

# Feasibility and clinical utility of comprehensive genomic profiling of hematological malignancies

Suguru Fukuhara<sup>1</sup>  | Yuji Oshikawa-Kumade<sup>2,3</sup>  | Yasunori Kogure<sup>2</sup>  |  
 Sumito Shingaki<sup>2</sup>  | Hirokazu Kariyazono<sup>2,3</sup> | Yoshiya Kikukawa<sup>2,3</sup> | Junji Koya<sup>2</sup>  |  
 Yuki Saito<sup>2,4</sup>  | Mariko Tabata<sup>2,5</sup>  | Kota Yoshifuji<sup>2,6</sup> | Kota Mizuno<sup>2,7</sup> |  
 Akiko Miyagi-Maeshima<sup>8</sup>  | Hiromichi Matsushita<sup>9</sup>  | Masanaka Sugiyama<sup>10</sup>  |  
 Chitose Ogawa<sup>10</sup> | Yoshihiro Inamoto<sup>11</sup>  | Takahiro Fukuda<sup>11</sup> | Masato Sugano<sup>12</sup>  |  
 Nobuhiko Yamauchi<sup>13</sup> | Yosuke Minami<sup>13</sup>  | Makoto Hirata<sup>14</sup>  | Teruhiko Yoshida<sup>14</sup>  |  
 Takashi Kohno<sup>15</sup>  | Shinji Kohsaka<sup>16</sup>  | Hiroyuki Mano<sup>16</sup>  | Yuichi Shiraishi<sup>17</sup> |  
 Seishi Ogawa<sup>18</sup> | Koji Izutsu<sup>1</sup>  | Keisuke Kataoka<sup>2,7</sup> 

<sup>1</sup>Department of Hematology, National Cancer Center Hospital, Tokyo, Japan

<sup>2</sup>Division of Molecular Oncology, National Cancer Center Research Institute, Tokyo, Japan

<sup>3</sup>Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

<sup>4</sup>Department of Gastroenterology, Keio University School of Medicine, Tokyo, Japan

<sup>5</sup>Department of Urology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

<sup>6</sup>Department of Hematology, Graduate School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan

<sup>7</sup>Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan

<sup>8</sup>Department of Pathology, National Cancer Center Hospital, Tokyo, Japan

<sup>9</sup>Department of Laboratory Medicine, National Cancer Center Hospital, Tokyo, Japan

<sup>10</sup>Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan

<sup>11</sup>Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

<sup>12</sup>Department of Pathology and Clinical Laboratories, National Cancer Center Hospital East, Kashiwa, Japan

<sup>13</sup>Department of Hematology and Oncology, National Cancer Center Hospital East, Kashiwa, Japan

<sup>14</sup>Genetic Medicine and Services, National Cancer Center Hospital, Tokyo, Japan

<sup>15</sup>Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan

<sup>16</sup>Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan

<sup>17</sup>Division of Genome Analysis Platform Development, National Cancer Center Research Institute, Tokyo, Japan

<sup>18</sup>Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

## Correspondence

Keisuke Kataoka and Koji Izutsu, Division of Molecular Oncology, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.  
 Email: [kkataoka-ky@umin.ac.jp](mailto:kkataoka-ky@umin.ac.jp) (K.K.); [kizutsu@ncc.go.jp](mailto:kizutsu@ncc.go.jp) (K.I.)

## Abstract

Identification of genetic alterations through next-generation sequencing (NGS) can guide treatment decision-making by providing information on diagnosis, therapy selection, and prognostic stratification in patients with hematological malignancies. Although the utility of NGS-based genomic profiling assays was investigated in hematological malignancies, no assays sufficiently cover driver mutations, including

Suguru Fukuhara, Yuji Oshikawa-Kumade, and Yasunori Kogure contributed equally.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Cancer Science* published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.

**Funding information**

Otsuka Pharmaceutical Co., Ltd; National Cancer Center Research and Development Funds, Grant/Award Number: 30-A-1.

recently discovered ones, as well as fusions and/or pathogenic germline variants. To address these issues, here we have devised an integrated DNA/RNA profiling assay to detect various types of somatic alterations and germline variants at once. Particularly, our assay can successfully identify copy number alterations and structural variations, including immunoglobulin heavy chain translocations, *IKZF1* intragenic deletions, and rare fusions. Using this assay, we conducted a prospective study to investigate the feasibility and clinical usefulness of comprehensive genomic profiling for 452 recurrently altered genes in hematological malignancies. In total, 176 patients (with 188 specimens) were analyzed, in which at least one alteration was detected in 171 (97%) patients, with a median number of total alterations of 7 (0–55). Among them, 145 (82%), 86 (49%), and 102 (58%) patients harbored at least one clinically relevant alteration for diagnosis, treatment, and prognosis, respectively. The proportion of patients with clinically relevant alterations was the highest in acute myeloid leukemia, whereas this assay was less informative in T/natural killer-cell lymphoma. These results suggest the clinical utility of NGS-based genomic profiling, particularly for their diagnosis and prognostic prediction, thereby highlighting the promise of precision medicine in hematological malignancies.

**KEYWORDS**

comprehensive genomic profiling, hematological malignancy, next-generation sequencing, precision medicine, somatic alteration

## 1 | INTRODUCTION

With the advent of next-generation sequencing (NGS), large-scale studies have delineated the entire landscape of genetic alterations in various types of human cancers. These efforts have broadened our knowledge of driver alterations and have revealed the molecular mechanisms of oncogenesis, bringing the promise of cancer precision medicine.<sup>1,2</sup> These discoveries have clinical implications in the diagnosis of specific subtypes defined by recurrent alterations and the prognostic prediction of patient outcome.<sup>3</sup> More importantly, some alterations can guide the use of molecularly targeted therapy and predict the resistance to specific inhibitors, highlighting the relevance of genomic testing in clinical oncology.<sup>4</sup>

Hematological malignancies are diagnosed and classified using morphology, immunophenotype, and genetic alterations. A diverse array of genetic alterations underlies their pathogenesis, as exemplified by more than 250 recurrent alterations listed in the latest WHO classification.<sup>5</sup> In addition, reflecting their diversity and the presence of rare disease types, there have been many genetic studies in hematological malignancies, which have identified novel genetic alterations even recently.<sup>6–8</sup> Moreover, a non-negligible proportion of patients with myeloid neoplasms, such as acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), have pathogenic germline variants in leukemia-predisposing genes, such as *RUNX1* and *DDX41*,<sup>6,9</sup> illuminating the potential necessity of genomic testing for germline susceptibility in hematological malignancies.

A variety of somatic alterations, including single nucleotide variants (SNVs) and insertions/deletions (indels), copy number alterations (CNAs), and structural variations (SVs), are present in hematological malignancies.<sup>5</sup> Among them, recurrent SVs (also called as rearrangements), such as those involving immunoglobulin heavy chain (IGH), are characteristic of specific subtypes. These include chromosomal translocations, inversions, duplications, and deletions, resulting in aberrant expression of oncogenes (activating SV), or generation of fusion transcripts (fusion-generating SV). Several conventional methods, such as G-banding karyotyping, FISH, and RT-PCR, have been utilized to detect these SVs. However, these methods are designed to identify individual SVs, and the spectrum of SVs that can be evaluated by them is still limited.

Recent studies investigated the feasibility and utility of NGS-based genomic profiling in hematological malignancies, which enables simultaneous examination of many somatic alterations at once.<sup>10–12</sup> However, its potential may be underestimated because these studies did not sufficiently cover driver genes listed in the WHO classification as well as recently discovered ones. In addition, germline controls and/or RNA panel were lacking, and several disease types, including T/natural killer-cell non-Hodgkin lymphoma (T/NK-NHL), were not well investigated in these studies. Therefore, to address these issues, here we devised an integrated DNA/RNA profiling assay that can simultaneously detect various types of somatic alterations and germline variants in 452 genes recurrently altered in various hematological malignancies. Using this assay, we have

performed a prospective hospital-based cohort study to investigate the potential of NGS-based comprehensive genomic profiling in 188 specimens of hematological malignancies.

## 2 | MATERIALS AND METHODS

### 2.1 | Workflow of clinical sequencing

We performed a prospective cohort study to evaluate the feasibility and clinical utility of integrated DNA/RNA profiling assay using tumor and germline specimens. The workflow is divided into four parts: (i) patient enrollment/specimen collection, (ii) library preparation, (iii) sequencing and bioinformatic analysis, and (iv) assessment of clinical relevance (Figure 1A). DNA and RNA were independently processed for library preparation and hybrid selection.

### 2.2 | Patients and specimens

Patients aged 1 year or older who were diagnosed with untreated or relapsed/refractory hematological malignancies according to the WHO classification<sup>5</sup> and would intend to receive chemotherapy and/or hematopoietic stem cell transplantation (HSCT) were enrolled. The availability of tumor specimens with tumor content of 5% or more was required ( $\geq 20\%$  was recommended). Fresh or formalin-fixed paraffin-embedded (FFPE) specimens of bone marrow (BM), peripheral blood (PB), fluid aspirate, lymph node, or tumor tissues were collected. Buccal swab was obtained as a matched germline control. The study was approved by the National Cancer Center Institutional Review Board, and all patients and/or their legal guardians (when minors were enrolled) provided written informed consent for this study.

### 2.3 | Design of integrated DNA/RNA profiling assay

We designed a hybridization capture-based DNA/RNA profiling assay to detect SNVs, indels, SVs, CNAs, and fusions (Tables S1–S3). These include almost all driver alterations listed in the Japanese Society of Hematology (JSH) Guideline for Genomic Testing in Hematological Malignancies (2021 release) (JSH Genome Guideline; <http://www.jshem.or.jp/genomgl/>) (Tables S4–S6), which covers almost all alterations listed in the WHO classification<sup>5</sup> and recently discovered ones in hematological malignancies. The DNA panel covered (i) entire coding sequences (CDSs) of 321 frequently mutated genes, (ii) eight non-coding regions affected by recurrent activating SVs, including IGH translocations, *GATA2/MECOM*, and *CD274/PDCD1LG2* truncations, (iii) immunoglobulin (IG) and T-cell receptor (TCR) genes, (iv) HTLV-1 provirus sequence, (v) the *miR-15a/16-1* regions, and (vi) 1296 single nucleotide polymorphism (SNP) regions to evaluate genome-wide copy number and detect intragenic deletions

and duplications, including those involving *IKZF1* and *KMT2A (MLL)* (Table S1). The RNA panel was designed to detect (i) CDSs of 44 genes targeted by recurrent fusions, (ii) CDSs and untranslated regions of 32 genes targeted by recurrent activating SVs, and (iii) IG/TCR genes (Table S2). To increase the sensitivity to detect fusions, we have extended our previous method<sup>13</sup> to design probes for not only fused exons and their adjacent exons but also junction sequences of 134 well-known fusions (Figure 1B; Table S3). The performance of our assay was assessed for (i) 233 mutations in five cell lines detected at an allele frequency of 0.05 or more by amplicon sequencing with QIAseq Human Myeloid Neoplasms Panel (Qiagen), and (ii) 35 SVs/fusions and *IKZF1* intragenic deletion in 31 cell lines described in previous reports. In addition, using clinical specimens, fusions were evaluated using 13 different RT-PCR tests and five different FISH tests, and rearrangements and deletion of 17p (del17p) using eight different FISH tests.

### 2.4 | Library preparation and sequencing

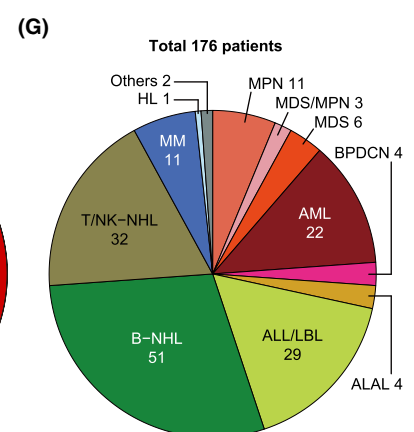
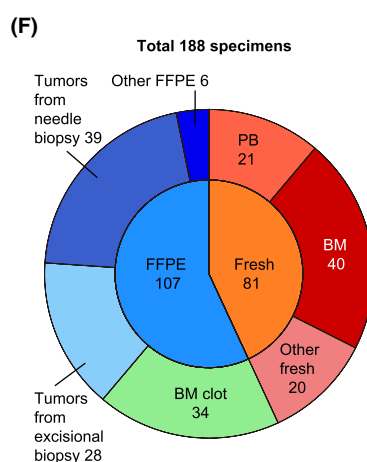
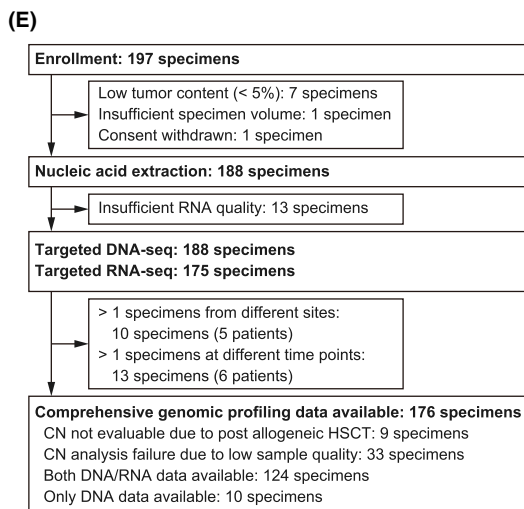
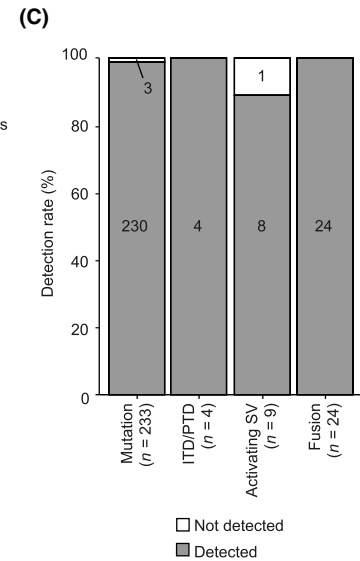
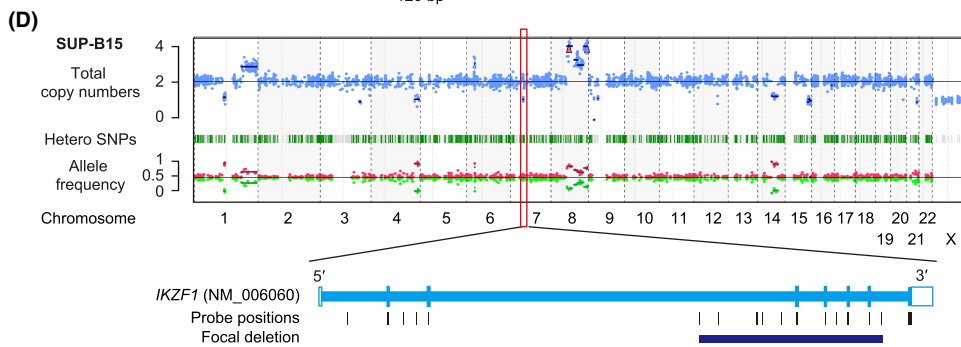
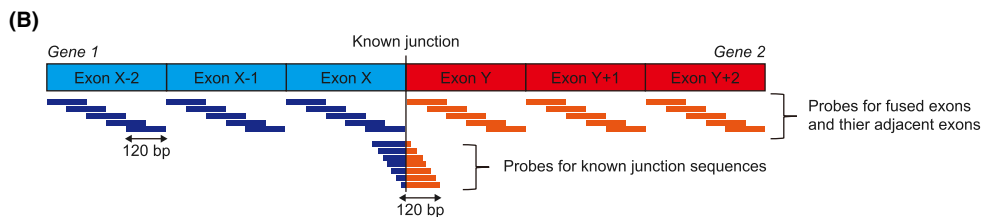
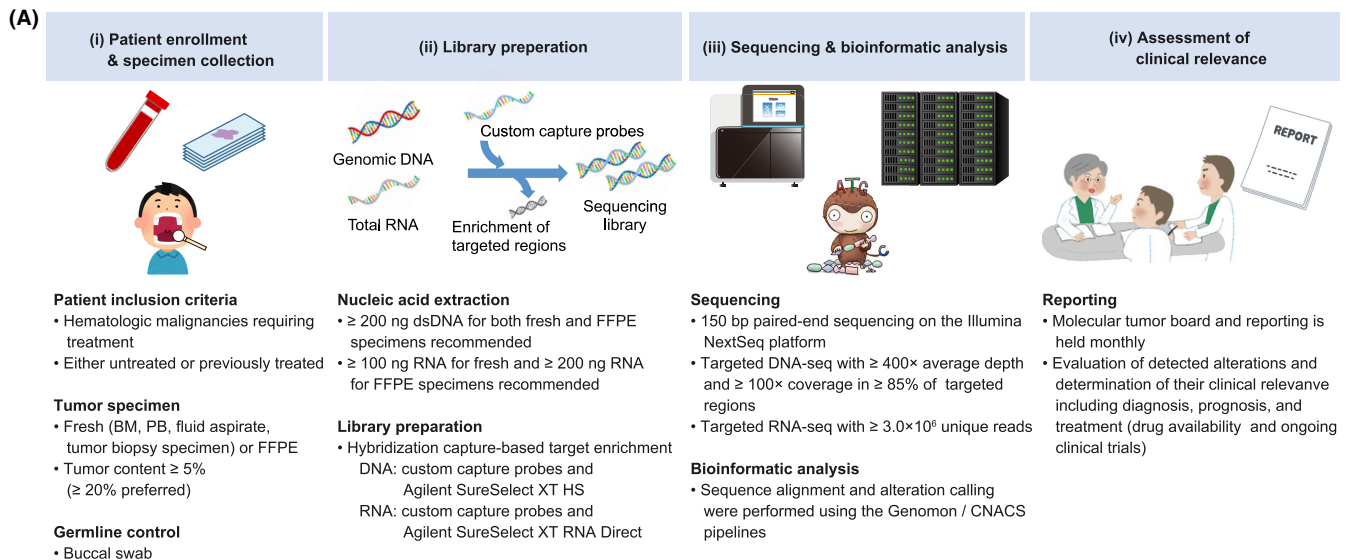
Fresh tumor and buccal germline DNA were extracted using the QIAamp DNA Blood Mini kit (Qiagen) or the Maxwell RSC Blood DNA Kit (Promega). FFPE tumor DNA was extracted using the GeneRead DNA FFPE kit (Qiagen) and its quality was evaluated with Agilent 4200 TapeStation (Agilent Technologies). Fresh and FFPE tumor RNA were extracted using the QIAamp RNA Blood Mini Kit (Qiagen) or the Maxwell RSC simplyRNA Blood Kit (Promega) and the RNeasy FFPE Kit (Qiagen) or the Maxwell RSC RNA FFPE Kit (Promega), respectively. Library preparation for targeted DNA sequencing (DNA-seq) was performed using a custom SureSelect library (Agilent Technologies), as previously described.<sup>14,15</sup> Library preparation for targeted RNA sequencing (RNA-seq) was also performed using a custom SureSelect library with SureSelect XT RNA direct according to the manufacturer's instructions. These libraries were sequenced using the NextSeq 500 (Illumina) to generate 150 bp paired-end reads to  $\geq 400\times$  average depth for DNA-seq and  $\geq 3$  million uniquely mapped reads for RNA-seq either in-house or at RIKEN GENESIS. Quality control results were compared using a two-sided Welch's *t*-test.

### 2.5 | Mapping and mutation detection

For targeted DNA-seq data, sequence alignment and mutation calling were performed using the Genomon pipeline version 2.6.2 (<https://github.com/Genomon-Project/>), as previously described.<sup>14,15</sup> Sequencing reads were mapped to the custom reference genome consisting of 1000 genomes Reference Genome Sequence hs37d5 with the HTLV-1 genome (AB513134). Putative somatic mutations with (i) EBCall<sup>16</sup>  $p < 0.01$ , (ii) four or more variant reads, and (iii) allele frequency  $\geq 0.05$  were adopted and filtered by excluding (i) synonymous SNVs, (ii) variants only present in unidirectional reads, and (iii) variants occurring in repetitive genomic regions. These candidate

mutations were further filtered, unless they were listed 10 or more times in the COSMIC database version 70 or *ASXL1* (NM\_015338.5) c.1927dupG, by removing known germline variants (i) observed in NCBI dbSNP build 131, or (ii) observed at a frequency  $\geq 0.0001$  in any

of the following datasets: the 1000 Genomes Project (October 2014 release), NCBI dbSNP build 131, National Heart, Lung, and Blood Institute Exome Sequencing Project 6500, the Human Genome Variation Database (version 2.00), or the Exome Aggregation



**FIGURE 1** Overview of the study using comprehensive genomic profiling of patients with hematological malignancies. (A) Schema of the prospective hospital-based cohort study. (B) Strategy for capturing fusions by targeted RNA sequencing (RNA-seq). (C) Detection rate of known alterations using cell lines according to alteration type. (D) Representative result of copy number (CN) analysis (top) and zoom-in view for an *IKZF1* deletion (bottom) in SUP-B15 cell line. (E) Flow diagram showing enrollment, nucleic acid extraction, and sequencing. (F) Distribution of tissues and specimen types in all 188 specimens. (G) Distribution of disease types in 176 patients. ALAL, acute leukemias of ambiguous lineage; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone marrow; B-NHL, B-cell non-Hodgkin lymphoma; BPDCN, blastic plasmacytoid dendritic cell neoplasm; DNA-seq, DNA sequencing; FFPE, formalin-fixed, paraffin-embedded; HL, Hodgkin lymphoma; HSCT, hematopoietic stem cell transplantation; ITD, internal tandem duplication; LBL, lymphoblastic lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPN, myeloproliferative neoplasm; PB, peripheral blood; PTD, partial tandem duplication; SNP, single nucleotide polymorphism; SV, structural variation; T/NK-NHL, T/natural killer-cell non-Hodgkin lymphoma

Consortium r0.3.1. Finally, all detected mutations were manually checked by Integrative Genomics Viewer (IGV).<sup>17</sup>

## 2.6 | SV and CNA detection

SVs and CNAs were detected using the Genomon pipeline and the CNACS algorithm, respectively, as previously described.<sup>14,15</sup> Putative SVs were manually curated and further filtered by removing those (i) with fewer than five supporting reads in tumor; (ii) with allele frequency in tumor <0.02; or (iii) present in in-house pooled normal specimens. SV breakpoints were visually inspected using IGV. Candidate focal CNAs (shorter than half a chromosome arm) were assessed in genomic regions where sequencing coverage was sufficient in unmatched control samples, and then manually reviewed. Among genes covered by the DNA panel, 374 and 193 genes (170 for deletions and 23 for amplifications) listed in the JSH Genome Guideline were analyzed for SVs and focal CNAs (Table S6).

## 2.7 | Fusion detection

For targeted RNA-seq data, sequence alignment and fusion calling were performed using the Genomon RNA pipeline version 2.6.2, with a slight modification of those used for poly-A RNA-seq.<sup>14,15</sup> Candidate fusions with 10 or more supporting reads in tumor were filtered by excluding (i) endogenous IG/TCR recombination; and (ii) those detected in in-house pooled normal specimens. Finally, mapping errors were removed by visual inspection with IGV. As *IGH/DUX4* and *STIL-TAL1* fusions are difficult to detect, these fusions were individually reviewed.

## 2.8 | Definition and interpretation of clinically relevant alterations

Clinical evidence levels (A–D) were assigned to each genetic alteration in terms of diagnosis, treatment, and prognostic prediction for each disease subtype at the time of NGS-based genomic profiling according to the JSH Genome Guideline (Table S6), and driver alteration was defined as those with clinical evidence in each disease subtype. Germline variants involving 59 genes responsible for (i) hematological diseases with a germline predisposition proposed in

the JSH Genome Guideline, and/or (ii) hereditary cancers for which the American College of Medical Genetics and Genomics (ACMG)<sup>18</sup> recommends reporting of incidental and secondary findings were evaluated (Table S1). The molecular tumor board comprised of a multidisciplinary team interpreted the sequencing results, including evaluation of somatic alterations and germline variants, and determination of their clinical relevance based on the evidence level assigned for individual alterations.

## 3 | RESULTS

### 3.1 | Design and validation of integrated DNA/RNA profiling assay

We designed NGS-based comprehensive genomic profiling using tumor and germline specimens. Integrated DNA/RNA analysis with elaborated panel design can provide increased breadth and improved sensitivity to detect not only fusion-generating SVs but also activating SVs, which causes aberrant expression of oncogenes (Figure 1B, Tables S1–S3). As the excellent performance of targeted DNA-seq for detecting SNVs and CNAs has been established (Table S7),<sup>14,15</sup> we then evaluated the analytical accuracy for fusion-generating and activating SVs by integrated DNA/RNA analysis (Figure 1C, Table S8). We detected all 24 known fusions, three *FLT3*-internal tandem duplications (ITDs), and 1 *KMT2A*-partial tandem duplication (PTD), with no false positive call observed. We also detected eight of nine activating SVs, consisting of *IGH* translocations (including *IGH/MYC*, *IGH/BCL2*, *IGH/CCND1*, *IGH/CRLF2*, and *IGH/DUX4*) and *GATA2/MECOM* rearrangement. In addition, we confirmed successful detection of an intragenic deletion of *IKZF1*<sup>19</sup> (Figure 1D). These observations suggest acceptable sensitivity for various kinds of SVs and intragenic deletions.

### 3.2 | Quality evaluation of NGS-based genomic profiling

Between 2019 and 2021, 197 specimens from 185 patients were enrolled in our prospective cohort study (Figure 1A,E). Nine specimens were excluded, and the remaining 188 specimens were submitted to further analysis (Table S9). The specimens consisted of 81 fresh and 107 FFPE specimens, including tumors from excisional



biopsy ( $n = 28$ ) and needle biopsy ( $n = 39$ ) (Figure 1F). The median average depth and 100× coverage of DNA-seq were 1022× (range, 143–1723×) and 0.98 (0.58–0.99) in tumors, and 942× (129–1371×) and 0.97 (0.56–0.99) in normal tissues, respectively. Fresh specimens tended to show a higher depth than FFPE specimens, which included all six (4%) specimens with low coverage (<0.85), whereas no difference was observed across tissues and biopsy procedures (Figure S1A,B). Except for nine specimens collected after allogeneic HSCT, genome-wide copy number was evaluable in 142 (79%) of 179 specimens, which contained all fresh specimens but only 64% of FFPE specimens (Figures 1E and S1C). As the amount of input RNA was too small in 13 (7%) specimens, the remaining 175 specimens were analyzed by targeted RNA-seq (Figures 1E and S1D). The read number was higher in fresh than FFPE specimens, with a median of  $7.1 (0.9\text{--}13.3) \times 10^6$  (Figure S1E). DNA integrity number was lower in FFPE specimens stored for 1 year or longer than those stored for less than 1 year ( $p < 0.01$ ) (Figure S1F). The median turnaround time (TAT), defined as time from specimen receipt to molecular tumor board, was 50.5 (21–167) days.

### 3.3 | Somatic alterations detected by NGS-based genomic profiling

Among the 188 specimens, five patients submitted two specimens from different sites (10 specimens), and six patients submitted two or more specimens at different time points (13 specimens). Therefore, a total of 176 patients with various backgrounds were analyzed (Figure 1G, Tables 1 and S9). The integrated DNA/RNA profiling detected 1746 somatic alterations in 296 genes and at least one alteration in 171 patients (97%) (Figure 2A, Tables S10–S13). The median number of alterations per specimen was 7 (0–55), with 10 or more alterations detected in 38% of patients. The most frequently altered gene was *TP53* (19%), followed by *CREBBP* (15%), *CDKN2A/B* (13%), *KMT2D* (13%), and *NRAS* (11%) (Figures 2B and S2).

Targeted DNA-seq detected 1027 mutations (SNVs and indels), including 451 driver and 576 passenger mutations, in 238 genes, and at least one mutation was detected in 159 (90%) patients (Figures 2A; Table S10). The median number of mutations per specimen was four (0–44), including one (0–19) of driver mutations. The most frequently mutated gene was *TP53* (18%), followed by *CREBBP* (11%), *NRAS* (11%), *TET2* (10%), and *KMT2D* (10%) (Figures 2B and S2).

Combined targeted DNA-seq and RNA-seq identified 121 SVs, including 35 fusion-generating, 51 activating, and 27 disrupting SVs as well as five *KMT2A*-PTDs and three *FLT3*-ITDs, and at least one SV was detected in 95 (54%) patients (Figure 2A, Table S11). Fusions included five *PICALM-MLL10*, five *BCR-ABL1*, three *RUNX1-RUNX1T1*, and five *KMT2A*-related fusions (Figures 2B and S2). Among them, 21 fusions were evaluated by RT-PCR or FISH, which were all positive. The negative and positive predictive values of our assay were all 100% for *FLT3*-ITDs and fusions. Activating SVs mainly consisted of IGH translocations, such as 15 IGH/*BCL2*, 12 IGH/*CCND1*, and

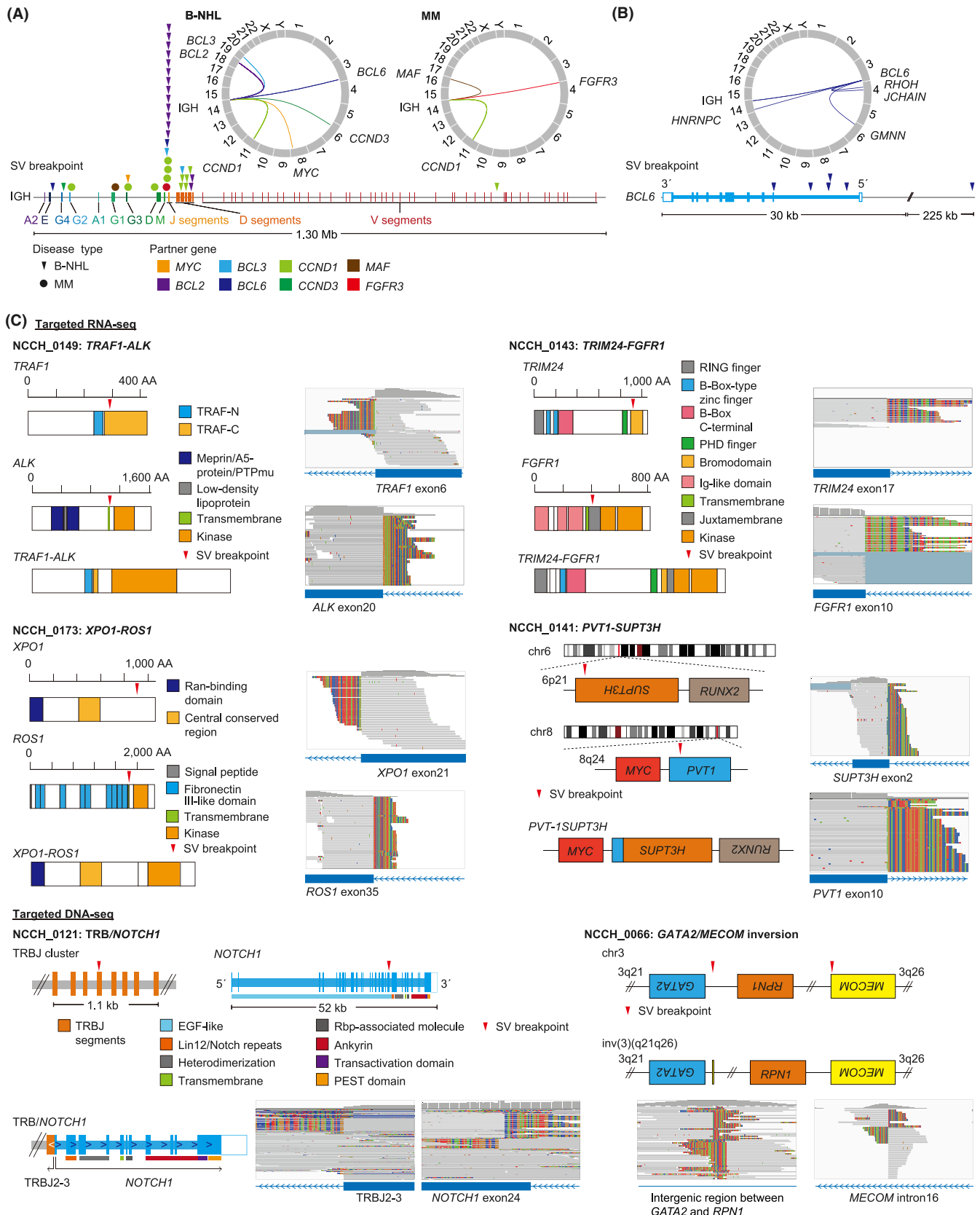
TABLE 1 Characteristics of 176 patients with hematological malignancies

Characteristic	Number (%)
Number of patients	176
Disease type	
AML	22 (13)
ALL/LBL	29 (16)
ALAL	4 (2)
BPDCN	4 (2)
MPN	11 (6)
MDS/MPN	3 (2)
MDS	6 (3)
B-NHL	51 (29)
T/NK-NHL	32 (18)
HL	1 (1)
MM	11 (6)
Others	2 (1)
Sex	
Male	111 (63)
Female	65 (37)
Age, years	
0–14	14 (8)
15–39	39 (22)
40–64	61 (35)
≥65	62 (35)
Disease status	
Primary	104 (59)
Relapsed/refractory	72 (41)

Abbreviations: ALAL, acute leukemias of ambiguous lineage; ALL/LBL, acute lymphoblastic leukemia/lymphoblastic lymphoma; AML, acute myeloid leukemia; B-NHL, B-cell non-Hodgkin lymphoma; BPDCN, blastic plasmacytoid dendritic cell neoplasm; HL, Hodgkin lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPN, myeloproliferative neoplasm; T/NK-NHL, T/natural killer-cell non-Hodgkin lymphoma.

two IGH/*BCL6* translocations, but also included two TCR translocations (Figures 2B and S2). The IGH translocations mainly occurred adjacent to DJ segments in B-cell NHL (B-NHL) and in the switch regions of constant genes in multiple myeloma (MM) (Figure 3A). All SV partners of *BCL2* and *CCND1* were IGH (Figure 3A, Table S11). Although IGH was the most common partner of *MYC* (50%) and *BCL6* SVs (33%), multiple additional partners were identified (Figure 3B, Table S11). Other activating SVs involved *REL*, *NOTCH1*, and *CD274*. Among them, 23 SVs, including IGH translocations, were assessed by FISH, in which 21 were positive, but two (one IGH/*MYC* and one non-IGH/*MYC*) were negative. When FISH was negative ( $n = 30$ ), no corresponding SVs were detected by NGS, producing negative and positive predictive values of 94% and 100% for activating SVs, respectively.





**FIGURE 3** Detection of activating and fusion-generating structural variations (SVs) in clinical specimens from patients with hematological malignancies. (A) Distribution of breakpoints and partners of immunoglobulin heavy chain (IGH) translocations in B-cell non-Hodgkin lymphoma (B-NHL; triangle) and multiple myeloma (MM; circle). (B) Distribution of breakpoints and partners of *BCL6* SVs. (C) Rare fusions and SVs detected in this study. Red triangles represent SV breakpoints. AA, amino acid; DNA-seq, DNA sequencing; EGF, epidermal growth factor; Ig, immunoglobulin; PEST, proline, glutamic acid, serine, and threonine; PTP, protein tyrosine phosphatase; Rbp, Recombination signal binding protein; RING, really interesting new gene; RNA-seq, RNA sequencing; TRAF, tumor necrosis factor receptor-associated factor



In 134 patients in which genome-wide copy numbers were evaluable, 322 arm-level CNAs (163 amplifications and 159 deletions), and 277 focal CNAs (34 amplifications and 242 deletions) were identified, with a median number of CNAs per patient of three (Figure 2A, Tables S12 and S13). We identified arm-level or focal del17p involving *TP53* in 21 patients. Among them, eight were assessed by FISH, of which six were positive. Taken together, these findings suggest that our integrated DNA/RNA profiling can detect a variety of somatic alterations with adequate sensitivity.

### 3.4 | Extensive heterogeneity of somatic alterations in our cohort

The entire cohort showed extensive heterogeneity of somatic alterations, while reflecting the genetic landscape of each disease type reported by previous genetic studies<sup>20–24</sup> (Figure S2). As confirmed in cell lines, integrated DNA/RNA profiling successfully identified one *GATA2/MECOM* inversion and five *KMT2A*-PTDs (Figure 3C, Table S11). In addition to well-known disease-defining SVs and fusions, our assay detected various kinds of SVs and fusions, such as *PVT1-SUPT3H* fusion and *DUSP22* rearrangement characteristic of blastic plasmacytoid dendritic cell neoplasm (BPDCN) and *ALK*-negative anaplastic large cell lymphoma, respectively. Moreover, we identified rare IG/TCR-involving SVs, including *IGH/BCL3* and *TRB/NOTCH1*, as well as kinase fusions, such as *TRAF1-ALK*, *TRIM24-FGFR1*, and *XPO1-ROS1*, which can be a promising therapeutic target (Figure 3C).

### 3.5 | Differences in somatic alterations across disease types

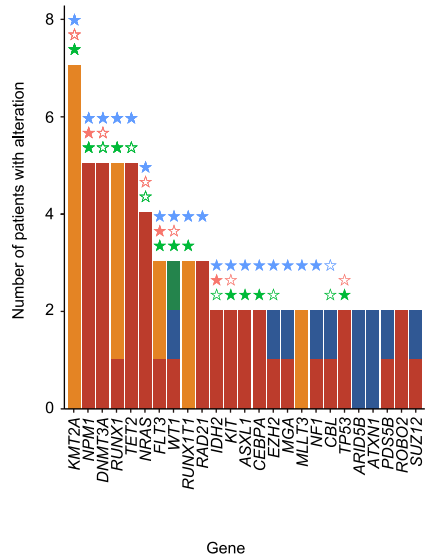
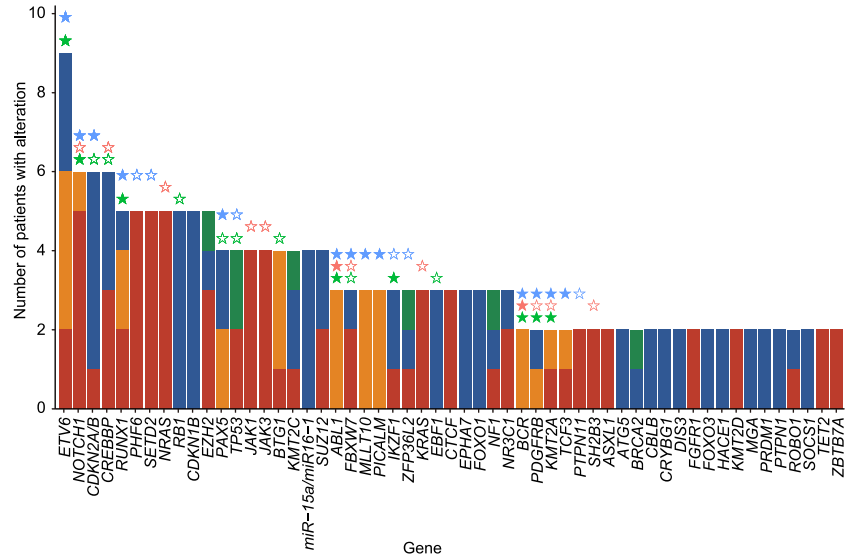
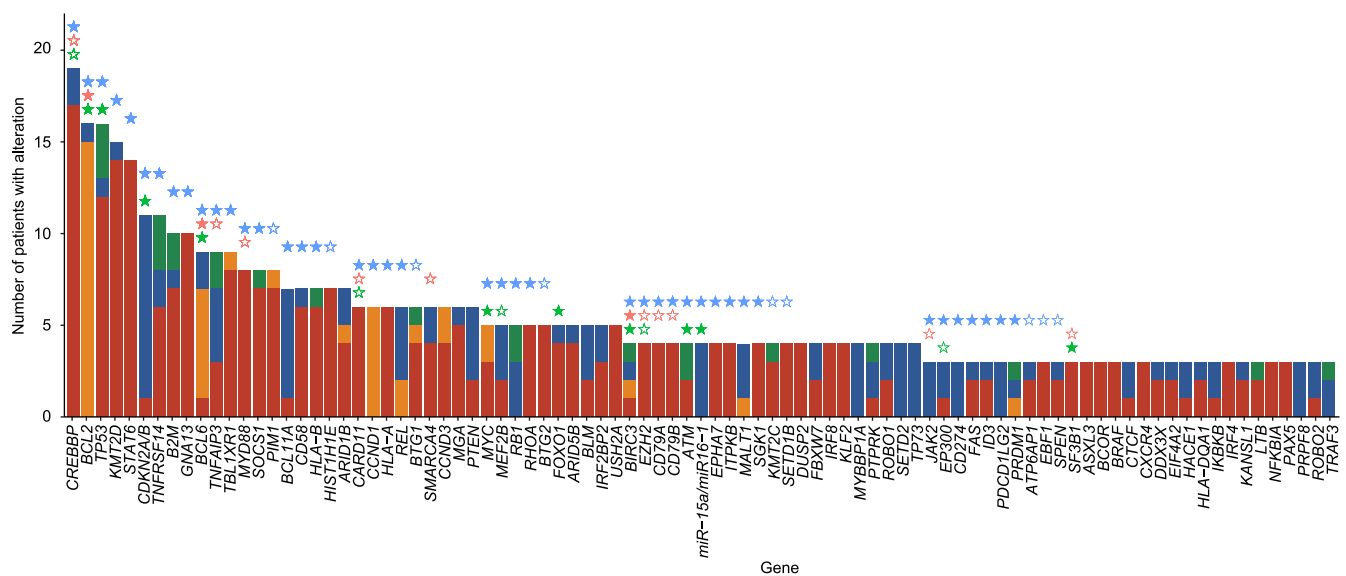
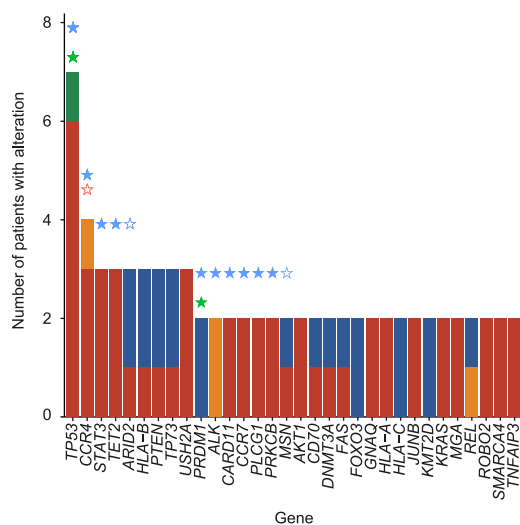
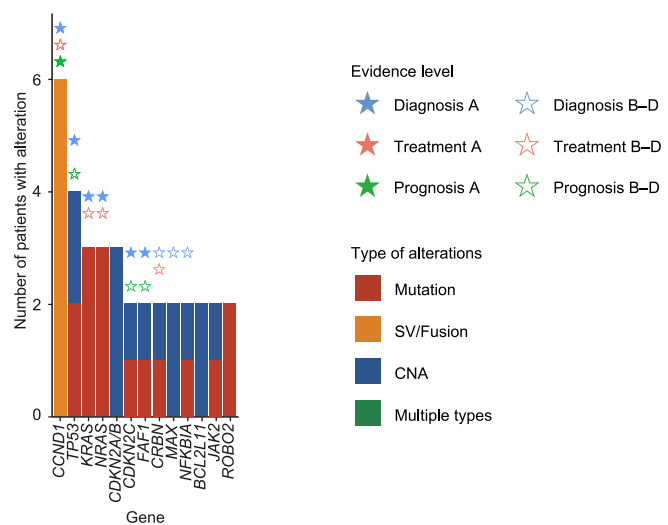
Disease-specific frequencies revealed quite different genetic profiles and associated clinical evidence across disease types (Figures 4 and 5, Table S14). These frequencies were comparable or slightly higher than those reported by the FoundationOne Heme panel,<sup>10</sup> demonstrating the promising performance of our assay (Tables S15–S17). *KMT2A* (32%), *NPM1* (23%), *DNMT3A* (23%), *RUNX1* (23%), and *TET2* (23%) were frequently altered in AML ( $n = 22$ ) (Figure 4A). Among them, 95%, 91%, and 95% of patients harbored at least one genetic alteration with clinical evidence for diagnosis, treatment, and prognosis, respectively (Figure 5). In addition to those already detected by other conventional methods, integrated DNA/RNA profiling identified many subtype-defining alterations, including four *NPM1*, one biallelic *CEBPA*, and one *RUNX1* mutation, one *GATA2/MECOM* inversion, and one *DDX41* germline variant. According to the risk stratification by the European LeukemiaNet,<sup>25</sup> seven (32%) patients with normal cytogenetics ( $n = 6$ ) or no cytogenetics data available ( $n = 1$ ) changed prognostic risk category (four from intermediate to favorable, two from intermediate to adverse, and one from not available to intermediate risk), due to the presence of four *NPM1*, two *ASXL1*, and one biallelic *CEBPA* mutations. Potential targetable alterations included three *FLT3*-ITDs and kinase domain mutations (*FLT3*

inhibitor, level A) and two *IDH1/2* mutations (*IDH1/2* inhibitor, level D). In addition, *DNMT3A* mutation was associated with anthracycline resistance (level B) in five patients.<sup>26</sup>

*ETV6* (31%), *NOTCH1* (21%), *CDKN2A/2B* (21%), and *CREBBP* (21%) were frequently mutated in acute lymphoblastic leukemia/lymphoblastic lymphoma (ALL/LBL) ( $n = 29$ ) (Figure 4B). Among them, 83%, 76%, and 76% of patients harbored at least one genetic alteration with clinical evidence for diagnosis, treatment, and prognosis, respectively (Figure 5). In addition to already detected ones, several subtype-defining fusions were identified, including *KMT2A-MAML2*, *ZNF384-EP300*, *ETV6-ABL1*, and *ATF7IP-PDGFRB* ( $n = 1$  for all). The patients harboring the latter two *ABL*-class gene rearrangements were reclassified into Philadelphia chromosome-like (Ph-like) ALL. The diagnosis of one T-ALL patient changed into myeloid/lymphoid neoplasms with eosinophilia due to the identification of *ETV6-LYN* fusion (NCCH\_0023), which was also the case in one mixed-phenotype acute leukemia patient with *TRIM24-FGFR1* fusion (NCCH\_0143). In addition to subtype-defining alterations, two *IKZF1* deletions and six *NOTCH1* mutations were associated with worse prognosis in B- and T-ALL/LBL, respectively. In addition to the aforementioned fusions, including one *ABL1* fusion (*ABL* inhibitor, level C) and one *PDGFRB* fusion (*PDGFRB* inhibitor, level C), potential targetable alterations included those activating kinase and cytokine receptor signaling, such as one *SH2B3* (*JAK* inhibitor, level C) and one *FLT3* (*FLT3* inhibitor, level C) mutations.

Frequent alterations in B-NHL ( $n = 51$ ) included *CREBBP* (37%), *BCL2* (31%), *TP53* (31%), *KMT2D* (29%), and *STAT6* (27%) (Figure 4C). Among them, 94%, 53%, and 63% of patients harbored at least one genetic alteration with clinical evidence for diagnosis, treatment, and prognosis, respectively (Figure 5). Four patients changed diagnoses based on genome profiling: one from MALT lymphoma to follicular lymphoma (FL) due to *IGH/BCL2* translocation (NCCH\_0039), one from low-grade B-NHL to Waldenström macroglobulinemia (WM) due to *MYD88* mutation (NCCH\_0002), one from primary effusion lymphoma-like lymphoma to recurrence of primary central nervous system lymphoma due to *MYD88*, *CD79B*, and *PIM1* mutations (NCCH\_0090), and one from high-grade B-cell lymphoma, not otherwise specified (HGBL, NOS) to HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangement (NCCH\_0180). Different alterations were associated with prognosis in different B-NHL subtypes, according to the subtype-specific prognostication systems, such as LymphGen classification in diffuse large B-cell lymphoma (DLBCL),<sup>27</sup> the m7-FLIPI clinicogenetic model in FL,<sup>28</sup> and the iwCLL guidelines in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).<sup>29</sup> Due to the paucity of targeted therapy in B-NHL, only four patients had druggable alterations, including three *EZH2* mutations (*EZH2* inhibitor, level C) in FL and one *MYD88* mutation (*BTK* inhibitor, level C) in WM. In addition, four *MYD88* and three *CD79B* mutations were reported to predict sensitivity to *BTK* inhibitor in DLBCL (level C).

Frequent alterations in T/NK-NHL ( $n = 32$ ) included *TP53* (22%), *CCR4* (13%), *STAT3* (9%), *TET2* (9%), and *ARID2* (9%) (Figure 4D). Among them, 53%, 13%, and 19% of patients harbored at least one genetic alteration with clinical evidence for diagnosis, treatment,

**(A) AML (n = 22)****(B) ALL/LBL (n = 29)****(C) B-NHL (n = 51)****(D) T/NK-NHL (n = 32)****(E) MM (n = 11)**

## Evidence level

- ★ Diagnosis A
- ★ Diagnosis B-D
- ★ Treatment A
- ★ Treatment B-D
- ★ Prognosis A
- ★ Prognosis B-D

## Type of alterations

- Mutation
- SV/Fusion
- CNA
- Multiple types

**FIGURE 4** Spectrum of altered genes in clinical specimens from patients with hematological malignancies, by disease type. (A–E) Number of somatic alterations in patients diagnosed with (A) acute myeloid leukemia (AML), (B) acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma (LBL), (C) B-cell non-Hodgkin lymphoma (B-NHL), (D) T/natural killer-cell non-Hodgkin lymphoma (T/NK-NHL), and (E) multiple myeloma (MM). Stars represent the evidence level assigned to genes according to the Japanese Society of Hematology Genome Guideline in each disease type. In case of >1 alteration type detected, they are considered multiple for tumor suppressor or functionally unknown genes, whereas only one major alteration type is counted for oncogenes. CNA, copy number alteration; SV, structural variation

and prognosis, respectively (Figure 5), demonstrating a smaller proportion of patients harboring clinically relevant alterations in T/NK-NHL. In two patients suspected of peripheral T-cell lymphoma with follicular helper T-cell phenotype, no somatic alterations were detected, leading to a change of diagnosis to reactive lymphoid hyperplasia (NCCH\_136 and NCCH\_139). These observations suggest the relevance of NGS-based genomic profiling to determine clonality in mature T-cell neoplasms. The only therapeutically relevant alteration was *CCR4* mutations (*CCR4* inhibitor, level D) in four patients.<sup>30</sup>

*CCND1* (55%), *TP53* (36%), *KRAS* (27%), *NRAS* (27%), and *CDKN2A/B* (27%) were frequently altered in MM ( $n = 11$ ) (Figure 4E). Among them, 100%, 73%, and 73% of patients harbored at least one genetic alteration with clinical evidence for diagnosis, treatment, and prognosis, respectively (Figure 5). According to International Myeloma Working Group molecular classification,<sup>31</sup> eight patients were classified into four subtypes, including hyperdiploidy ( $n = 2$ ), *IGH/CCND1* ( $n = 4$ ), *IGH/FGFR3* ( $n = 1$ ), and *IGH/MAF* ( $n = 1$ ). According to the Stratification for Myeloma and Risk-Adapted Therapy (mSMART 3.0),<sup>32</sup> seven patients had high-risk genetic abnormalities (such as *del17q* and *1q* gain), including one double-hit (*IGH/MAF* and *TP53* mutation) and one triple-hit (*IGH/FGFR3*, *del17p*, and *1q* gain) patients. Regarding treatment, six patients had *IGH/CCND1* (*BCL2* inhibitor, level B) and two patients had *CRBN* mutations, known as resistant mutation to immunomodulatory drugs (level C).

### 3.6 | Clinical utility in diagnosis, treatment, and prognostic prediction

In total, 145 (82%), 86 (49%), and 102 (58%) patients harbored at least one clinically relevant somatic alteration for diagnosis, treatment, and prognosis, of which 76%, 12%, and 44% were supported by level A evidence, respectively (Figure 5). Particularly, most (65%) of the relevant alterations for treatment were of potential clinical significance (evidence level C or D). Although clinically relevant alterations mainly consisted of mutations, SVs/fusions accounted for a substantial proportion of the relevant alterations for treatment (Figure S3). However, only one received targeted therapy based on the recommendation by the molecular tumor board, who had an *ETV6-ABL1* fusion and was treated with dasatinib (*ABL* inhibitor) as off-label use (NCCH\_0094). These differences were consistent across disease types, suggesting that comprehensive genomic profiling is informative for diagnosis followed by prognostic prediction, whereas its utility is inadequate for therapy selection (Figure 5). Regardless of clinical purpose, patients with AML had the highest proportion of

clinically relevant alterations (particularly with level A evidence), followed by those with ALL/LBL, B-NHL, and MM, whereas their proportion was limited in T/NK-NHL, suggesting the different degrees of utility of comprehensive genomic profiling across disease types.

### 3.7 | Detection of cancer-related germline variants

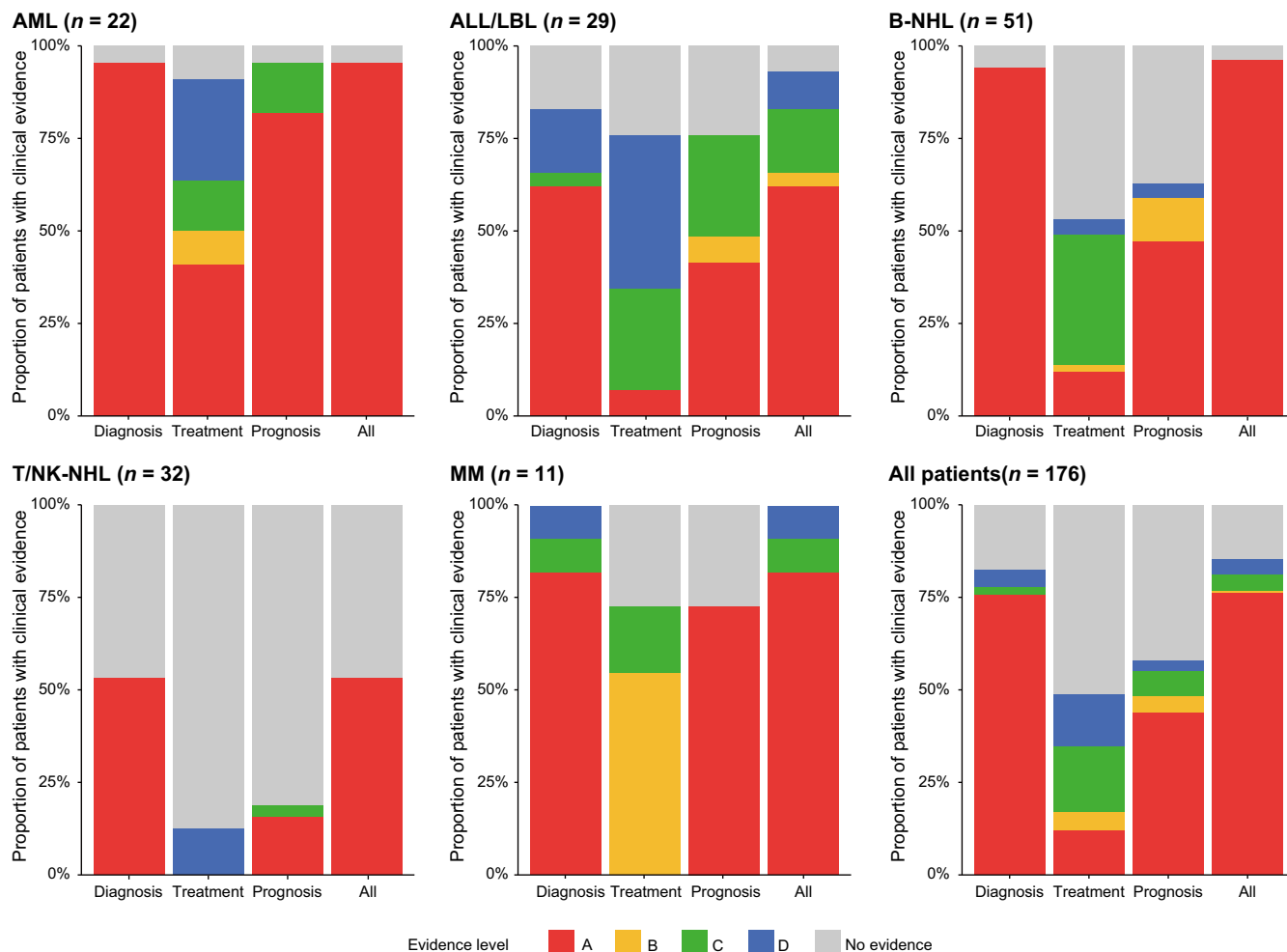
Germline variants causing hereditary cancers were identified in six (3%) patients, all of which were of level A evidence (Table S18). Among them, one AML patient harbored a *DDX41* germline variant and one AML, one MDS, and three ALL/LBL patients harbored deleterious *BRCA1* or *BRCA2* germline variants associated with hereditary breast and ovarian cancers. All six patients were referred for genetic counseling, and three of them requested confirmatory testing and were validated to have the variants.

### 3.8 | Multi-site and multi-timepoint analyses

Multi-site and multi-timepoint analyses were performed for five and six patients, respectively (Table S9). In one patient with a history of essential thrombocythemia who developed myeloid sarcoma, an *MPL* mutation was found in both BM and sarcoma specimens, while several alterations, including *RUNX1* mutation and *7q* deletion, were detected only in sarcoma specimens, suggesting that these alterations contribute to the clonal evolution (Figure S4A). In one CLL patient analyzed before and after BTK inhibitor therapy, a *PLCG2* mutation, known to confer BTK inhibitor resistance (level D), was detected only after progression (Figure S4B). These results suggest that serial analysis is helpful to uncover somatic alterations underlying therapeutic resistance.

## 4 | DISCUSSION

Through a prospective hospital-based cohort study, we demonstrate the feasibility and clinical utility of integrated DNA/RNA profiling for hematological malignancies in the clinical setting. Our study substantiates that this assay enables comprehensive genomic profiling of clinical specimens as a single test, including the assessment of a variety of SVs and CNAs, such as IG translocations, *IKZF1* intragenic deletion, and rare fusions. Particularly, comprehensive genomic profiling is clinically relevant in diagnosis and prognostic prediction, although the usefulness in guiding the use of molecularly targeted therapy is still limited. A larger proportion of patients with myeloid



**FIGURE 5** Clinical utility of comprehensive genomic profiling in hematological malignancies. Proportion of patients harboring at least one somatic alteration with indicated clinical evidence level according to the Japanese Society of Hematology Genome Guideline in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma (LBL), B-cell non-Hodgkin lymphoma (B-NHL), T/natural killer-cell non-Hodgkin lymphoma (T/NK-NHL), and multiple myeloma (MM), and for all patients

neoplasms can benefit from this assay, whereas it is less informative for T/NK-NHL patients.

Consistent with previous studies,<sup>10–12</sup> targeted DNA/RNA-seq is feasible not only for fresh but also for FFPE specimens regardless of the collection methods, except for those collected more than one year before the analysis. However, CNA assessment is still difficult for more than one-third of FFPE specimens, suggesting that fresh specimens are recommended for certain disease types in which CNAs are clinically relevant. Although our assay successfully identified various SVs and fusions, the detection rate of *MYC* SVs was insufficient, probably due to the presence of non-IG partner and widespread distribution of *MYC* breakpoints.<sup>33</sup>

Comprehensive genomic profiling is most informative for diagnosis. First, our assay can identify various subtype-defining alterations, such as fusions in AML and ALL/LBL, and *IGH* translocations in aggressive B-NHL and MM. In addition, several alterations, including

*PVT1-SUPT3H* in BPDCN, are rare but characteristic of a specific disease type. Second, our assay can evaluate the clonality, which can help distinguishing non-neoplastic from neoplastic diseases, particularly in patients suspected of MDS, myeloproliferative neoplasm (MPN), and peripheral T-cell lymphoma. Third, the analysis of buccal swab specimens allows precise identification of germline variants responsible for both leukemia predisposition and other hereditary diseases, including *DDX41* mutations. Such germline variants may influence transplant candidacy and donor selection, although appropriate strategies for their management and genetic counseling have not been established.

The identification of various kinds of kinase fusions provides important therapeutic information, particularly in Ph-like ALL<sup>34</sup> and other kinase fusion-mediated malignancies, as they are potential targets of molecular-based therapies. In addition, our assay can detect resistant alterations to specific inhibitors, such as *PLCG2* mutation

in CLL and *CRBN* mutation in MM.<sup>35,36</sup> Serial sampling may provide additional therapeutically relevant information, given the mutational differences between diagnosis and relapse.<sup>37–39</sup> Although the currently targetable alterations are inadequate for hematological malignancies, comprehensive genomic profiling can serve as a platform for identifying appropriate candidate individuals for clinical trials investigating molecularly targeted therapies.

Comprehensive genomic profiling can help predict patient prognosis and determine transplant candidacy, particularly in AML and ALL/LBL. In addition, such genetic information may be useful to consider the timing of therapeutic intervention in MDS, MPN, and indolent lymphomas. As clinical evidence for the utility of genetic alterations to predict prognosis is still limited, additional clinicogenetic studies are required.

In our study, TAT was approximately 50 days, which prevents the use of the profiling result for determining induction therapy for acute leukemia. Therefore, it is essential to shorten TAT and combine with conventional methods to treat rapidly progressing malignancies. As the JSH Genome Guideline assigns clinical evidence at the gene level rather than at the position or alteration level, some clinical evidence can be assigned to alterations with uncertain significance, such as *BCL2* and non-L265P *MYD88* mutations. Thus, discussion in the molecular tumor board is needed to avoid misinterpretation. As only a small number of patients were enrolled in our study, large-scale real-world data are warranted to validate our findings. Despite these challenges, comprehensive genomic profiling can open a new era of precision medicine in hematological malignancies.

## ACKNOWLEDGMENTS

This work was supported by Otsuka Pharmaceutical Co., Ltd. and National Cancer Center Research and Development Funds (30-A-1). Otsuka Pharmaceutical Co., Ltd. played a role in the design, analysis, and interpretation of the data and in the preparation, review, and approval of the manuscript, and as such are included in the author list. No honoraria or payments were made for authorship. We thank F. Ueki, Y. Hokama, Y. Ito, and M. Sagou for technical assistance. Nucleic acid extraction, library preparation, and sequencing were supported by RIKEN GENESIS and Fundamental Innovative Oncology Core at the National Cancer Center. The supercomputing resources were provided by the Human Genome Center, The Institute of Medical Science, The University of Tokyo. The image of sequencer is from TogoTV (©2016 DBCLS TogoTV / CC-BY-4.0).

## DISCLOSURE

S.F. has received research funding from Chugai Pharmaceutical Co., Ltd. Y.O.-K., H.K., and Y.Kikukawa are employees of Otsuka Pharmaceutical Co., Ltd. Spouse of Y.O.-K. is an employee of Taiho Pharmaceutical Co., Ltd. H.M. has received research funding from Sekisui Medical Co., Ltd. T.K. has received honoraria from Eli Lilly Japan K.K. and research funding from Chugai Pharmaceutical Co., Ltd and Sysmex Corp. S.K. has received a patent royalty from Konica Minolta Inc. H.M. has received a patent royalty from Konica Minolta

Inc. and research funding from Konica Minolta Inc. Y.S. has received research funding from Otsuka Pharmaceutical Co., Ltd. S.O. has received a patent royalty from Cordia Therapeutics Inc., research funding from Cordia Therapeutics Inc. and Otsuka Pharmaceutical Co., Ltd., and has accepted researchers from Cordia Therapeutics Inc. K.I. has received honoraria from Janssen Pharmaceutical K.K. and Ono Pharmaceutical Co., Ltd., and research funding from Otsuka Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., AstraZeneca K.K., AbbVie Inc., Incyte Corp., Bristol Myers Squibb K.K., Novartis Pharma K.K., Bayer Yakuhin, Ltd., Pfizer Japan Inc., Janssen Pharmaceutical K.K., Yakult Honsha Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Ono Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Beigene, Ltd., and Genmab K.K. K.K. has received research funding from Otsuka Pharmaceutical Co., Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., and Chordia Therapeutics Inc., has received scholarship endowments from Eisai Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Takeda Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Mochida Pharmaceutical Co., Ltd., JCR Pharmaceuticals Co., Ltd., and Asahi Kasei Pharma Corp., and has accepted researchers from Otsuka Pharmaceutical Co., Ltd. The other authors declare no competing financial interests. H.M. is a Deputy Editor-in-Chief, and T.Y., T.K., and K.K. are Associate Editors of *Cancer Science*.

## ETHICS STATEMENT

The study was approved by the National Cancer Center Institutional Review board. All patients and/or their legal guardians (when minors were enrolled) provided written informed consent for this study.



## AUTHOR CONTRIBUTIONS

S.F., K.I., and K.K. designed the clinical study. Y.O.-K., Y.Kogure, S.S., Y.S. and K.K. designed the integrated DNA/RNA profiling assay. T.K., S.K., H.M., and S.O. assisted the assay design. H.K. and Y.Kikukawa performed experiments. Y.O.-K., Y.Kogure, Y.Kikukawa, Y.S., and K.K. performed sequencing data analyses. J.K., Y.S., M.T., K.Y., and K.M. assisted experiments and data analyses. S.F., A.M.-M., H.M., M.Sugiyama, C.O., Y.I., T.F., M.Sugano, N.Y., Y.M., and K.I. provided clinical specimens. M.H. and T.Y. interpreted germline variants. S.F., Y.O.-K., Y.Kogure, and K.K. generated figures and tables and wrote the manuscript. K.I. and K.K. led the entire project. All authors participated in discussions and interpretation of the data and results.

## ORCID

Suguru Fukuhara  <https://orcid.org/0000-0002-2423-9978>  
 Yuji Oshikawa-Kumade  <https://orcid.org/0000-0003-1959-6038>  
 Yasunori Kogure  <https://orcid.org/0000-0003-1158-5131>  
 Sumito Shingaki  <https://orcid.org/0000-0002-4249-5000>  
 Junji Koya  <https://orcid.org/0000-0002-0605-1226>  
 Yuki Saito  <https://orcid.org/0000-0003-2686-9604>  
 Mariko Tabata  <https://orcid.org/0000-0002-8013-7327>  
 Akiko Miyagi-Maeshima  <https://orcid.org/0000-0001-7251-7920>



Hiroichi Matsushita  <https://orcid.org/0000-0002-1629-6630>  
 Masanaka Sugiyama  <https://orcid.org/0000-0003-0691-0331>  
 Yoshihiro Inamoto  <https://orcid.org/0000-0003-4881-0427>  
 Masato Sugano  <https://orcid.org/0000-0003-2695-8172>  
 Yosuke Minami  <https://orcid.org/0000-0003-0863-0739>  
 Makoto Hirata  <https://orcid.org/0000-0002-9994-9958>  
 Takashi Kohno  <https://orcid.org/0000-0002-5371-706X>  
 Shinji Kohsaka  <https://orcid.org/0000-0001-8651-6136>  
 Hiroyuki Mano  <https://orcid.org/0000-0003-4645-0181>  
 Koji Izutsu  <https://orcid.org/0000-0001-9129-8057>  
 Keisuke Kataoka  <https://orcid.org/0000-0002-8263-9902>

## REFERENCES

- Chang K, Creighton CJ, Davis C, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* 2013;45:1113-1120.
- The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. *Nature.* 2020;578:82-93.
- Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. *Cell.* 2013;155:27-38.
- McDermott U. Next-generation sequencing and empowering personalised cancer medicine. *Drug Discov Today.* 2015;20:1470-1475.
- Swerdlow SH, Campo E, Harris NL, et al. *WHO classification of tumours of haematopoietic and lymphoid tissues.* Revised 4th Edition ed. International Agency for Research on Cancer; 2017.
- Yasuda T, Tsuzuki S, Kawazu M, et al. Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. *Nat Genet.* 2016;48:569-574.
- Seki M, Kimura S, Isobe T, et al. Recurrent SPI1 (PU.1) fusions in high-risk pediatric T cell acute lymphoblastic leukemia. *Nat Genet.* 2017;49:1274-1281.
- Kogure Y, Kameda T, Koya J, et al. Whole-genome landscape of adult T-cell leukemia/lymphoma. *Blood.* 2022;139(7):967-982.
- Klco JM, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. *Nat Rev Cancer.* 2021;21:122-137.
- He J, Abdel-Wahab O, Nahas MK, et al. Integrated genomic DNA/RNA profiling of hematologic malignancies in the clinical setting. *Blood.* 2016;127:3004-3014.
- McKerrell T, Moreno T, Pongstingl H, et al. Development and validation of a comprehensive genomic diagnostic tool for myeloid malignancies. *Blood.* 2016;128:e1-e9.
- Yasuda T, Sanada M, Nishijima D, et al. Clinical utility of target capture-based panel sequencing in hematological malignancies: A multicenter feasibility study. *Cancer Sci.* 2020;111:3367-3378.
- Kohsaka S, Tatsuno K, Ueno T, et al. Comprehensive assay for the molecular profiling of cancer by target enrichment from formalin-fixed paraffin-embedded specimens. *Cancer Sci.* 2019;110:1464-1479.
- Watatani Y, Sato Y, Miyoshi H, et al. Molecular heterogeneity in peripheral T-cell lymphoma, not otherwise specified revealed by comprehensive genetic profiling. *Leukemia.* 2019;33:2867-2883.
- Kataoka K, Nagata Y, Kitanaka A, et al. Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat Genet.* 2015;47:1304-1315.
- Shiraishi Y, Sato Y, Chiba K, et al. An empirical Bayesian framework for somatic mutation detection from cancer genome sequencing data. *Nucleic Acids Res.* 2013;41:e89.
- Robinson JT, Thorvaldsdóttir H, Winckler W, et al. Integrative genomics viewer. *Nat Biotechnol.* 2011;29:24-26.
- Manickam K, McClain MR, Demmer LA, et al. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021;23:2029-2037.
- Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *New England Journal of Medicine.* 2009;360:470-480.
- Grinfeld J, Nangalia J, Baxter EJ, et al. Classification and personalized prognosis in myeloproliferative neoplasms. *N Engl J Med.* 2018;379:1416-1430.
- Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med.* 2016;374:2209-2221.
- Gu Z, Churchman ML, Roberts KG, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. *Nat Genet.* 2019;51:296-307.
- Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N Engl J Med.* 2018;378:1396-1407.
- Walker BA, Mavrommatis K, Wardell CP, et al. Identification of novel mutational drivers reveals oncogene dependencies in multiple myeloma. *Blood.* 2018;132:587-597.
- Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129:424-447.
- Guryanova OA, Shank K, Spitzer B, et al. DNMT3A mutations promote anthracycline resistance in acute myeloid leukemia via impaired nucleosome remodeling. *Nat Med.* 2016;22:1488-1495.
- Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. *Cancer Cell.* 2020;37(551-568):e514.
- Pastore A, Jurinovic V, Kridel R, et al. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol.* 2015;16:1111-1122.
- Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood.* 2018;131:2745-2760.
- Sakamoto Y, Ishida T, Masaki A, et al. CCR4 mutations associated with superior outcome of adult T-cell leukemia/lymphoma under mogamulizumab treatment. *Blood.* 2018;132:758-761.
- Fonseca R, Bergsagel PL, Drach J, et al. International Myeloma Working Group molecular classification of multiple myeloma: Spotlight review. *Leukemia.* 2009;23:2210-2221.
- Mikhael JR, Dingli D, Roy V, et al. Management of newly diagnosed symptomatic multiple myeloma: Updated Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART) consensus guidelines 2013. *Mayo Clin Proc.* 2013;88:360-376.
- Lieber MR. Mechanisms of human lymphoid chromosomal translocations. *Nat Rev Cancer.* 2016;16:387-398.
- Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med.* 2014;371:1005-1015.
- Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med.* 2014;370:2286-2294.
- Kortum KM, Mai EK, Hanafiah NH, et al. Targeted sequencing of refractory myeloma reveals a high incidence of mutations in CRBN and Ras pathway genes. *Blood.* 2016;128:1226-1233.
- Ding L, Ley TJ, Larson DE, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature.* 2012;481:506-510.



38. Waanders E, Gu Z, Dobson SM, et al. Mutational landscape and patterns of clonal evolution in relapsed pediatric acute lymphoblastic leukemia. *Blood Cancer Discov.* 2020;1:96-111.
39. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature.* 2015;526:525-530.

**How to cite this article:** Fukuhara S, Oshikawa-Kumade Y, Kogure Y, et al. Feasibility and clinical utility of comprehensive genomic profiling of hematological malignancies. *Cancer Sci.* 2022;113:2763-2777. doi: [10.1111/cas.15427](https://doi.org/10.1111/cas.15427)

#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.