with a high TMB

To examine the ability of the NCC Oncopanel test to evaluate the TMB, we used the NCC Oncopanel test to examine 20 additional cancer cases in which the TMB had been measured in previous studies by whole exome sequencing.[24-26] We then compared the TMB values generated by the 2 assays. The TMB values (the number of somatic mutations per megabase after subtracting germline variations detected in the corresponding peripheral blood DNA) generated by the NCC Oncopanel test showed a strong correlation ($R^2 = 0.98$) with those by whole exome sequencing, indicating that the NCC Oncopanel test is more appropriate than other gene panel tests[4] as a tool for evaluating the TMB (Figure 3). Among the 187 cases for which gene profiling data were available, 17 (9.1%) showed high TMB values according to a recently proposed threshold (10 or more mutations/Mb).[32-34] These 17 cases included melanoma, non-small-cell lung cancer, and colorectal cancer, and are thus consistent with a recent report of tumor types with a high TMB[34] (Table S4). In particular, 8 cases with a TMB value of more than 20 mutations/Mb had endogenous or exogenous risk factors linked to a high TMB. Mismatch repair deficiency, an endogenous factor causing a high TMB,[34] occurred in 2 of 8 cases that harbored loss of function mutations (a somatic P415 fs mutation and a germline Q341* mutation) in the *MSH2* gene. Temozolomide, a mutagenic alkylate agent,[35] was used to treat 1 case of glioma and the tumor sample obtained after treatment was subjected to the NCC Oncopanel test. The remaining 4 patients had been considered exposed to exogenous mutagenic factors (ie, UV light and cigarette smoke).[36] A prospective clinical trial study showed that a high TMB phenotype (defined by 10 or more mutations/Mb) is a biomarker for responses to immune checkpoint blockade therapy;[32] therefore, a high TMB was defined as evidence level 1B for drug selection. Among the 17 high TMB cases, 2 had been judged as negative for original actionable gene aberrations. Thus, taking high TMB into account meant that the fraction of cases with actionable gene aberrations was 59.4% (111/187).

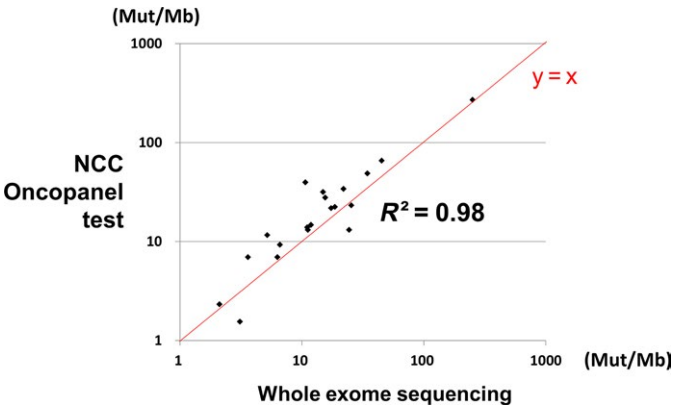

FIGURE 3 Assessment of tumor mutation burden by the NCC Oncopanel test in a cohort of Japanese patients with solid tumors who had completed standard chemotherapy. Comparison of tumor mutation burden measured by whole exome sequencing vs that by NCC Oncopanel testing. Tumor mutation burden (mutations [Mut]/Mb) was measured in 20 samples assessed previously by whole exome sequencing, and the results were compared. The NCC Oncopanel test assessed matched tumor and nontumor samples. The line $y = x$ is plotted in red

Evidence levels 1A-3A in the Clinical Practice Guidelines for Next Generation Sequencing in Cancer Diagnosis and Treatment[9] correspond to evidence levels A-C in the guidelines published by the Association for Molecular Pathology, ACMG, American Society of Clinical Oncology, and College of American Pathologists.[22] Therefore, the same percentage (ie, 59.4%) of cases was also judged as positive for aberrations based on evidence levels A-C; ie, they had clinically significant gene aberrations.

3.4 | Drug treatment according to actionable gene aberrations

Drug treatment according to actionable gene aberrations detected by the NCC Oncopanel test was examined as of May 31, 2018, ie, approximately 1 year after enrollment of the last case. In total, 25 (13.4%) cases received molecular-targeted drugs in accordance with their identified gene aberrations (Table 2). A number of cases (n = 19, 76.0%) received therapy with drugs that were not approved for their particular tumor. Among these, 15 (60.0%) received investigational drugs after enrollment into clinical trials matched to their gene aberrations, and the remaining 4 (16.0%) received kinase inhibitory drugs approved for treatment of different tumor types in Japan (ie, off-label use). The remaining 6 (24%) cases were prescribed PMDA-approved molecular-targeted drugs. By contrast, 86 cases with actionable gene aberrations (including a high TMB) did not receive genomically matched therapies. Among these, 9 cases were dead or had poor performance status at the time that the results were returned. For the majority of the remaining cases (n = 77), there were no available/accessible genomically matched clinical trials or drugs.

3.5 | Diagnosis and prognosis based on actionable gene aberrations

The results of gene profiling using the NCC Oncopanel test were also used for diagnosis and prognosis. Germline mutations causing hereditary cancers were identified in 6/187 (3.2%) patients (Table 3). All were defined at evidence level 1 for diagnosis. The diagnoses were as follows: hereditary breast and ovarian cancer based on deleterious *BRCA1* or *BRCA2* mutations (n = 4), Lynch syndrome based on a deleterious *MSH2* mutation (n = 1), and Li-Fraumeni syndrome based on a deleterious *TP53* mutation (n = 1). Subsequently, 3 patients received genetic counseling from Genetic Medicine and Services at NCCH. Two

TABLE 2 Cancer cases that received molecular-targeted therapy according to their actionable gene aberrations (n = 25)

No.	TOP-GEAR ID	Tumor type	Age (years)	Gender	AYA	Rare cancer	Actionable gene aberration	Drug	Drug type
1	5022	Ovarian cancer	37	F	Y	Y	*KRAS* mutation	Pan-RAF inhibitor	Investigational drug
2	5025	Colorectal cancer	69	M	n	n	*KRAS* mutation	Pan-RAF inhibitor	Investigational drug
3	5010	Colorectal cancer	44	M	n	n	*BRAF* mutation	Pan-RAF inhibitor	Investigational drug
4	5058	Pancreatic cancer	47	M	n	n	*KRAS* mutation	Pan-RAF inhibitor	Investigational drug
5	5004	Pancreatic cancer	58	F	n	n	*KRAS* mutation	ERK inhibitor	Investigational drug
6	5054	Esophageal carcinoma	61	M	n	n	*FGFR2* amplification	FGFR2 inhibitor	Investigational drug
7	5017	Soft tissue sarcoma (Malignant cardiac tumor)	28	F	Y	Y	*MDM2* amplification	HDM2 inhibitor	Investigational drug
8	5130	Soft tissue sarcoma (Liposarcoma)	54	F	n	Y	*MDM2* amplification	HDM2 inhibitor	Investigational drug
9	5076	Non-small-cell lung cancer	67	M	n	n	Tumor mutation burden high	Immunocheckpoint inhibitor	Investigational drug
10	5160	Non-small-cell lung cancer	42	M	n	n	Tumor mutation burden high	Immunocheckpoint inhibitor	Investigational drug
11	5078	Non-small-cell lung cancer	67	F	n	n	*CCDC6-RET* fusion	Alectinib	Investigational drug
12	5164	Breast cancer	35	F	Y	n	*HER2* amplification	HER2 ADC	Investigational drug
13	5215	Biliary cancer	68	M	n	n	*HER2* amplification	HER2 ADC	Investigational drug
14	5227	Tumors of unknown primary site	65	F	n	Y	*PIK3CA* mutation	TORC1/2 inhibitor	Investigational drug
15	5208	Apocrine adenocarcinoma	70	M	n	Y	*FGFR2-CLIP1* fusion	FGFR inhibitor	Investigational drug
16	5060	Inflammatory myofibroblastic tumor	51	M	n	Y	*CLTC-ALK* fusion	Alectinib	Off-label use
17	5219	Mastocytoma	39	M	Y	Y	*KIT* mutation	Imatinib	Off-label use
18	5003	Non-small-cell lung cancer	36	M	Y	n	*CCDC6-RET* fusion	Lenvatinib	Off-label use
19	5077	Histiocytic sarcoma	18	M	Y	Y	*MAP2K1* mutation	Trametinib	Off-label use
20	5098	Non-small-cell lung cancer	46	M	n	n	*EML4-ALK* fusion	Alectinib	Approved drug
21	5041	Non-small-cell lung cancer	51	F	n	n	*EGFR* mutation (p.V769_D770insGQR)	Afatinib	Approved drug
22	5162	Non-small-cell lung cancer	54	F	n	n	*EGFR* mutation (p.E746_T751delinsI)	Afatinib	Approved drug
23	5109	Non-small-cell lung cancer	64	F	n	n	*EGFR* mutation (p.S752_I759del)	Gefitinib	Approved drug
24	5115	Non-small-cell lung cancer	35	M	Y	n	*CD74-ROS1* fusion	Crizotinib	Approved drug
25	5071	Melanoma	60	M	n	Y	Tumor mutation burden high	Nivolumab	Approved drug

ADC, antibody-drug conjugate; AYA, adolescent and young adult (15-39 years); F, female; FGFR, fibroblast growth factor receptor; HDM2, human double minute 2 homolog; HER2, human epidermal growth factor receptor 2; M, male; TOP-GEAR, Trial of Onco-Panel for Gene-profiling to Estimate both Adverse events and Response during cancer treatment; TORC1/2, target of rapamycin complex 1/2; Y, yes; n, no.

TABLE 3 Germline mutations detected in 6 patients with solid tumor who had undergone standard chemotherapy

No.	TOP-GEAR ID	Tumor type	Age	Gender	Gene	Germline mutation (nucleotide change, effect)	ClinVar	Genetic counseling
1	5018	Cardiac angiosarcoma	38	M	*MSH2*	c.C1120T, p.Q374X	RCV000076043	Done
2	5126	Ovarian cancer	48	F	*BRCA1*	c.T188A, p.L63X	RCV000077499	Done
3	5110	Ovarian cancer	64	F	*BRCA1*	c.4338_4339insAGAA, p.Q1447 fs*16	–	Not yet
4	5158	Breast cancer	36	F	*BRCA2*	c.5574_5577del, p.I1859 fs	RCV000168442	Not yet
5	5019	Breast cancer	61	F	*BRCA2*	c.517-2A>T, splicing	–	Done
6	5161	Thymic cancer	46	F	*TP53*	c.833_834insT, p.P278 fs	–	Not yet

–, not registered; F, female; M, male; TOP-GEAR, Trial of Onco-Panel for Gene-profiling to Estimate both Adverse events and Response during cancer treatment.

dedifferentiated liposarcomas showed amplification of *MDM2*, a biomarker (evidence level 2) for diagnosis of this tumor type. Therefore, these results supported pathological diagnosis of these tumors. In addition, a hotspot *IDH1* mutation (R132H) was detected in 2 glioma cases. This is a biomarker (evidence level 2) for predicting a good prognosis.

4 | DISCUSSION

Here, we present the results of a prospective study designed to analyze 114 cancer-related genes using the NCC Oncopanel test. The test, including bioinformatics analysis, was carried out in a quality-assured laboratory at NCCH. Among the 230 analyzed cases, gene profiling data were obtained for 187 (81.3%). Corresponding peripheral blood DNAs were used to accurately address somatic and germline mutations, as well as TMBs. The 187 samples comprised surgical or biopsy FFPE specimens used in daily clinics and covered more than 30 cancer types, including rare cancers. Approximately half of the specimens (n = 112, 48.7%) were obtained from hospitals in Japan other than NCCH. The success rate was similar to that reported for other gene panel tests undertaken at CLIA-assured laboratories (80%-85%)[2,37] in the USA.

At least 1 genetic aberration was detected in 83.4% of analyzed cases, and 59.4% had actionable gene aberrations, including a high TMB. This result is also comparable with those reported by prospective studies in the USA that used different gene panel tests; these tests detected actionable gene aberrations in approximately half of cases examined (40%-60%).[3,38] Thus, we conclude that the NCC Oncopanel test is feasible in the clinical setting in Japan. Reasons for test failure included DNA of low quality/quantity and tissue cross-contamination. Tissue cross-contamination was detected in 3.9% of the study samples; this was a major pre-analytical issue as recently discussed.[39] This rate of our study is consistent with a recent report indicating that 3% of cases showed clinically significant (ie, more than 5%) levels of cross-contamination during routine clinical sequencing.[39] In our study, most of the cross-contaminated tissue samples yielded poor quality and/or low yields of DNA (Table S5). Some tumor samples with poor-quality DNA also failed due to low read depth. In fact, DNAs from tumor samples stored for long periods (more than 3 years) often yielded poor-quality DNA; therefore, selecting tumor specimens appropriate for NGS (ie, fresh and large samples) as well as careful laboratory processing is critical for accurate and robust analysis using the NCC Oncopanel test.

The percentage of carcinoma cases with actionable gene aberrations related to drug selection was greater (65.5%) than that of sarcoma cases (33.3%). These percentages for all types of tumor will be increased in future by developing drugs that target currently "undruggable" alterations, such as deleterious mutations in SWI/SNF chromatin regulator genes,[40-42] which are detected in tumors such as sarcoma. In addition, we classified several detected mutations in currently druggable genes as "variants of unknown significance" due to lack of biological and clinical evidence. Annotation of those variants of unknown significance will also increase the percentage of patients with detected gene aberrations linked to molecular-targeted therapy.

Aside from identifying druggable gene aberrations, the gene panel test proved useful for diagnosis (6 hereditary tumors and 2 liposarcomas) and prognosis (2 gliomas). Detection of germline mutations in cancer-predisposing genes provides doctors with valuable information about hereditary cancers. Detection of typical gene aberrations in a few cases facilitated diagnosis or prognosis assessment by treating physicians.

The NCC Oncopanel test led to drug treatment according to actionable gene aberrations in 25 cases (13.4%). These included 7 cases of adolescent and young adult (aged 15-39 years) and rare cancers (Table 2). The prognosis for adolescent and young adult and rare cancers has improved more slowly than that for other groups; therefore, efficient therapeutic regimens for these cancers are needed urgently.[43] Drug treatment according to gene panel test results will facilitate development of drugs by promoting drug repositioning and clinical trials. Unfortunately, at present, identification of actionable gene aberrations related to drug selection does not mean that the patient receives treatment with a therapeutic agent specific for his/her aberration. Indeed, there was a large difference between the percentage of patients with actionable gene aberrations (59.4%) and the percentage that received therapy with a drug targeting that aberration (13.4%). Unfortunately, there were no available/accessible genomically matched clinical trials or drugs for the majority of patients with actionable gene aberrations. A recent prospective cohort study in the USA revealed that only 11% of patients receiving the MSK-IMPACT gene panel test were subsequently enrolled on genomically matched clinical trials.[3] The gaps between the number of patients with actionable mutations and those receiving genomically matched therapy indicate the need to develop drugs targeting new genes covering not only druggable kinase genes but also nonkinase genes such as epigenomic and transcriptional regulator genes, which are often mutated in a variety of tumors.[1,44] Developing drugs that target such currently undruggable molecules will be of great help.

The NCC Oncopanel test has recently been approved by the PMDA in the SAKIGAKE program of the MHLW[45] (OncoGuide NCC Oncopanel System) and will be reimbursed by the national insurance system. After implementation, several challenges will remain. First, the amount of cancer genomic data increases daily; therefore, the significance of gene aberrations with respect to therapy, diagnosis, and prognosis requires continuous re-evaluation. Clinical oncologists and molecular tumor board members must keep up-to-date with information about actionable gene aberrations and investigational drugs. The cancer knowledge database being established by the Center for Cancer Genomics and Advanced Therapeutics at the NCC, Japan, will be a great help (https://www.ncc.go.jp/en/information/2018/0601/index.html). Second, the NCC Oncopanel test analyzes both tumor and nontumor DNA; therefore, germline mutations will be identified. Germline mutations responsible for hereditary disease are present in a small percentage of East Asians.[46] Therefore, appropriate annotation of germline mutations and subsequent genetic counseling, coupled with a total care package, must be undertaken by each hospital. Routine performance of gene panel tests will improve patient experiences in oncology clinics and promote drug development.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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