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Clinical impact of a cancer genomic profiling test using an in-house comprehensive targeted sequencing system

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Abstract

Precision medicine is a promising strategy for cancer treatment. In this study, we developed an in-house clinical sequencing system to perform a comprehensive cancer genomic profiling test as a clinical examination and analyzed the utility of this system. Genomic DNA was extracted from tumor tissues and peripheral blood cells collected from 161 patients with different stages and types of cancer. A comprehensive targeted amplicon exome sequencing for 160 cancer-related genes was performed using next-generation sequencing (NGS). The sequencing data were analyzed using an original bioinformatics pipeline, and multiple cancer-specific gene alterations were identified. The success rate of our test was 99% (160/161), while re-biopsy was required for 24% (39/161) of the cases. Potentially actionable and actionable gene alterations were detected in 91% (145/160) and 46% (73/160) of the patients, respectively. The actionable gene alterations were frequently detected in PIK3CA (9%), ERBB2 (8%), and EGFR (4%). High tumor mutation burden (TMB) (≥10 mut/Mb) was observed in 12% (19/160) of the patients. The secondary findings in germline variants considered to be associated with hereditary tumors were detected in 9% (15/160) of the patients. Seventeen patients (11%, 17/160) were treated with genotype-matched therapeutic agents, and the response rate was 47% (8/17). The median turnaround time for physicians was 20 days, and the median survival time after the initial visit was 8.7 months. The results of the present study prove the feasibility of implementing in-house clinical sequencing as a promising laboratory examination technique for precision cancer medicine.

1 | INTRODUCTION

Implementation of genomic sequencing assays for clinical cancer analyses is a recent development in the field of cancer treatment.[1,2] The wider availability of next-generation sequencing (NGS) has promoted the analysis of genomic biomarkers in recent years. Precision medicine based on genomic biomarkers has especially advanced in the treatment of lung adenocarcinoma.[3] Genomic testing for *EGFR* mutations,[4] *ALK* fusions,[5] *ROS1* fusions,[6] and *BRAF* mutations[7] is universally utilized as a laboratory examination, and the molecular targeted therapies against these gene alterations are accepted as one of the standard treatments for patients with lung cancer. Moreover, microsatellite instability (MSI)-high or deficient mismatch repair (dMMR) for programmed cell death 1 (PD-1) inhibitor[8] and *NTRK* fusions for TRK inhibitor[9,10] are utilized as tumor-agnostic genomic biomarkers.

While traditional anticancer drugs act on all rapidly dividing normal and cancerous cells, new approaches have shifted to gene alteration strategies in pursuit of potential therapeutic targets with enhanced specificity. Therefore, it is essential to undertake genotype-matched clinical trials, such as basket and umbrella trials, to confirm the utility of precision cancer medicine.[11-13] Some large basket trials aimed at studying different cancer types, like TAPUR (Targeted Agent and Profiling Utilization Registry) trial (NCT02693535) and NCI-MATCH (National Cancer Institute-Molecular Analysis for Therapy Choice) trial (NCT02465060), are currently in progress and are expected to result in genotype-matched treatment strategies.[14,15]

Alternatively, comprehensive cancer genomic profiling service providers, such as MSK-IMPACT[16,17] and FoundationOne,[18] have innovated routine laboratory investigation techniques for a variety of biological samples in clinical settings to assist everyday cancer care for different types of cancer. In the present study, we developed a novel, in-house clinical sequencing system called "Clinical Sequencing System in Hokkaido University Hospital for Cancer Individualized Medicine (CLHURC)" and launched an outpatient department from April 2016 that is specialized in clinical sequencing for patients with cancer. Our division implements precision medicine in clinical practice and explores suitable individualized treatment strategies, including referral enrollment for appropriate clinical trials.

Sequencing systems for molecular characterization of cancers have been increasingly developed in recent years; however, the clinical utility of in-house comprehensive cancer genomic profiling systems for patients with cancer, as a part of medical services, has never been reported in detail. Therefore, in this study, we report the utility of our in-house clinical sequencing system as a potential tool for precision cancer medicine in clinical practice.

2 | MATERIALS AND METHODS

2.1 | Clinical Sequencing System in Hokkaido University Hospital for Cancer Individualized Medicine (CLHURC)

In April 2016, we launched an outpatient division specialized for sequencing samples obtained from cancer patients. Since then, we have started operating an original in-house clinical sequencing system called CLHURC. All working processes of genome sequencing were completed in our hospital. We received tumor specimens from patients with different types of cancer, who agreed to undergo a comprehensive cancer genomic profiling test, with mainly formalin-fixed paraffin-embedded (FFPE) blocks or 10-20 pieces of undyed 5 μm pathology specimens. In re-biopsy cases, we collected specimens that were fixed using the PAXgene Tissue System (Qiagen) that is intended for high quality nucleic acid purification, to achieve high success rates in sequencing. In general, the quality of nucleic acids extracted from PAXgene-fixed paraffin-embedded specimens is better than that of nucleic acids extracted from FFPE specimens.[19]

In our system, genomic DNA was extracted and purified from tumor tissues as well as peripheral blood mononuclear cells (PBMCs) obtained from patients. The minimum amount of DNA extracted was 50 ng. We checked the quality of DNA based on the DNA integrity number (DIN) score, which was calculated using the Agilent 2000 TapeStation (Agilent Technologies), and DNA libraries were prepared for genome sequencing if the quality of DNA had a DIN score above 3.1. Subsequently, we performed a targeted amplicon exome sequencing for 160 cancer-related genes using the Illumina MiSeq sequencing platform (Illumina) (Table S1). All the above processes were performed in an Internatinal Organization for Standardization (ISO) 15189-certified laboratory at our hospital.

2.2 | Bioinformatics analysis

Genome annotation and curation for analyzing the sequencing data were performed using an original bioinformatics pipeline called GenomeJack (Mitsubishi Space Software) (http://genomejack.net/english/index.html) within three working days. In the GenomeJack pipeline, mapping of the NGS reads to the human reference genome (UCSC human genome 19) was performed using Burrows-Wheeler Aligner,[20] and the reads were realigned with ABRA.[21] For identification of single nucleotide variants (SNVs), SAMtools[22] was used to pile up the sequencing reads and defective SNVs showing conflict between pairwise reads were abandoned. The criteria for

calling mutations are as follows. We determined the noise distributions arising from random sequencing errors. Each mutation was evaluated by binomial test ($P < 0.05$) to reduce random sequencing errors. We called somatic mutations by comparing the number of mismatch bases in tumor with normal control using Fisher's exact test ($P < 0.001$). Possible germline SNPs were excluded using Single Nucleotide Polymorphism Database (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/), the ExAC database (https://gnomad.broadinstitute.org/), Human Genetic Variation Database (HGVD) (http://www.hgvd.genome.med.kyoto-u.ac.jp/), and the ToMMo 2KJPN database (https://jmorp.megabank.tohoku.ac.jp/ijgvd/). The copy number of each gene was calculated as the median value of all the sequencing reads covering the target genes and compared with the median value of control samples. In calling copy number alterations (CNAs), we defined more than three-fold copy number increases as "gain" and less than two-fold decreases as "loss." We identified cancer-specific somatic gene alterations, such as SNVs, insertions/deletions (Indels), and CNAs. Moreover, tumor mutation burden (TMB) was measured as a potential biomarker for immunotherapy. In our test, TMB was defined as the number of nonsynonymous and synonymous mutations in the target regions per megabase of tumor genome (the total size of targeted region in our test was 0.74 Mb), and high TMB was defined as at least 10 mutations per megabase (≥10 mut/Mb). The TMB values measured by our system showed a strong correlation ($R^2 = 0.92$) with those assessed by whole exome sequencing in the comparative test using 46 trial samples. We chose 10 mut/Mb as a threshold of high TMB, which has been recently proposed by reports about the association between TMB and the efficacy of immune checkpoint inhibitors.[23] All the detected gene alterations in 160 cancer-related genes were annotated and curated using the COSMIC database (https://cancer.sanger.ac.uk/cosmic), the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), the CIViC database (https://civicdb.org/home), SnpEff,[24] and the Clinical Knowledgebase (CKB) database (https://ckb.jax.org/).

2.3 | Definition of actionable gene alteration

We added a level of evidence to each detected gene alteration based on procedures outlined in the following: "A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists"[25] and "Clinical Practice Guidance for Next-generation Sequencing in Cancer Diagnosis and Treatment (Edition 1.0)."[26]

In "A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists," it cites the following levels of evidence for each gene alteration: Level A, biomarkers that predict response or resistance to US Food and Drug Administration (FDA)-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors; Level B, biomarkers that predict response or resistance to a therapy based on well-powered studies with consensus from experts in the field, or have diagnostic and/or prognostic significance of certain diseases based on well-powered studies with expert consensus; Level C, biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different tumor type (ie, off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies; and Level D, biomarkers that show plausible therapeutic significance based on preclinical studies, or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus.

In "Clinical Practice Guidance for Next-generation Sequencing in Cancer Diagnosis and Treatment (Edition 1.0)," it cites the following levels of evidence for each gene alteration: Level 1A, biomarker (gene mutation) that is approved by regulatory authority as a companion diagnostic for the said cancer type; Level 1B, biomarker (gene mutation) that is approved by the FDA as a companion diagnostic (or complementary diagnostic) for the said cancer type, or biomarker (gene abnormality), with which consistent results have been obtained to support clinical usefulness of an anticancer agent in a prospective clinical study with biomarker-based patient selection, or in meta-analysis data for the specific cancer; Level 2A, biomarker (gene abnormality), with which results have been obtained to support clinical usefulness of an anticancer agent in subgroup analysis of a prospective clinical study for the said cancer type; Level 2B, biomarker (gene abnormality) approved by regulatory authority for other cancer type(s), or with which results have been obtained to support clinical usefulness of an anticancer agent; Level 3A, biomarker (gene abnormality), for which a correlation with clinical usefulness of an anticancer agent has been reported in scientific knowledge-based case reports, etc.; Level 3B, biomarker (gene abnormality), with which a correlation with therapeutic efficacy of an anticancer agent has been reported by pharmacodynamic evaluation in vitro and in vivo; and Level 4, gene abnormality that is known to be involved in cancer.

We defined gene alterations that had evidence levels A–C in "A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists" or evidence levels 1A–3A in "Clinical Practice Guidance for Next-generation Sequencing in Cancer Diagnosis and Treatment (Edition 1.0)" as actionable gene alterations. Furthermore, we defined gene alterations that had evidence levels A–D in the former or evidence levels 1A–3B in the latter as potentially actionable gene alterations.

2.4 | Panel of genome experts (expert panel)

The analysis reports were discussed and reviewed by a panel of genome experts including medical oncologists, molecular oncologists, pathologists, medical geneticists, clinical laboratory technicians, bioinformaticians, genetic counselors, pharmacists, and nurses. The final judgement on actionable gene alterations was made by a panel of genome experts. The final report, including

information regarding the recommended treatments based on genomic profiling, was confirmed after approval of the panel, after which the report was returned to physicians and patients. The expected turnaround time of this system was approximately 14 days.

2.5 | Patients

In this system, we included patients who were diagnosed with any type of cancer and who had an Eastern Cooperative Oncology Group (ECOG) performance status score of 0-2, regardless of age, staging, or treatment history. We targeted the outpatients who visited our division between April 2016 and April 2017. Written informed consent was obtained from all patients regarding the use of genomic and clinical data for research. The study protocol was approved by the ethics committee of Hokkaido University Hospital (016-0260).

2.6 | Reporting secondary findings from germline variants

In our system, secondary findings in germline variants could be identified by comparing the genomic profile of tumor tissues with that of PBMCs. Therefore, we instituted a policy based on patients' consent to disclose secondary findings revealed by comprehensive cancer genomic profiling test. If the patients agreed to receive the germline information, such information was disclosed only if the detected germline variants were confirmed as pathogenic or likely pathogenic by the global cancer genome database, such as ClinVar, and were also listed in the recommendations of the American College of Medical Genetics and Genomics (ACMG) for reporting incidental findings in clinical exome and genome sequencing.[27]

2.7 | Statistical analyses

Survival analyses for estimating the median survival time was calculated using the Kaplan-Meier method considering the time-to-event period, ie, from the time of initial visit to death. All statistical analyses were undertaken using the IBM SPSS Statistics software (version 21.0 for Windows; IBM).

3 | RESULTS

3.1 | Patient characteristics

Between April 2016 and April 2017, 169 patients with different types of cancer enquired for comprehensive genome sequencing at our division. Of these patients, 161 applied for a comprehensive cancer genomic profiling test. The characteristics of 161 cancer patients are summarized in Table 1. Most of the patients had an ECOG performance status score of 0 or 1, which was an ideal indicator for undergoing the

comprehensive cancer genomic profiling test that would help to decide their future course of treatment. Although the majority of patients had unresectable tumors, a small number of patients presented with cancer in the early stage. The top five primary sites of cancer were colorectal (18%), pancreas (13%), breast (10%), stomach (7%), and non-small cell lung cancer (NSCLC) (6%) (Table 2). Moreover, most patients had received chemotherapy for their cancer and many patients had one or more standard chemotherapy sessions pending at the time of their initial visit. Upon enquiring each patient's desire to know secondary findings at the time of the initial visit, only one-third of the patients indicated that they wanted to know about secondary findings from germline information.

3.2 | Results of genome sequencing

In our sequencing system, the quality and quantity of DNA were checked before the library was constructed for genome sequencing. Re-biopsy was required for patients whose samples yielded insufficient or poor-quality DNA. Although re-biopsy was required for 24% (39/161) of the patients, the comprehensive cancer genomic profiling test was carried out successfully in almost all patients and was complemented by re-biopsy. We sequenced genomic DNA from 160 (99%, 160/161) patients with different types of cancer, with a mean sequencing depth of 860× (range, 518-3250×). The mean tumor

TABLE 1 Characteristics of 161 patients recruited in the study

Characteristics	Patients (N = 161)
Age, years	
Median (range)	65 (2-85)
Sex, n (%)	
Male	83 (52)
Female	78 (48)
ECOG performance status, n (%)	
0	106 (66)
1	34 (21)
2	19 (12)
3	2 (1)
UICC stage, n (%)	
Non-stage IV or recurrence	20 (12)
Stage IV or recurrence	141 (88)
Prior treatment, n (%)	
Yes	137 (85)
No	24 (15)
Unadministered standard chemotherapy, n (%)	
Yes	118 (73)
No	43 (27)

[a] UICC (Union for International Cancer Control) TNM Classification of Malignant Tumors, 8th Edition.

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

TABLE 2 Tumor types among 161 patients recruited in the study. (A) Primary sites. (B) Detail of the other sites of tumors

(A)

Primary sites	Patients (N = 161), n (%)
Colorectal	29 (18)
Pancreas	21 (13)
Breast	16 (10)
Stomach	11 (7)
NSCLC	10 (6)
Endometrial	9 (6)
Biliary	9 (6)
Ovary	8 (5)
Head and neck	6 (4)
Esophagus	6 (4)
Others	36 (22)

(B)

Primary sites	Patients (n = 36)
Urothelial	4
Neuroendocrine carcinoma	4
Kidney	3
Sarcoma	3
Small intestine	3
Liver	2
Acute lymphocytic leukemia	1
Cervix	1
Inflammatory myofibroblastic tumor	1
Intraductal papillary neoplasm of the bile duct	1
Mantle cell lymphoma	1
Mesothelioma	1
Mixed acinar-neuroendocrine carcinoma	1
Peritoneal	1
Polycythemia vera	1
Prostate	1
Seminoma	1
Skin	1
Solid pseudopapillary neoplasm	1
Solitary fibrous tumor	1
Thymoma	1
Unknown primary	1
Vagina	1

Abbreviation: NSCLC, non-small cell lung cancer.

cellularity of the samples, calculated by their pathological review, was 46% (range, 5%-90%; Figures S1 and S2, Tables S2-S5).

Potentially actionable gene alterations were detected in 91% (145/160) of the patients, and they were frequently detected in *TP53*

TABLE 3 Potentially actionable gene alterations found in 160 patients who underwent cancer genomic profiling test. (A) Potentially actionable gene mutations. (B) Potentially actionable copy number alterations. (C) Potentially actionable gene alterations (gene mutations + copy number alterations)

(A)

Top 10 genes	Patients (n = 160), n (%)
TP53	86 (53)
KRAS	41 (26)
APC	37 (23)
SMAD4	11 (7)
FBXW7	10 (6)
ARID1A	9 (6)
BRAF	7 (4)
PIK3CA	7 (4)
PTEN	7 (4)
BRCA2	6 (4)

(B)

Top 14 genes	Copy numbers	Patients (n = 160), n (%)
BRCA2	Loss	15 (9)
MYC	Amplification	14 (9)
MLH1	Loss	12 (8)
ATM	Loss	11 (7)
ERBB2	Amplification	10 (6)
TP53	Loss	10 (6)
BRCA1	Loss	8 (5)
FBXW7	Loss	8 (5)
PIK3CA	Amplification	8 (5)
ARID1A	Loss	7 (4)
CHEK2	Loss	7 (4)
FLT3	Amplification	7 (4)
JAK2	Amplification	7 (4)
KRAS	Amplification	7 (4)

(C)

Top 10 genes	Patients (n = 160), n (%)
TP53	88 (55)
KRAS	46 (29)
APC	26 (16)
BRCA2	20 (12)
ATM	16 (10)
FBXW7	16 (10)
ARID1A	15 (9)
PIK3CA	15 (9)
MYC	14 (9)
BRCA1	13 (8)

TABLE 4 Actionable gene alterations found in 160 patients who underwent cancer genomic profiling test. (A) Actionable gene mutations. (B) Actionable copy number alterations. (C) Actionable gene alterations (gene mutations + copy number alterations)

(A)

Top 11 genes	Agents	Patients (n = 160), n (%)
PIK3CA	PI3K/AKT/MTOR inhibitor	7 (4)
ATM	PARP inhibitor, platinum	5 (3)
BRAF	BRAF inhibitor	5 (3)
BRCA1	PARP inhibitor, platinum	5 (3)
BRCA2	PARP inhibitor, platinum	5 (3)
KRAS G12C	KRAS G12C inhibitor	5 (3)
AKT1	AKT/MTOR inhibitor	3 (2)
EGFR	EGFR inhibitor	3 (2)
ERBB2	HER2 inhibitor	2 (1)
ESR1	Estrogen blocker	2 (1)
PBRM1	PD-1/PD-L1 inhibitor	2 (1)

(B)

Top six genes	Copy numbers	Agents	Patients (n = 160), n (%)
BRCA2	Loss	PARP inhibitor, platinum	10 (6)
PIK3CA	Amplification	PI3K/AKT/MTOR inhibitor	8 (5)
CDK4	Amplification	CDK4/6 inhibitor	4 (3)
EGFR	Amplification	EGFR inhibitor	4 (3)
MDM2	Amplification	MDM2 inhibitor	2 (1)
MET	Amplification	MET inhibitor	1 (1)

(C)

Top 13 genes	Agents	Patients (n = 160), n (%)
PIK3CA	PI3K/AKT/MTOR inhibitor	15 (9)
ERBB2	HER2 inhibitor	12 (8)
EGFR	EGFR inhibitor	7 (4)
ATM	PARP inhibitor, platinum	5 (3)
BRAF	BRAF inhibitor	5 (3)
BRCA1	PARP inhibitor, platinum	5 (3)
BRCA2	PARP inhibitor, platinum	5 (3)
KRAS G12C	KRAS G12C inhibitor	5 (3)
CDK4	CDK4/6 inhibitor	4 (3)
AKT1	AKT/MTOR inhibitor	3 (2)
ESR1	Estrogen blocker	2 (1)
MDM2	MDM2 inhibitor	2 (1)
PBRM1	PD-1/PD-L1 inhibitor	2 (1)

(55%, 88/160), *KRAS* (29%, 46/160), *APC* (16%, 26/160), and *BRCA2* (12%, 20/160; Table 3). On the contrary, actionable gene alterations were detected in 46% (73/160) of the patients, and they were frequently detected in *PIK3CA* (9%, 15/160), *ERBB2* (8%, 12/160), and *EGFR* (4%, 7/160) (Table 4, Tables S6-S8). The median TMB calculated from our pipeline was 5.4 mut/Mb (range, 1.3-120.8 mut/Mb). In our cohort, 11% (19/160) of the patients were TMB-high (≥10 mut/Mb) (Figure 1, Table 5). The distribution of the maximum evidence level for gene alterations detected in each patient is shown in Table 6.

On the basis of genomic information, 18% (28/160) of the patients received some kind of treatment after genome sequencing (Table S9). Most importantly, only 11% (17/160) of the patients received genotype-matched treatments based on the actionable gene alterations. This indicates that approximately three-quarters of patients who had tumors with actionable gene alterations could not eventually undergo genotype-matched treatments, mainly because of the deterioration in their physical condition and the inaccessibility of off-label drugs. Detailed information regarding genotype-matched treatments is shown in Table 7. Tumor assessment related to the best overall response rate was analyzed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Accordingly, the response and disease control rate of the genotype-matched treatments in our cohort were 47% (8/17) and 76% (13/17), respectively. The median turnaround time from initial visit for physicians and patients was 20 days (range, 9-65 days) and 28 days (range, 10-78 days), respectively. Moreover, the median survival time from the time of initial visit to death (post-sequencing survival time) was 8.7 months (95% confidence interval, 5.0-12.4 months).

3.3 | Secondary findings in germline variants

Secondary findings were detected in tumor tissues as well as PBMCs of 9% (15/160) of the patients (Table S10). Detailed information regarding secondary findings considered to be associated with hereditary tumors is shown in Table 8. If germline variants were detected in the genes listed in the ACMG recommendations[27] of secondary findings, and the patients agreed to be informed, the detail was shared with the patients after genetic counseling.

4 | DISCUSSION

In this study, we report the clinical utility of an in-house targeted amplicon sequencing system for cancer patients. Clinical implementation of precision cancer medicine has been considered a promising treatment strategy for cancer, and a variety of studies in experimental settings have been performed and reported. However, only few reports have focused on an in-house clinical sequencing system as a laboratory examination. Our cohort consisted of patients with different types of cancer who were willing for genotype-matched treatments in clinical practice. The sequencing reports were returned within a reasonable turnaround time with a very high success rate of sequencing, and we concluded that our in-house sequencing system was feasible and promising. However, undertaking in-house comprehensive cancer
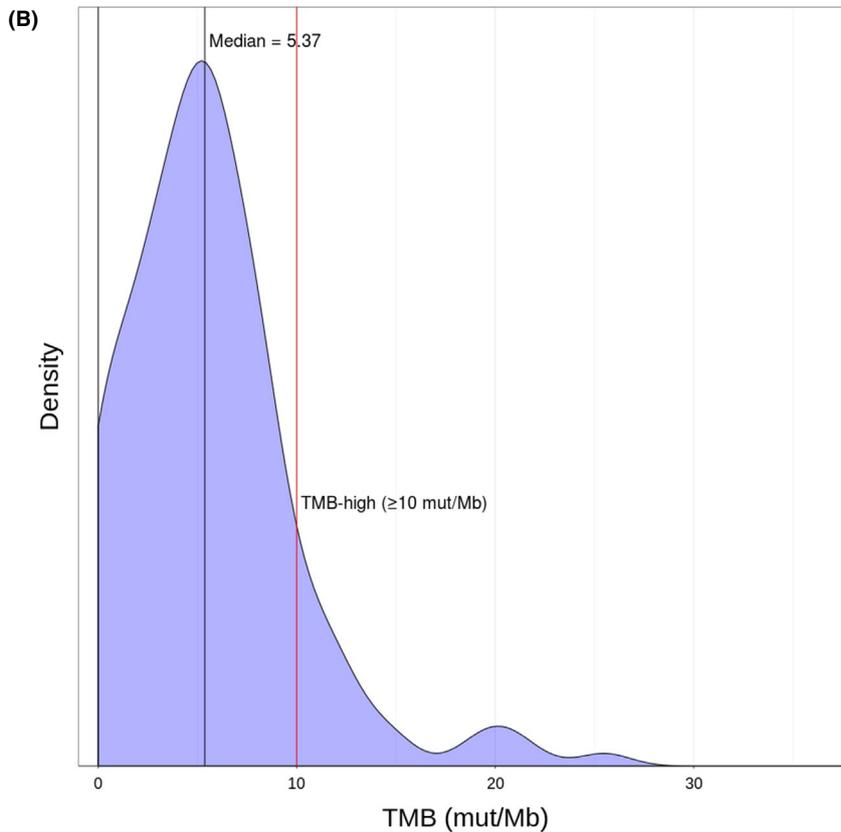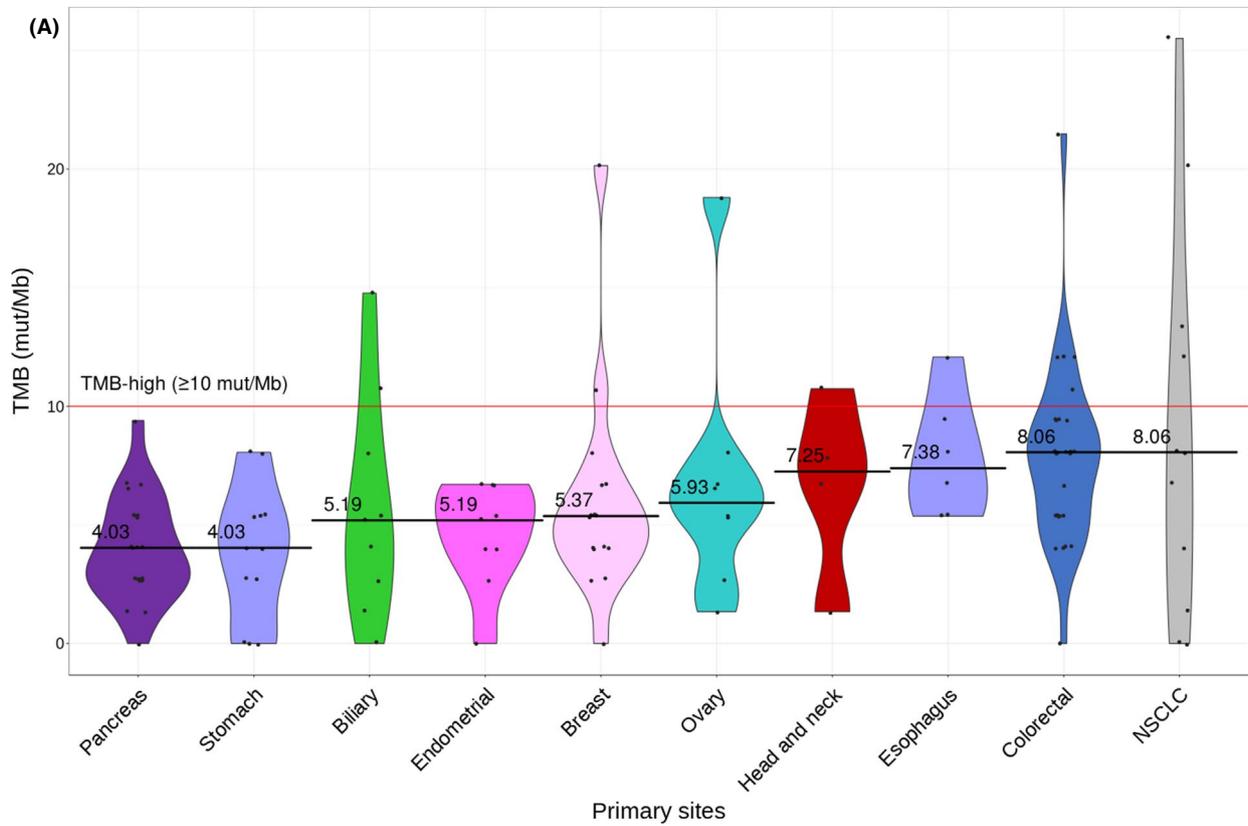
FIGURE 1 Tumor mutation burden (TMB) profile of 160 cancer patients. A, TMB spectrum across tumor types. Violin plots show the distribution of TMB for the top 10 tumor types. Tumor types are arranged from right to left with decreasing median TMB. The width of each plot indicates the frequency of cases with a given TMB. The red line indicates the threshold for cases with TMB-high status (10 mutations [mut]/Mb). B, Distribution of TMB observed across all patients. NSCLC, non-small cell lung cancer.

TABLE 5 Patients with high tumor mutation burden (TMB; ≥10 mut/Mb)

Patients	Primary sites	Smoking history (Brinkman index)	TMB (mut/Mb)
CLHURC-148	Neuroendocrine carcinoma	0	120.8
CLHURC-093	NSCLC	1840	25.5
CLHURC-004	Colorectal	0	21.5
CLHURC-133	NSCLC	380	20.1
CLHURC-136	Breast	0	20.1
CLHURC-059	Ovary	0	18.8
CLHURC-138	Biliary	0	14.8
CLHURC-151	Small intestine	0	14.8
CLHURC-058	NSCLC	3060	13.4
CLHURC-073	Colorectal	0	12.1
CLHURC-095	Colorectal	440	12.1
CLHURC-104	Colorectal	800	12.1
CLHURC-130	Esophagus	1800	12.1
CLHURC-154	NSCLC	940	12.1
CLHURC-042	Head and neck	1260	10.7
CLHURC-043	Neuroendocrine carcinoma	430	10.7
CLHURC-074	Breast	100	10.7
CLHURC-076	Biliary	0	10.7
CLHURC-113	Colorectal	1750	10.7

Abbreviation: NSCLC, non-small cell lung cancer.

genomic profiling tests in real-world medical settings remains challenging, mainly due to the need for highly qualified staff and the high cost involved.

A recent large cohort study on 10 000 patients with cancer using the MSK-IMPACT platform reported that 37% of the patients harbored actionable gene alterations, but only 11% of the patients were subsequently enrolled in genotype-matched clinical trials.[28] In addition, two Japanese retrospective studies that analyzed samples from 85 and 230 cancer patients using the OncoPrime and NCC Oncopanel platforms, reported the identification of actionable gene mutations in 86% and 59% of the patients, respectively, but only 13% of the patients assessed by either platform received subsequent treatments based on the genomic information.[29,30] In our cohort, actionable gene alterations were detected in 46% of the patients, and 11% of the patients eventually received the genotype-matched treatments. The definition of actionable gene alterations differs with every platform; however, the rate of patients who eventually opted for genotype-matched treatments was 10%-20% in all platforms. This range is indicative of the current performance of precision cancer medicine using comprehensive cancer genomic profiling, despite the number of targeted cancer-related genes in the cancer panel.

The turnaround time of genome sequencing is an important factor when implementing comprehensive cancer genomic profiling

into clinical practice. The Japanese retrospective study using the OncoPrime and NCC Oncopanel platforms reported that the median turnaround time for physicians was 40 and 32 days, respectively.[29,30] In our cohort, the median turnaround time for physicians was 20 days. Although the expected turnaround time of our system was approximately 14 days, it took an additional six days to convene a panel of genome experts. In our system, the entire process of genome sequencing, including extraction of DNA from tumor samples, NGS assay, and genomic analysis, was performed in our hospital. During cancer genome sequencing using pathological tissue samples, the process is mainly divided into three parts: pre-analysis, analysis, and post-analysis. The advantages of an in-house sequencing system depend to a large extent on the pre-analysis part. Pre-analysis is the process of handling of tumor samples, consisting of collecting and checking specimens, extraction of nucleic acids, checking the quality and quantity of nucleic acids, and NGS library preparation. In our in-house sequencing system, all of the processes have been performed by full-time experts within a week just after the patients agreed to take the examination. Therefore, these steps contributed to shortening of turnaround time, and the short turnaround time with a very high success rate of sequencing was achieved because the in-house processing was performed as a laboratory examination. In addition, in our case, the median post-sequencing survival time was 8.7 months, which was sufficient to provide opportunities to patients for receiving genotype-matched treatments. However, to maximize the benefits of precision cancer medicine, it is essential to develop an efficient system, which is accessible for cancer patients and facilitates enrollment of such patients into genotype-matched clinical trials.

The therapeutic efficacy of precision cancer medicine remains unknown. A previous prospective randomized controlled phase II trial (the SHIVA trial), designed to evaluate molecularly targeted therapy based on tumor molecular profiling vs. conventional therapy for advanced cancer reported that the use of molecularly targeted agents outside their indications did not improve progression-free survival compared with treatments at physician's choice in heavily pretreated patients with cancer.[31] These results provide important insights as they indicate that off-label use of molecularly targeted agents should not be readily encouraged, even if they were recommended by comprehensive cancer genomic profiling tests. The response rate and disease control rate of the genotype-matched treatments in our cohort were 47% and 76%, respectively. Furthermore, a previous Japanese comprehensive screening study of targeted molecules by NGS in patients with cancer reported that the response disease control rates of the genotype-matched phase I clinical trial were 33% and 78%, respectively.[32] The available evidence and our data support the therapeutic efficacy of precision cancer medicine. Additional confirmatory, prospective, genotype-matched trials are required to validate the clinical utility of precision cancer medicine.

In clinical interpretation of sequencing data, the management of secondary findings is a pivotal issue. In our system, information of secondary findings was disclosed to the patients only after genetic counseling regardless of whether secondary findings were detected

TABLE 6 Distribution of the maximum evidence level for gene alterations detected in each patient. (A) Evidence levels according to "A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists". (B) Evidence levels according to "Clinical Practice Guidance for Next-generation Sequencing in Cancer Diagnosis and Treatment (Edition 1.0)"

(A)

Evidence levels	Evidence level classifications	Patients (n = 160), n (%)
A	Biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors	13 (8)
B	Biomarkers that predict response or resistance to a therapy based on well-powered studies with consensus from experts in the field, or have diagnostic and/or prognostic significance of certain diseases based on well-powered studies with expert consensus	13 (8)
C	Biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different tumor type (ie, off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies	47 (29)
D	Biomarkers that show plausible therapeutic significance based on preclinical studies, or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus	72 (45)
None	None of the above	15 (9)

(B)

Evidence levels	Evidence level classifications	Patients (n = 160), n (%)
1A	Biomarker (gene mutation) that is approved by regulatory authority as a companion diagnostic for the said cancer type	7 (4)
1B	Biomarker (gene mutation) that is approved by FDA as a companion diagnostic (or complementary diagnostic) for the said cancer type, or biomarker (gene abnormality), with which consistent results have been obtained to support clinical usefulness of an anticancer agent in a prospective clinical study with biomarker-based patient selection, or in meta-analysis data for the specific cancer	6 (4)
2A	Biomarker (gene abnormality), with which results have been obtained to support clinical usefulness of an anticancer agent in subgroup analysis of a prospective clinical study for the said cancer type	13 (8)
2B	Biomarker (gene abnormality) approved by regulatory authority for other cancer type(s), or with which results have been obtained to support clinical usefulness of an anticancer agent	14 (9)
3A	Biomarker (gene abnormality), for which a correlation with clinical usefulness of an anticancer agent has been reported in scientific knowledge-based case reports, etc	33 (21)
3B	Biomarker (gene abnormality), with which a correlation with therapeutic efficacy of an anticancer agent has been reported by pharmacodynamic evaluation in vitro and in vivo	45 (28)
4	Gene abnormality that is known to be involved in cancer	27 (17)
None	None of the above	15 (9)

Abbreviation: FDA, Food and Drug Administration.

or not. Most of the patients did not want to receive genetic counseling at that time and only one-third of the patients wished to be informed about secondary findings, which seemed to be lower than expected. In our cohort, secondary findings in germline variants considered to be associated with hereditary tumors were identified in 9% (15/160) of all the patients. In particular, secondary findings listed in the cancer-related genes of the ACMG recommendations were identified in 6% (10/160) of all patients. Based on previous reports, the prevalence of pathogenic or likely pathogenic variants across all the ACMG recommendations was approximately 0.8%-5%.[33-37] Thus, our finding strongly supports these results and paves the way for our future subject aiming at establishing a standard

procedure to manage secondary findings, including interpretation of variant of unknown significance (VUS).

The present study had some limitations. First, our cancer panel targeting 160 cancer-related genes did not cover fusion genes, and therefore we might have underestimated the detection rates of actionable gene alterations. In particular, gene fusions such as *ALK*, *FGFR*, *NTRK*, *RET*, and *ROS1* can be rational actionable targets, although we overlooked the small numbers of patients with these gene fusions.[38] Second, it is very difficult to launch an in-house clinical sequencing system in every hospital. Not only is the preparation and maintenance of an NGS system costly, but a team of medical experts and trained personnel is required to carry out

TABLE 7 Detailed information regarding genotype-matched treatments

Patients	Primary sites	Targeted gene alterations	Treatments	Best responses
CLHURC-030	Breast	*PIK3CA* H1047R	Everolimus + S-1	PD
CLHURC-048	NSCLC	*EGFR* E746_S752V	Gefitinib	CR
CLHURC-051	Urothelial	*ERBB2* amp	Trastuzumab	PR
CLHURC-074	Breast	*AKT1* E17K	AKT inhibitor + fulvestrant	SD
CLHURC-081	Breast	*AKT1* E17K	AKT inhibitor	SD
CLHURC-083	Ovary	*PIK3CA* amp	Everolimus	SD
CLHURC-096	Breast	*ERBB2* amp	Trastuzumab + carboplatin	PD
CLHURC-100	Stomach	*BRCA1* E1754G	Capecitabine + cisplatin + trastuzumab	PR
CLHURC-110	Esophagus	*ERBB2* amp	Capecitabine + cisplatin + trastuzumab	PR
CLHURC-133	NSCLC	*ERBB2* G778_P780dup	Trastuzumab	PR
CLHURC-136	Breast	*PIK3CA* amp	Everolimus + exemestane	PD
CLHURC-141	Head and neck	*ERBB2* S310Y	Trastuzumab + docetaxel	PR
CLHURC-142	Endometrial	*PIK3CA* H1047R	Everolimus + letrozole	SD
CLHURC-148	Neuroendocrine carcinoma	TMB-high (120.8 mut/Mb)	PD-1 inhibitor	CR
CLHURC-149	Vagina	Germline *BRCA2* G3355Vfs*28	Olaparib	SD
CLHURC-150	Esophagus	*BRCA2* K2538T	5-Fluorouracil + cisplatin	PR
CLHURC-157	Colorectal	*BRCA2* S2670L	mFOLFOX6	PD

Abbreviations: CR, complete response; mFOLFOX6, 5-fluorouracil, leucovorin, and oxaliplatin; NSCLC, non-small cell lung cancer; PR, partial response; SD, stable disease; TMB, tumor mutation burden.

TABLE 8 Detailed information regarding secondary findings in germline variants considered to be associated with hereditary tumors

Patients	Primary sites	Sex	Age, years	Genes	AA changes	Phenotypes	Family histories
CLHURC-012	Breast	Female	33	*PTEN*	R130*	*PTEN* hamartoma tumor syndrome	Breast: grandmother, grandaunt ×2; Colorectal: grandfather
CLHURC-025	Kidney	Male	37	*FH*	N415Kfs*37	Hereditary leiomyomatosis and renal cell carcinoma syndrome	NSCLC: grandmother; Stomach: grandfather
CLHURC-027	NSCLC	Female	62	*PMS2*	K580*	Lynch syndrome	Stomach: father; Unknown primary: grandmother
CLHURC-038	Sarcoma	Female	41	*TP53*	R196*	Li-Fraumeni syndrome	None
CLHURC-039	Breast	Female	44	*BRCA2*	T630Nfs*6	Hereditary breast and ovarian cancer	Breast: grandmother ×2; Prostate: father; Colorectal: sister; Stomach: uncle
CLHURC-040	Pancreas	Male	67	*ATM*	Q2729*	Ataxia telangiectasia	Breast: mother, sister ×3; Head and neck: sister
CLHURC-044	Ovary	Female	54	*BRCA1*	E1148Rfs*7	Hereditary breast and ovarian cancer	Unknown primary: grandmother, grandfather ×2; Colorectal: uncle
CLHURC-053	Kidney	Male	30	*FH*	V84Dfs*5	Hereditary leiomyomatosis and renal cell carcinoma syndrome	Kidney: father, grandfather; Pancreas: uncle; Breast: uncle; Childhood cancer: aunt
CLHURC-081	Breast	Female	50	*BRCA1*	Q1447Rfs*22	Hereditary breast and ovarian cancer	Breast: mother, aunt ×2; Ovary: mother; Pancreas: aunt; Stomach: father
CLHURC-096	Breast	Female	52	*BRCA1*	L63*	Hereditary breast and ovarian cancer	Colorectal: uncle
CLHURC-134	Skin	Male	42	*ATM*	E376Ifs*2	Ataxia telangiectasia	Unknown primary: grandfather
CLHURC-136	Breast	Female	38	*BRCA2*	D252Vfs*24	Hereditary breast and ovarian cancer	Breast: mother; Endometrial: aunt
CLHURC-149	Breast	Female	67	*BRCA2*	G3355Vfs*28	Hereditary breast and ovarian cancer	Liver: mother
CLHURC-152	Breast	Female	44	*BRCA1*	Q81*	Hereditary breast and ovarian cancer	Breast: mother, sister
CLHURC-153	Cervix	Female	56	*BRIP1*	c.508-1G > A	Hereditary breast and ovarian cancer	Breast: aunt

Abbreviations: AA, amino acid; NSCLC, non-small cell lung cancer

and interpret genome sequencing. Thus, to promote genome medicine nationwide, an outsourcing system can be helpful. In an in-house system, the number of inspections in each sequencing run is limited, and therefore, the cost of genome sequencing using an in-house system is higher than an outsourcing system in general. An outsourcing system using a high-capacity NGS can process a lot of samples in each sequencing run, and then it can reduce the cost per patient. However, it takes some time to start a sequencing run until a certain number of samples are collected, and therefore, in general, the turnaround time of an outsourcing system is longer than an in-house system. We have to consider the balance between turnaround time and cost in developing a clinical sequencing system. Therefore, we are developing a novel and low-cost clinical sequencing system with a short turnaround time, which can be used by nationwide clinical facilities to outsource the cancer genomic profiling test of their patient samples. Finally, our in-house clinical sequencing was performed in an ISO 15189-certified laboratory at our hospital. Comprehensive cancer genomic profiling tests as a clinical examination should essentially be carried out in laboratories that are certified in quality control for genome sequencing, such as the Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists-Laboratory Accreditation Program (CAP-LAP)-certified laboratories.

In conclusion, the present study proves the feasibility of using an in-house clinical sequencing system as a promising laboratory examination technique in clinical practice. Although outsourcing of cancer genomic profiling tests is currently predominant, in-house systems are preferable, especially in terms of safe and secure handling of clinical specimens and short turnaround time, and to facilitate research and development. As the cost for genome sequencing drops and genomic medicine gains popularity for cancer treatment, it is anticipated that in-house clinical sequencing systems will be soon implemented as a clinical laboratory test, particularly in core hospitals for cancer genomic medicine.

CONFLICT OF INTEREST
The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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