Clinical utility of target capture-based panel sequencing in hematological malignancies: A multicenter feasibility study

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Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALL, acute lymphoblastic leukemia; CNV, copy-number variation; CSeq, clinical sequencing; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell; ITD, internal tandem duplication; JSH, Japanese Society of Hematology; ML-DS, myeloid leukemia associated with Down syndrome; MLPA, multiplex ligation-dependent probe amplification; MM, multiple myeloma; NGS, next-generation sequencing; PAF, potentially actionable finding; PPA, positive percent agreement; ROI, region of interest; UPD, uniparental disomy; VAF, variable allele frequency; WHO, World Health Organization.

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In the last decade, NGS technologies have revolutionized the understanding of the cancer genome through many genomic studies, which revealed recurrent driver mutations shared across different human cancers or that are specific to a certain type of cancer. These studies also clarified the complexity of intra-tumor and inter-tumor clonal structures and the process of clonal evolution from benign to malignant states. NGS technologies have been further applied toward clinical management for cancer patients, leading to molecular diagnosis, precise prognostic stratification, and identification of molecular target therapy. Accurate, fast, and cost-effective target-enrichment NGS panel tests have accelerated a large-scale practical implementation of precision medicine for patients with cancer.

Hematology has been the vanguard of genomic medicine since the 1980s, when Southern blot analysis was used to aid the diagnosis of lymphoma. Notably, identification of the BCR-ABL1 and PML-RARA fusion genes caused a paradigm shift in molecularly targeted therapies against CML and acute promyelocytic leukemia, and aided in making remarkable progress in long-term outcomes. It is also apparent that genetic classification has become quite developed in the field of hematology, and many genetic subtypes are listed in among 2017 classifications by the WHO. Recent comprehensive genomic studies further demonstrated the existence of patients with new genetic subtypes of morphologically diagnosed AML, ALL, and DLBCL. Based on the disease subtypes or useful genetic markers, risk-based stratified treatment has been practiced more commonly when managing patients with hematological malignancies, suggesting that genomic information is indispensable for clinical practice with these disorders.

Although relatively large numbers of reports have shown the utility of panel sequencing for solid tumors, it remains unclear...
whether it is an effective approach with clinical benefit for hematological malignancies. In particular, few reports have discussed the value of panel testing in diagnostic and prognostic assessment, which are likely to provide useful information especially for patients with hematological malignancies. Therefore, we developed a DNA-panel testing method for hematological malignancies that can simultaneously detect various types of gene alterations including single-nucleotide variants (SNVs), insertions/deletions (indels), CNVs, and immunoglobulin heavy chain locus (IGH) translocations. We then performed a prospective multicenter feasibility clinical sequencing (CSeq) study and assessed the clinical utility of the panel testing in a unified way across 96 patients with AML, ALL, MM, or DLBCL.

2 | MATERIALS AND METHODS

2.1 | Patients

To evaluate the feasibility of target capture-based panel testing for hematological malignancies, adult patients with AML (n = 25), pediatric patients with AML or ALL (n = 25), adult patients with MM (n = 25), and adult patients with DLBCL (n = 25) were enrolled in the CSeq study. The patient samples were collected as ancillary studies, which were designated as the CS-17-CSeq arm of the CS-17 study conducted by Japan Adult Leukemia Study Group for adult AML, the CSeq-17 arm of the JPLSG-CHM14 study conducted by the Japan Pediatric Leukemia/Lymphoma Study Group for pediatric leukemia, the MM-15-CSeq arm of the JSH-MM-15 prospective observational study conducted by JSH for MM, and the Lymphoma-CSeq study for DLBCL. Most patients were registered for the study with an initial diagnosis, except for 13 relapsed pediatric patients. The studies were approved by the ethics committees at all participating institutions. Informed consent was obtained from all patients or guardians when children were enrolled. This study was conducted in accordance with the Declaration of Helsinki. Before genetic testing was conducted, patients were asked whether they wanted to receive a report describing any germline mutations that might be found.

2.2 | Hybridization-based targeted CSeq assay

To detect somatic SNVs, indels, CNVs, and IGH translocations with clinical or preclinical relevance in managing hematological malignancies, we designed a capture panel consisting of the entire coding regions of 330 genes [which included frequently mutated genes in hematological malignancies or targetable molecules in cancer (Table S1)], some IGH regions (Figure S1), and 1179 single-nucleotide polymorphism baits. Tumor specimens were prepared from bone marrow (AML, ALL, and MM) or freshly resected tumor tissues (DLBCL), and subjected to DNA extraction. To enrich the tumor cells from patients with MM, CD138-positive cells were selected before DNA extraction, using CD138 MicroBeads (Miltenyi Biotec).

We used normal sample pairs (buccal mucosa or peripheral blood) as controls to discriminate between somatic and germline mutations. Sequencing libraries were prepared from 50-200 ng DNA using the SureSelect XT reagent (Agilent Technologies) according to the manufacturer’s instructions and subjected to NGS from both ends with the MiSeq or HiSeq2500 platform (Illumina).

2.3 | Bioinformatics analysis

We considered the NGS to be successful if the average sequence depth in the tumor sample was above 300×, based on a previous report. Using tumor cells and matched normal tissue, mutation calling was performed using the Genomon2 pipeline (https://genomon.readthedocs.io/ja/latest/), as previously described. Putative somatic mutations with (i) a Fisher exact test P-value of <.01, (ii) a VAF in the tumors of >.05, (iii) a sequencing depth in the tumor of ≥50 were adopted, and filtered by excluding (i) synonymous SNVs or noncoding variants and (ii) variants only present in unidirectional reads. The remaining variants were interrogated for evidence that they were present at significantly higher VAFs than expected for errors (P ≤ 10−3), for which the statistical significance was evaluated by empirical Bayesian mutation calling, as previously described.

To detect IGH translocations and tandem duplication, we used Genomon-SV (https://github.com/Genomon-Project/GenomonSV), and searched for known variants by manual curation. Breakpoints of candidate alterations were inspected visually using the Integrative Genomics Viewer tool (http://software.broadinstitute.org/software/igv/). Candidate FLT3-internal tandem duplication (FLT3-ITD) calls were validated by PCR analysis, and the FLT3-ITD allelic ratio was determined as previously reported.

CNVs were detected using the CNACS algorithm (https://github.com/papaemmelab/toil_cnacs). Candidate focal CNVs (shorter than half of a chromosome arm, except for 17p deletions involving TP53) were manually reviewed and further filtered by removing the regions showing detection with <3 capture probes, as described previously. Gene amplification was defined as increase in the number of copies of a gene to more than 4 copies, and hyperdiploidy was defined as the presence of >50 chromosomes.

2.4 | Germline mutations

We defined reportable germline mutations in 22 genes, as follows: 16 genes (APC, BRCA1, BRCA2, MSH2, MSH6, NF2, PMS2, PTEN, RB1, RET, STK11, TP53, TSC1, TSC2, VHL, and WT1) in the panel were responsible for hereditary cancers for which American College of Medical Genetics and Genomics (ACMG) recommends reporting as incidental or secondary findings, and 6 genes (ANKRD26, CEBPA, DDX41, ETV6, GATA2, and RUNX1) were associated with myeloid neoplasms with a germline predisposition that were proposed as a diagnostic category by the WHO in the 2017 classification scheme.

Germline variants in all 22 genes were analyzed as described above,
except for determining Fisher exact test P-values, which were omitted because a single analysis was performed. Putative variants were further filtered based on known variants listed in the 1000 Genomes Project (October 2014 release); the National Center for Biotechnology Information dbSNP database (build 131); Exome Aggregation Consortium (ExAC); the Human Genome Variation Database (April 2016 release); and 3.5KJPN (the ToMMo Japanese reference panel).26

2.5 Analytical validation

We used 22 cell lines and 4 standard reference materials (HD728, HD731, HD753, and HD829; Horizon Discovery) for sensitivity analysis. For these specimens, the regions of interest (ROIs) for analytical validation were defined as follows: regions involving common somatic mutations that were verified by orthogonal methods including MLPA, information in the literature, the Catalog of Somatic Mutations in Cancer (version 87)27 or Cancer Cell Line Encyclopedia28 database, or manufacturer’s validation data. As a result, the ROI comprised 89 genetic regions, and the PPA was evaluated for 26 specimens. For the analytical validation assays, the same analytical filter used for the germline mutation analysis was adopted, although the filter of tumor VAF >.05 was removed. For specificity analysis, 2 normal specimens from the Genome in a Bottle Consortium (RM8391 and RM8393) were used, and the sequencing specificity was determined for the same ROI, as verified by performing MLPA or searching the literature.29

2.6 Analysis of clonality

To analyze the clonality of lymphoid malignancies, we designed a capture-based NGS panel consisting of coding regions for IGH, IGK, IGL, TRA, TRB, TRD, and TRG (M. Sanada, Y. Iijima, manuscript in preparation). The clonality was assessed using the Vidjil pipeline.30 Candidate clonal rearrangements were validated by PCR analysis.

2.7 Molecular tumor board for hematological malignancies

The multicenter molecular tumor board was composed of multidisciplinary members, and meetings were held once or twice a month to interpret the sequencing results of all patients, with the goal of identifying PAF. The tumor board included a hematological specialist, pathologists, genome researchers, bioinformaticians, medical geneticists, and genetic counselors. Before the molecular tumor board was established, clinically important information, such as age, sex, diagnoses, and leukemia-associated translocation, was collected. Board members discussed analytical validity, clinical validity, and the clinical utility of the sequencing results. Based on the significance of clinical decision making, we categorized genetic alterations into 4 levels (Level A to Level D) according to standard guidelines for evidence-based categorization of somatic variants.23 In the process of curating of genetic alterations, professional guidelines, or crucial reports (Table S2) were used as a reference. The clinical utility was assessed regardless of the patients’ disease stage, clinical history, and accessibility to clinical trials for unapproved drugs. Candidate germline mutations were also reviewed by board members, and decisions were made as to whether to present the results to the patients.

2.8 Definition of PAF

For the purposes of this study, we defined a PAF as any genomic finding obtained by the CSeq assay that was capable of providing (a) a disease subtype or change in diagnosis (evidence level A or B),31 (b) a risk category (evidence level A or B), or (c) a targetable molecular aberration (evidence level C or above).

3 RESULTS

3.1 Analytical validation of the CSeq assay

We first evaluated the analytical performance of the CSeq assay. The sensitivity of our assay, as determined from the PPA of the ROI, was 95.6% for SNVs (65/68), 100% for short indels (10/10), 100% for large indels (7/7), 100% for CNVs (13/13), 72.2% for IGH translocations (13/18), and 93.3% overall for all variants combined (113/121) (Tables S3 and 1). The PPA for variants with a VAF of approximately 5% – the threshold set for SNVs and indels – was 93.3% (42/45, Table S3). These results were considered acceptable, except for the sensitivity of detecting IGH translocations. Specificity analysis was performed for 2 normal samples, and the CSeq assay showed no false-positive variant calls in the ROIs (Table 1).

3.2 Feasibility of the CSeq assay

From November 2017 to April 2019, 100 patients with hematological malignancies (0 y old to 87 y old) were enrolled in this study. Four patients were excluded from further analysis due to misdiagnosis (n = 3) or patient death before registration (n = 1). We performed target capture sequencing for DNA isolated from the remaining 96 patients (Table 2). The average sequence depth in each tumor sample was 597 × (range, 357 ×-837 ×), and all cases showed a depth above the set threshold (300 ×), making the sequencing success rate 100% (Figure S2).

The median turnaround time, defined as the interval between the date of sample shipping and the date of returning the analysis report, was 41 d (range, 21-80 d), which was 1 or 2 wk longer than our anticipated timeline. The primary reason for the delay was the
waiting period for the next molecular tumor board meeting, which usually took place approximately 2–3 wk after completion of the bioinformatic analysis.

Disease-specific mutational landscapes for AML, ALL, MM, and DLBCL are presented in Figure 1 in terms of the major driver mutations, and a full listing of the observed gene mutations is provided in Table S4. At least 1 genetic alteration was identified in 93 of the 96 (96.9%) cases. The common driver alterations found with each disease were as follows: NPM1 mutations (n = 7) and Del (7q) (n = 7) in AML, CDKN2A deletion (n = 7) and IKZF1 deletion (n = 7) in ALL, Del(13q) (n = 12) and hyperdiploidy (n = 10) in MM, and CD79A/B mutations (n = 7) and MYD88 mutations (n = 7) in DLBCL.

In addition, the CSeq assay also detected FLT3-ITD (n = 5) and IGH (n = 2) in AML, KMT2A-AFF1 (n = 1) and KMT2A-MLLT3 (n = 1) in AML, BCR-ABL1 (n = 2) in ALL, KMT2A-AFF1 (n = 1) in MM, and RUNX1-RUNX1T1 (n = 1) in DLBCL. These results highlight the importance of comprehensive genetic profiling in the management of hematologic malignancies and the potential for personalized treatment strategies.

### Table 1: Summary of analytical validation of CSeq assay

| Mutation type | Sample | Orthogonal methods | No. of analytical variants | True positive (TP) | False negative (FN) | PPA (%) TP/TP + FN |
|---------------|--------|-------------------|----------------------------|--------------------|---------------------|-------------------|
| SNV           | Cell line, Standard material | Database (CCLE, COSMIC) | 68 | 65 | 3 | 95.6 |
| Short indel   |        |                   | 10 | 10 | 0 | 100 |
| Large indel   |        |                   | 7 | 7 | 0 | 100 |
| CNV           | MLPA   |                   | 18 | 18 | 0 | 100 |
| Translocation | Cell line | Literature | 18 | 13 | 5 | 72.2 |

### Table 2: Clinical information of the patients enrolled in CSeq study

| Characteristics | No. (n = 96) |
|-----------------|--------------|
| Age, y          |               |
| 0-4             | 9            |
| 5-14            | 11           |
| 15-39           | 8            |
| 40-64           | 27           |
| 65-79           | 30           |
| ≥80             | 11           |
| Gender          |               |
| Male            | 54           |
| Female          | 42           |
| Disease status  |               |
| Primary         | 83           |
| Relapse         | 13           |
| Diagnosis       |               |
| Multiple myeloma| 94           |
| Diffuse large B-cell lymphoma | 57 |
| Acute myeloid leukemia | 20 |
| Acute lymphoblastic leukemia | 11 |
| B-cell leukemia | 15 |
| T-cell leukemia | 13 |
| Other           | 24           |
| BCR-ABL1        | 23           |
| RUNX1-RUNX1T1   | 22           |
| KMT2A-AFF1      | 22           |
| KMT2A-MLLT3     | 22           |
| TCF3-HLF        | 22           |
| BCR-ABL1        | 22           |
| B-cell leukemia | 15           |
| T-cell leukemia | 13           |
| Other           | 24           |

**Abbreviations:** CCLE, Cancer Cell Line Encyclopedia; COSMIC, Catalog of Somatic Mutations in Cancer; MLPA, multiplex ligation-dependent probe amplification; SNV, single nucleotide variant.
translocations including \(\text{IGH-CCND1} (n = 4)\), \(\text{IGH-BCL6} (n = 4)\), \(\text{IGH-NSD2} (n = 3)\), and \(\text{IGH-MYC} (n = 3)\).

One of the purposes of this study was to estimate the prevalence of patients with PAF. The CSeq assay identified PAF in 26 of 30 patients (86.7%) with AML, 11 of 17 patients with ALL (64.7%), 20 of 24 patients (83.3%) with MM, and 22 of 25 patients (88.0%) with DLBCL (Figure 1). Thus, a total 79 of 96 patients with hematological malignancies (82.2%) had PAF, demonstrating the high clinical efficacy of this assay.

### 3.3 Clinical utility of the CSeq assay in drug selection

We identified actionable alterations leading to drug selection in 44 cases, most of which were considered as being of preclinical significance \((n = 36, \text{evidence level C})\) (Figure 2 and Table S5). These alterations \((n \geq 3)\) and potential modes of targeted therapy (evidence level C) included RAS pathway mutations \((n = 13, \text{BRAF and MEK inhibition})\), \(\text{CD79A/79B mutations} (n = 7, \text{PKC inhibition})\), \(\text{MYD88 mutations} (n = 4, \text{BTK inhibition})\), \(\text{TP53 mutations} (n = 4, \text{DNMT1 inhibition})\), and \(\text{CREBBP mutations} (n = 3, \text{HDAC inhibition})\). Two types of alterations, namely \(\text{FLT3-ITD} (n = 5, \text{FLT3 inhibition})\) and \(\text{IDH1/2 mutations} (n = 5, \text{IDH1/2 inhibition})\) were identified with a clinical evidence of level A in 8 patients with AML (Table S5).

### 3.4 Clinical diagnostic utility of the CSeq assay

Based on information from diagnostic guidelines and some crucial reports (Table S1), we tried to divide morphologically diagnosed disease into molecular subtypes after discussing the validity at the molecular tumor board meeting. Through our analysis, AML, 1 of the diseases with the most advanced molecular diagnosis, could be subclassified into 6 subtypes\(^{12,14,32}\): AML with \(\text{NPM1} (n = 7)\), AML with mutated chromatin, RNA-splicing genes, or both \((n = 5)\), AML with \(\text{TP53} (n = 5)\), AML with \(\text{CREBBP} (n = 3)\), and AML with \(\text{IDH1/2} (n = 3)\).
both (n = 4), AML with biallelic CEBPA mutations (n = 3), myeloid leukemia associated with Down syndrome (ML-DS, n = 2), and myeloid neoplasms with DDX41 germline mutations (n = 2) (Figure 3 and Table S4).

We also analyzed the diagnostic utility of CSeq analysis for patients with ALL, MM, and DLBCL. CSeq analysis revealed sub-type-defining events in 4 cases with B-ALL (iAMP21; n = 1, IGH-DUX4; n = 1, high-hyperdiploidy; n = 1, and KMT2A-MLLT3; n = 1). For patients with MM, 4 groups including hyperdiploidy (n = 8) and IGH-CCND1 (n = 4), IGH-NSD2 (n = 3), and IGH-MAFB (n = 1) translocations were identified, based on our analysis of CNVs and IGH translocations. We also classified DLBCL into 5 subtypes16; the MCD type (n = 6), the BN2 type (n = 3), the EZB type (n = 3), the high-grade B-cell lymphoma type (n = 2), and the N1 type (n = 1) (Figure 3 and Table S4). These results for DLBCL were almost consistent with pathological findings in cell of different origins independently analyzed by a pathologist, since 5 of 6 MCD types were non-GCB (germinal center B-cell) types and 2 of 3 EZB type were GCB types (Table S4).16

Interestingly, the CSeq assay leads to diagnostic changes in 4 cases. In 1 case with morphologically DLBCL, we identified both RHOA G17V and IDH2 R172W hot-spot mutations, which is strongly suggestive of angioimmunoblastic T-cell lymphoma (AITL).33 Confirmation of clonality involving both T-cell receptor and immunoglobulin production in tumors enabled us to diagnose this case as composite lymphoma (AITL and DLBCL; Figure S3).34 In 1 case initially diagnosed as B-ALL and 2 cases initially diagnosed as DLBCL, identification of key diagnostic alterations lead to a new diagnosis of Burkitt lymphoma/leukemia, high-grade B-cell lymphoma with MYC and BCL6 rearrangements, and high-grade B-cell lymphoma, not otherwise specified, respectively, according to the WHO's 2017 classification scheme (Figure 3 and Tables S4, S6).13

3.5 | Clinical utility of the CSeq assay in prognosis

The CSeq assay provided prognostic information with strong clinical evidence (level A or B) for 24 patients with AML (80.0%), 8 patients with ALL (47.0%), 19 patients with MM (79.1%), and 10 patients with DLBCL (40.0%) (Figure 2 and Table S4). The most frequent genetic alterations associated with prognosis in each disease were NPM1 mutations in AML (n = 7, favorable risk or intermediate risk), IKZF1 loss in ALL (n = 7, adverse risk), hyperdiploidy in MM (n = 8, standard risk), and TP53 mutations/del (17p) in DLBCL (n = 7, adverse risk).

3.6 | Cancer-related germline mutations

We identified 2 AML patients who harbored both a DDX41 germline mutation (A500fs) and a DDX41 somatic mutation (R525H), and another AML patient harboring a deleterious BRCA2 germline mutation, and these results were validated by Sanger sequencing (Table 3). The former patient was diagnosed as having myeloid leukemia with DDX41 mutations (evidence level A) and the latter was diagnosed as having a risk for developing hereditary breast and ovarian cancer (evidence level A). All 3 patients with germline mutations received genetic counseling for future cancer risks (Table 3).
No germline mutations were identified in patients with ALL, MM, and DLBCL.

### 3.7 Sequencing results that strongly influenced clinical actions

At 6 mo after registration, all patients were prospectively surveyed regarding their clinical course, treatment regimen, and participation in a clinical trial. For the patients with targetable molecular aberrations, we assessed whether treatment according to the CSeq reports was delivered or not. Although 44 patients received sequencing results regarding target therapy (evidence level A: 8 cases; evidence level C: 36 cases), no patients enrolled in clinical trials or received therapies based on sequencing results, except for 1 patient with an FLT3-ITD mutation that was already identified before registration (Table S5). Based on the prognostic information, 2 patients with AML underwent different treatment strategies; 1 patient selected chemotherapy rather than allogeneic stem cell transplantation; the other patient underwent a different treatment protocol (Table 3). Furthermore, although there is not enough clinical evidence in the management of DLBCL, the clinician also changed the treatment for 2 patients with DLBCL according to diagnostic or prognostic information; 1 patient underwent different treatment cycles, the other patient was given an additional radiation therapy. Patients diagnosed with composite lymphoma were treated and followed as having AITL, rather than DLBCL.

### 4 DISCUSSION

To assess the utility of genomic medicine in hematological malignancies, we performed a multicenter prospective study of capture-based panel sequencing for patients with these disorders. This prospective study demonstrated the feasibility of the CSeq assay in that it showed: (i) a high incidence of cases with PAF as assessed by standard criteria, (ii) a permissible turnaround time, and (iii) sequencing results likely to have high specificity. However, despite the growing availability of genomic medicine, 2 major issues need to be resolved. First, manual curation of sequencing results in the context of diagnostic, prognostic, and therapeutic value requires substantial work by the curator and causes a long waiting period before the final
| ID  | Diagnosis     | Clinical stage | Therapy options without sequencing | Actionable findings                                      | Clinical interpretation | Clinical actions                  |
|-----|---------------|----------------|------------------------------------|----------------------------------------------------------|-------------------------|-----------------------------------|
| CSeq-01 | AML           | Primary       | No genetic counseling              | DDX41 p.A500fs Myeloid neoplasms with germline            | Genotype counseling for  | DDX41 mutations                   |
| CSeq-09 | AML           | Primary       | No genetic counseling              | DDX41 p.A500fs Myeloid neoplasms with germline            | Genotype counseling for  | DDX41 mutations                   |
| CSeq-10 | AML           | Primary       | Allogeneic stem cell transplantation at first CR | Biallelic CEBPA mutations                                 | Favorable risk (Prognosis) | Continuation of cytotoxic chemotherapy without allogeneic stem cell transplantation |
| CSeq-11 | AML           | Primary       | Consolidation therapy containing multi-agent chemotherapy | Biallelic CEBPA mutations                                 | Favorable risk (Prognosis) | Change to consolidation therapy containing high-dose cytarabine alone |
| CSeq-14 | AML           | Primary       | No genetic counseling              | BRCA2 p.S1882X Risk for developing hereditary breast and ovarian cancer (Diagnosis) | Genotype counseling for | BRCA2 mutations                    |
| CSeq-73 | DLBCL         | Primary       | R-CHOP 6 cycles                    | TP53 p.E271K Adverse risk (Prognosis)                     | R-CHOP 8 cycles         |
| CSeq-77 | DLBCL         | Primary       | R-CHOP 8 cycles                    | MYD88 p.243N, CD79A p.190_202 del                        | MCD type (Diagnosis)    | Addition of radiation therapy for primary tumor site |
| CSeq-92 | DLBCL         | Primary       | Treatment for DLBCL                | RHOA p.G17V, IDH2 p.R172W Composite lymphoma (Diagnosis) | Treatment for both DLBCL and AITL |
a wide variety of recurrent chromosome rearrangements define different disease subtypes for ALL.\textsuperscript{1,5,42,43} Second, this assay is less sensitive in detecting IGH translocations (Table 1), which was probably caused by insufficient disposition of IGH capture probes or a mapping failure due to tandemly repeated sequences in the IGH regions. Combining this assay with RNA sequencing as well as existing laboratory tests would provide more excellent sequencing performance and improve clinical decision making for patients with hematological malignancies. Third, this assay is not suitable for evaluating clonal hematopoiesis of indeterminate potential (CHIP), a risk factor of hematological malignancies and cardiovascular diseases,\textsuperscript{3,44} because we used buccal mucosa as a control specimen for the patients with AML or ALL. Lastly, DLBCL tumor specimens were extracted not from formalin-fixed paraffin-embedded tissues but from freshly resected tumor tissues to ensure the sensitivity and specificity of this genomic analysis. This may differ from the actual clinical practice.

In conclusion, the CSeq assay enables detection of somatic and germline mutations in patients with hematological malignancies, which makes it a useful diagnostic and prognostic testing tool. Our findings suggest that using the panel test for hematological malignancies would be feasible, but further optimization of NGS analysis and developing system that allows easy access to unapproved drugs may improve treatment outcomes for patients with these disorders.

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DISCLOSURE

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

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