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Rapid and Automated Semiconductor-Based Next-Generation Sequencing for Simultaneous Detection of Somatic DNA and RNA Aberrations in Myeloid Neoplasms

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Evaluation of suspected myeloid neoplasms involves testing for recurrent, diagnostically and therapeutically relevant genetic alterations. Current molecular testing requires multiple technologies, different domains of expertise, and unconnected workflows, resulting in variable, lengthy turnaround times that can delay treatment. To address this unmet clinical need, we evaluated the Oncomine Myeloid Assay GX panel on the Ion Torrent Genexus platform, a rapid, integrated nucleic acid to report next-generation sequencing platform for detecting clinically relevant genetic aberrations in myeloid disorders. Specimens included synthetic DNA (101 targets) and RNA (9 targets) controls and real-world nucleic acid material derived from bone marrow or peripheral blood samples (40 patients). Ion Torrent Genexus results and performance indices were compared with those obtained from clinically validated genomic testing workflows in 2 separate clinical laboratories. The Ion Torrent Genexus identified 100% of DNA and RNA control variants. For primary patient specimens, the Ion Torrent Genexus reported 82 of 107 DNA variants and 19 of 19 RNA gene fusions identified on clinically validated assays, yielding an 80% overall detection rate. Reanalysis of exported, unfiltered Ion Torrent Genexus data revealed 15 DNA variants not called by the filtered on-board bioinformatics pipeline, yielding a 92% potential detection rate. These results hold promise for the implementation of an integrated next-generation sequencing system to rapidly detect genetic aberrations, facilitating accurate, genomics-based diagnoses and accelerated time to precision therapies in myeloid neoplasms. *(J Mol Diagn 2023, 25: 87—93; https://doi.org/10.1016/j.jmoldx.2022.11.005)*

The discovery of recurrent genetic alterations in myeloid neoplasms has improved diagnostic accuracy and expanded the targeted treatment options available to patients. This progress is especially pertinent in acute myeloid leukemia (AML), which bears a dismal 30.5% relative 5-year survival (Surveillance, Epidemiology, and End Results, *https://seer.cancer.gov/statfacts/html/amyl.html*, last accessed May 2, 2022). Not only have genetic studies allowed the recognition of AML subtypes defined by key genetic alterations, correlating with prognosis, genetic testing has also become a key tool for identifying residual or recurrent disease.[1—9] As a result, the current diagnostic workup of suspected AML involves evaluation of clinical, morphologic, immunophenotypic, cytogenetic, and molecular genetic aberrations.

Recently, a number of targeted therapies relying on the presence or absence of specific gene alterations have emerged with current National Comprehensive Cancer Network guidelines endorsing genetic testing for AML.[10,11]

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These drugs are used not only in the setting of relapsed or refractory disease but also as a component of induction chemotherapy in select settings. The successful implementation of these therapies relies on immediate knowledge of the leukemia's genetic features. These genetic alterations include large-scale chromosomal gains and losses, chromosomal translocations causing gene fusions, single-nucleotide variants (SNVs), insertions/deletions (indels), internal tandem duplications, and amplifications.

Unfortunately, the current diagnostic tools use multiple technologies, different domains of expertise, and unconnected workflows, resulting in markedly variable and lengthy turnaround times (TATs). Next-generation sequencing (NGS) is a powerful tool capable of identifying most of these alterations; however, current NGS platforms and bioinformatics bottlenecks represent significant barriers to an optimal and timely diagnosis, with TATs often exceeding 10 to 14 days, thereby delaying treatment decisions.[12] As a result, laboratories typically perform redundant testing, including standalone, single-gene sequencing, fluorescence *in situ* hybridization, and RT-PCR assays, to support a more rapid TAT for key variants.

To address these unmet clinical needs, the performance characteristics of the Oncomine Myeloid Assay GX panel on the Ion Torrent Genexus platform (Thermo Fisher Scientific, Waltham, MA) were evaluated. The Ion Torrent Genexus system leverages Ion semiconductor sequencing technology, which is based on the detection of pH changes, reflecting hydrogen ions that are released during DNA polymerization.[13] The reactions are run on a semiconductor chip (Ion Torrent GX5), which has a high-density array of micromachined wells wherein the biochemical process is scaled up in a massively parallel fashion. Given that the readout is the direct consequence of a biochemical reaction and no scanning or imaging is involved, the speed of base calling is rapid. Accordingly, this platform is well suited for rapid sequencing and exemplifies innovation wherein a chemical reaction is converted to a digital readout. The sequencing read lengths vary from 100 to 400 bases, and each chip generates 12 million to 15 million sequencing reads per lane or 48 million to 60 million reads per chip. The four-lane chip design offers the flexibility of performing sequencing one lane at a time.

Combined with the Ion Torrent Genexus system, the Oncomine Myeloid Assay GX panel permits simultaneous interrogation of 40 DNA target genes comprising 23 hotspots, 17 full genes (including *CALR*, *CEBPA,* and *TP53*) and 29 fusion driver genes with their fusion partners comprising >600 RNA fusion isoforms, and 5 expression target genes and 5 expression controls (Table 1).[14] The overall combination allows for a rapid (<24-hour nucleic acid to result TAT), integrated nucleic acid to report NGS platform for detection of clinically relevant genetic aberrations (SNVs, indels, and gene fusions) in a range of myeloid disorders, including AML, myeloproliferative neoplasms, myelodysplastic syndrome, chronic myelomonocytic leukemia, and juvenile myelomonocytic leukemia, among others.

Table 1 The 40 DNA Target Genes of the Oncomine Myeloid Assay

Hotspot genes				
ABL1	*BRAF*	***CBL***	*CSF3R*	***DNMT3A***
FLT3	*GATA2*	*HRAS*	***IDH1***	***IDH2***
JAK2	***KIT***	***KRAS***	*MPL*	***MYD88***
NPM1	***NRAS***	***PTPN11***	*SETBP1*	***SF3B1***
SRSF2	***U2AF1***	***WT1***		
Full genes				
ASXL1	***BCOR***	***CALR***	***CEBPA***	*ETV6*
EZH2	*IKZF1*	***NF1***	***PHF6***	***PRPF8***
RB1	***RUNX1***	***SH2B3***	***STAG2***	***TET2***
TP53	***ZRSR2***			
Fusion drivers				
ABL1	*ALK*	*BCL2*	*BRAF*	*CCND1*
CREBBP	*EGFR*	*ETV6*	*FGFR1*	*FGFR2*
FUS	*HMGA2*	*JAK2*	*KMT2A*	*MECOM*
MET	*MLLT3*	*MLLT10*	*MYBL1*	***MYH11***
NTRK3	*NUP214*	*PDGFRA*	*PDGFRB*	***RARA***
RBM15	***RUNX1***	*TCF3*	*TFE3*	
Expression genes				
BAALC	*MECOM*	*MYC*	*SMC1A*	*WT1*
Control genes				
EIF2B1	*FBXW2*	*PSMB2*	*PUM1*	*TRIM27*

The target genes comprised 23 hotspots, 17 full genes, 29 fusion driver genes with their fusion partners comprising >600 RNA fusion isoforms, and 5 expression target genes and 5 expression controls. Gene names in bold indicate those in which variants were identified during this study.

Materials and Methods

Characterization of Patients and Sample Acquisition

Clinical DNA and RNA samples were retrospectively identified from blood or bone marrow specimens that had already undergone nucleic acid extraction and genetic testing in a Clinical Laboratory Improvement Amendments (CLIA)–certified clinical laboratory following previously validated protocols. The search initially prioritized a variety of myeloid neoplasms, subsequently selecting lymphoid neoplasms to address those genes insufficiently covered by the myeloid neoplasm samples (eg, *TP53*). Another validation cohort of patient DNA and RNA samples was acquired from a collaborating, CLIA-certified laboratory, also extracted from patient blood and bone marrow using their clinically validated protocols. Finally, commercially available, synthetic DNA variant control samples with 101 targets (AcroMetrix Oncology Hotspot Control DNA, catalog number 969056; Thermo Fisher Scientific) as well as synthetic RNA variant control samples with 9 targets (Seraseq Myeloid Fusion RNA Mix, catalog number 0710-0407; SeraCare, Milford, MA) were selected to assess assay performance on a broader range of variants clinically relevant to myeloid neoplasia (Table 2). This study was granted an

institutional review board waiver for publication of data that was generated as a validation for clinical testing.

In-House Clinical Gene Fusion RT-PCR Testing

RNA was extracted from peripheral blood and bone marrow specimens with the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany). Extracted RNA was tested with RT-PCR protocols using the QuantiTect Multiplex PCR Kit (Qiagen). The protocols were clinically validated in their ability to qualitatively identify the following RNA fusion products: *RUNX1*::*RUNX1T1* (exon 5/exon 2), *CBFB*::*MYH11* (types A, D, and E), *PML*::*RARA* (intron 6, exon 6, and intron 3), and *BCR*::*ABL1* (p190 and p210).

In-House Clinical Hematolymphoid Neoplasm Gene Panel Sequencing and Variant Analyses

In-house clinical NGS testing was performed in a CLIA-certified genomics laboratory as previously reported.[15] Briefly, DNA was extracted from blood or bone marrow aspirate specimens using a DNA extraction kit (Qiagen). Libraries were prepared and normalized, then pooled for sequencing on a MiSeq instrument (Illumina, San Diego, CA). Fastq files generated from sequencing runs were processed through a custom in-house bioinformatics pipeline, yielding a set of variants that were interpretated and reported by a pathologist according to the clinically validated protocol. Variants deemed disease associated, probably disease associated, or variant of undetermined significance were reported, whereas variants deemed benign or likely benign were not reported. The laboratory-established limit of detection for the assay is 5%; however, variants observed at lower levels are reported as indeterminate if they were identified on the patient's prior sample and if all quality metrics were met.

Ion Torrent Genexus Sequencing via Oncomine Myeloid Assay

To maximize efficient reagent use, 30 ng of DNA and 15 ng of RNA from unique patients with nonoverlapping mutational profiles were paired and tested with the Oncomine Myeloid Assay (catalog number A47857; Thermo Fisher Scientific) according to the manufacturer's instructions. Briefly, a total volume of 25 μL of DNA and 15 μL of RNA per sample were dispersed into 96-well clear reaction plate with a barcode (catalog number 4483354; Applied Biosystems, Waltham, MA) and the plate was sealed with adhesive PCR plate foil (catalog number AB0626; Thermo Fisher Scientific). Then the sealed sample plate, the library and templating strips, primer pool tubes, Ion Torrent Genexus cartridge, and buffer bottles were loaded in the Ion Torrent Genexus Integrated Sequencer (Thermo Fisher Scientific). Library preparation and equalization, template preparation, and sequencing were performed automatically by the Ion Torrent Genexus sequencer. Ion Torrent Genexus software version 6.2.1 (Thermo Fisher Scientific) was used to set up the sequencing run and perform the postrun analysis. Review of identified variants was explored with the full range of built-in variant filters. Variants reported by Ion Torrent Genexus were reviewed against an in-house variant database, and those found to be benign or likely benign were excluded from further analysis.

Assay Comparison and Resolution of Assay Discrepancies

Analysis of concordance was limited to those genes and variants shared by both the clinical and OncoMine assays. Variants classified as benign were excluded from analysis, and all samples that failed Ion Torrent Genexus quality control (QC) metrics were excluded from the final analyses. Subsequent analysis of Ion Torrent Genexus sequencing data was performed by exporting the filtered and unfiltered variant call format data files and converting them from hg19 (Ion Torrent Genexus) to hg38 (clinical assays), ensuring no loss of variants through the conversion. The final converted, unfiltered data were processed through the in-house bioinformatics pipeline's variant callers, and the resulting variant list was used to resolve remaining discrepancies between assays. Statistical analysis of sequencing performance (including both initially and subsequently identified variants in Ion Torrent Genexus sequencing data) was performed with tidyverse package version 1.3.1 of RStudio software version 2022.02.3+492.pro3 (Prairie Trillium, Boston, MA) to produce descriptive statistics and calculate the variant allele fraction (VAF) correlation coefficient.

Results

QC Metrics

The Ion Reporter System onboard the instrument reported quality parameters for the samples in the study as follows: percent loading of chip; 92.4%, raw read accuracy; 98.8%, mean reads per lane; 23,165; base call accuracy, 98.6%; and mean amplicon length, 117 bp.

Control Sample Performance

The Ion Torrent Genexus accurately reported all 96 SNVs and all 5 indels from synthetic DNA control material, which included 16 unique genes, a 22-bp *FLT3* internal tandem duplication (ITD), and a 30-bp multiple nucleotide polymorphism in *KIT*. Similarly, 9 of 9 RNA control fusion variants were accurately reported, including 7 unique driver genes (Table 2).

Patient DNA Performance

For in-house primary patient blood- or marrow-derived DNA samples, samples from 12 patients with 10 unique hematolymphoid neoplasms were analyzed. The variants identified in these patients' specimens encompassed 31 unique genes shared by both the clinical and Oncomine assays (Figure 1 and Supplemental Table S1). With the use of only standard and extended built-in filters, the Ion Torrent Genexus reported 30 of 37 SNVs and 18 of 23 indels identified by the in-house, clinically validated assay, yielding an overall DNA variant detection rate of 80% for patient-derived samples. Reanalysis of unfiltered Ion Torrent Genexus sequencing data using in-house variant callers and manual analysis revealed 7 SNVs and 1 indel not called by the on-board rapid NGS bioinformatics pipeline, raising the overall sequencing detection rate to 93% with 4 undetected variants. Within the external patient validation material, the Ion Torrent Genexus reported 34 of 47 DNA variants (72%) with an additional 7 variants found in the unfiltered data (87% recovery). Of note, a single external sample failed Ion Torrent Genexus QC metrics and was excluded from analysis.

Overall, 82 of 107 clinically identified DNA variants (77%) were found on the Ion Torrent Genexus standard or extended filtered lists, increasing to 97 of 107 (91%) when reviewing unfiltered Ion Torrent Genexus data (Table 3). These variants included insertions as large as 75 bp (*FLT3*) and deletions as large as 55 bp (*RUNX1*). DNA variants were called at a mean VAF of 31.5%, ranging from 3.5% to 92.2%. Comparison of the VAF between methods yielded a Pearson correlation coefficient of $r^2 = 0.98$ (Figure 2).

Variants recovered from the Ion Torrent Genexus unfiltered data included variants in *ASXL1*, *DNMT3A*, *FLT3*, *KRAS*, *NRAS*, *PRPF8*, *PTPN11*, *RB1*, *STAG2*, *TET2*, *TP53*, and *ZRSR2*. Variants ultimately not identified in Ion Torrent Genexus sequencing data included variants in *ASXL1*, *CEBPA*, *FLT3*, *PHF6*, *TET2*, and *WT1* and were frequently indels (8 of 11), had a VAF <5% (7 of 11), and/or coexisted with additional variants in the same gene (6 of 11). Of those unidentified variants, only 1 had potentially significant, immediate clinical implications (isolated *FLT3* ITD, 165 bp, VAF of 2.1%). All 3 *IDH1*/*IDH2* variants were reported on Ion Torrent Genexus filtered lists. Beyond the clinically identified variants, however, Ion Torrent Genexus also identified 20 DNA variants not called by the clinical assays, including 8 SNVs and 12 indels, most commonly involving *BCOR* (Supplemental Table S1).

Patient RNA Performance

The Ion Torrent Genexus reported 10 of 10 gene fusions and was appropriately negative in 2 in-house patient-derived RNA samples, with an overall 100% concordance rate. Performance was similar on external patient RNA, with 7 of 7 fusions reported and an overall patient fusion detection rate of 100% (Table 3). Variants included t(8;21), t(15;17), and inv(16) with patient blast counts as low as 1% and a

Table 2 Control Samples Analyzed by the Ion Torrent Genexus Instrument

Gene	Mutations
ABL1	p.D276G, p.M351T, p.E355G, p.F359V, p.I293delinsMP
BRAF	p.V600E, p.L597R, p.N594G, p.N581S, p.G464V, p.R444W
CEBPA	p.L253R
DNMT3A	p.C911Y
EZH2	p.Y646F
FLT3	p.D839G, p.D835Y, p.A680V, p.V592A, p.F594_D600dup(x2), p.G831E, p.S451F
HRAS	p.Q61R, p.G12V, p.A59T
IDH1	p.R132H
IDH2	p.R172K, p.R140Q
KIT	p.A502_Y503dup, p.[N566=;N567=;Y568=;V569=;Y570=;I571=;D572=;P573=;T574=;Q575=;L576P], p.K509I, p.L576P, p.K642E, p.V654A, p.F469L, p.F506L, p.N512S
KRAS	p.G12D, p.T35I
MPL	p.S505N, p.W515L, p.A519T
NRAS	p.Q61R, p.G12D, p.A18T, p.G10E
PTPN11	p.D61Y, p.E69K, p.A72V, p.E76K, p.G503A, p.Q510K, p.P491L, p.T507A
RB1	p.E137Ter, p.L199Ter, p.R320Ter, p.R358Ter, p.R455Ter, p.R552Ter, p.R556Ter, p.R579Ter, p.L676FfsTer16, p.Q685Ter, p.K715Ter, p.N328D, p.W563R, p.L688P, p.C706F, p.D718G
TP53	p.Q375Ter, p.R342Ter, p.E339Ter, p.E331Ter, p.W327Ter, p.Q317Ter, p.R306Ter, p.E298Ter, p.W91Ter, p.E56Ter, p.E51Ter, p.P278L, p.R273H, p.R248Q, p.G245S, p.S241F, p.Y234C, p.Y220C, p.V216M, p.Y205C, p.Y163C, p.V157F, p.C135Y, p.L132R, p.S127F, p.?, p.R337C, p.G334V, p.V218E, p.A161T, p.L130V, p.R110L
Fusions	*BCR*(14)::*ABL1*(2), *ETV6*(4)::*ABL1*(2), *ETV6*(5)::*ABL1*(2), *FIP1L1*(11)::*PDGFRA*(12), *KAT6A*(17)::*CREBBP*(2), *PCM1*(23)::*JAK2*(12), *PML*(6)::*RARA*(3), *RUNX1*(3)::*RUNX1T1*(3), *TCF3*(16)::*PBX1*(3)

All the control genes were accurately reported by the instrument's built-in bioinformatics system. The SNV and indel samples consisted of 101 commercially available synthetic DNA controls. Fusion controls consisted of 9 commercially available synthetic RNA controls.

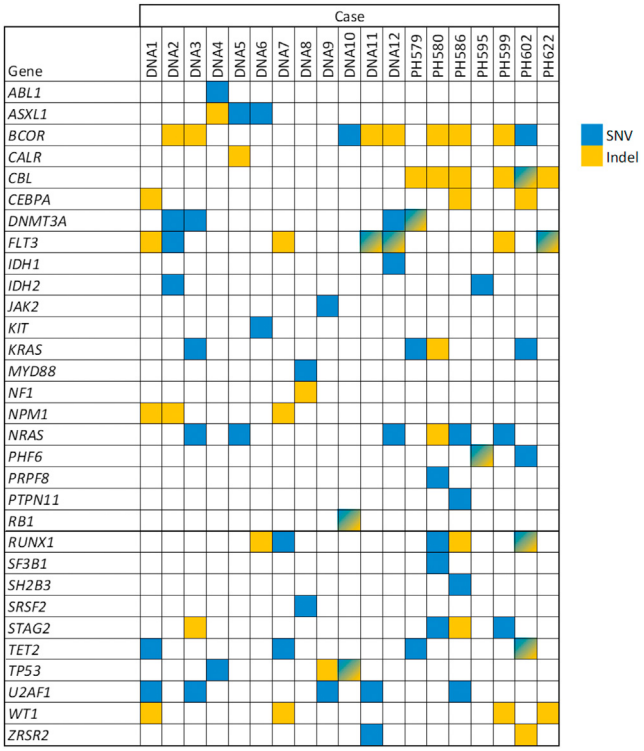

Figure 1 Co-occurrence plot of DNA alterations identified in patient samples by clinical or Ion Torrent Genexus sequencing assays by case and gene involved. **Blue** indicates the presence of a single-nucleotide variant (SNV) and **yellow** indicates the presence of an insertion, deletion, or deletion-insertion (indel). A **blue-yellow gradient** indicates the presence of both variant types in the gene of interest.

normalized copy number as low as 0.00142. Notably, one patient sample failed Ion Torrent Genexus QC metrics and was excluded from analysis. This sample contained a *BCR*::*ABL1* p190 fusion with a normalized copy number of 0.0002 (Supplemental Table S2). Additionally, one sample with clinical suspicion for low-level residual disease had <10 *PML*::*RARA* transcripts identified and was interpreted as indeterminate by RT-PCR, whereas the Ion Torrent Genexus result was negative.

Discussion

These findings provide early data supporting the feasibility and reliability of a rapid, clinical NGS assay for myeloid neoplasms. To assess accuracy of the platform, 101 variants from synthetic standards were evaluated. All 101 variants, which included SNVs and indels, were accurately detected (100% accuracy). Real-world samples were subsequently analyzed. With 17 of 18 real-world samples passing QC metrics, the assay demonstrated a 93% success rate on patient sample-derived nucleic acid material and reported 81% of variants identified by clinically validated assays. Among those 19% of variants unreported by the Oncomine/Ion Torrent Genexus assay, only one would have resulted in a significant clinical difference in patient management, namely the inappropriate withholding of anti-FLT3 therapy. All *IDH1*/*IDH2* variants and all therapeutically relevant gene fusions were reported accurately when present at diagnostic levels. No diagnostically relevant variants went unreported by the Ion Torrent Genexus assay. Overall sequencing metrics (VAF) were comparable between platforms.

Significant laboratory workflow benefits are noted with the Oncomine/Ion Torrent Genexus platform compared with the current testing methods. Given the rapid turnaround and breadth of coverage, the myeloid panel on the OncoMine/Ion Torrent Genexus platform effectively covered genomic variants detected using a variety of assays, including fluorescence *in situ* hybridization, RT-PCR, and NGS testing for diagnosis of myeloid disorders. The workflow permitted judicious use of frequently limited samples, which would otherwise be depleted due to exhaustion across different assays involved in the workup of myeloid neoplasms. With regard to the technical aspects of NGS, a standard NGS system requires an operator to complete nucleic acid extraction, shearing, nucleic acid QC, library preparation and normalization, sequencing, bioinformatics processing, and manual variant verification, interpretation, and reporting. In contrast, the evaluated Ion Torrent Genexus platform only requires an operator to extract and dilute nucleic acids, loading them with reagents onto the Ion Torrent Genexus instrument. The remaining templating, library preparation, sequencing, and first-pass analytics steps, including quality metrics and reporting, are integrated and automated within the systems workflow.

Table 3 Summary Statistics of Variant Performance by Nucleic Acid Material, Variant Type, and Gene of Interest

	No./total no. (%)	
Variant	Initial concordance	Recovered concordance
All variants	101/126 (80)	116/126 (92)
DNA variants	82/107 (77)	97/107 (91)
SNVs	50/67 (75)	64/67 (96)
Indels	32/40 (80)	33/40 (83)
RNA fusions	19/19 (100)	19/19 (100)
Key variants		
FLT3 ITD/indel	5/7 (71)	6/7 (86)
FLT3 D835	2/3 (67)	3/3 (100)
IDH1	1/1 (100)	1/1 (100)
IDH2	2/2 (100)	2/2 (100)
TP53	7/9 (78)	9/9 (100)
t(8;21)	4/4 (100)	4/4 (100)
t(9;22)	1/1 (100)	1/1 (100)
t(15;17)	7/7 (100)	7/7 (100)
inv(16)	5/5 (100)	5/5 (100)

Initial concordance describes those clinically identified variants reported on the Ion Torrent Genexus standard or extended filter lists. Recovered concordance describes the overall variant recovery rate after subsequent analysis of unfiltered Ion Torrent Genexus data.

Indels, insertions/deletions; ITD, internal tandem duplication; SNVs, single-nucleotide variants.

Limitations of Oncomine/Ion Torrent Genexus identified during this study include a few variants accurately sequenced by the platform but excluded in the tiered filtering protocol by the platform's on-board Ion Reporter bioinformatics pipeline. Accordingly, optimal implementation of the platform would include secondary use of a custom bioinformatics pipeline and database to improve frequency of accurate calls above and beyond default filters, an overall process that could still be performed with a more rapid TAT than a standard NGS tumor panel. Subsequent analysis of Ion Torrent Genexus sequencing data revealed most unreported variants within the unfiltered variant list. Unreported variants were particularly common in genes with multiple concurrent variants and at VAFs <5%. Furthermore, particular genes appeared more prone to underreported variants, namely *CEBPA* and *WT1*. Although 6 of 10 *FLT3* variants were initially filtered, 9 of 10 were ultimately identified in the sequencing data with only a single, 165-bp insertion unrecovered. For the RNA-based targets, although five target transcripts are included in the panel, they were not evaluated as a part of this study. These targets have the potential to identify less common variants typically found on conventional cytogenetics.

Given these considerations, Oncomine/Ion Torrent Genexus appears capable of accurate genetic testing with a rapid TAT, particularly in the setting of initial diagnosis. This capability has the potential to revolutionize the practice of hematopathologic diagnosis, allowing immediate, comprehensive genetic data to be available alongside morphologic and immunophenotypic data in rendering an initial diagnosis. With a comprehensive diagnosis available upfront, the potential for patient safety issues due to staggered result reporting is minimized. Furthermore, oncologists may be able to produce more targeted initial treatment plans and share more fully informed discussions of prognosis with patients, overall improving the treatment of myeloid neoplasms.

Clinical implementation of the Oncomine/Ion Torrent Genexus for routine genomic testing would conceivably be customized for the particular clinical practice setting in which it is being deployed. The proposed deployment in the authors' clinical setting entails export of sequencing data output (eg, binary alignment map or variant call format) to the laboratory's in-house custom pipeline for variant calling, examination of the results by dedicated expert variant reviewers, and final verification by attending faculty. Automation of the technical aspects of sequencing results in minimization of manual steps in analysis and workload improvements for laboratory personnel, without compromising of accuracy of variant calling and achievement of rapid (nucleic acid to result) TATs.

Automated and integrated workflow-based platforms that deliver clinically relevant results in <24 hours carry the

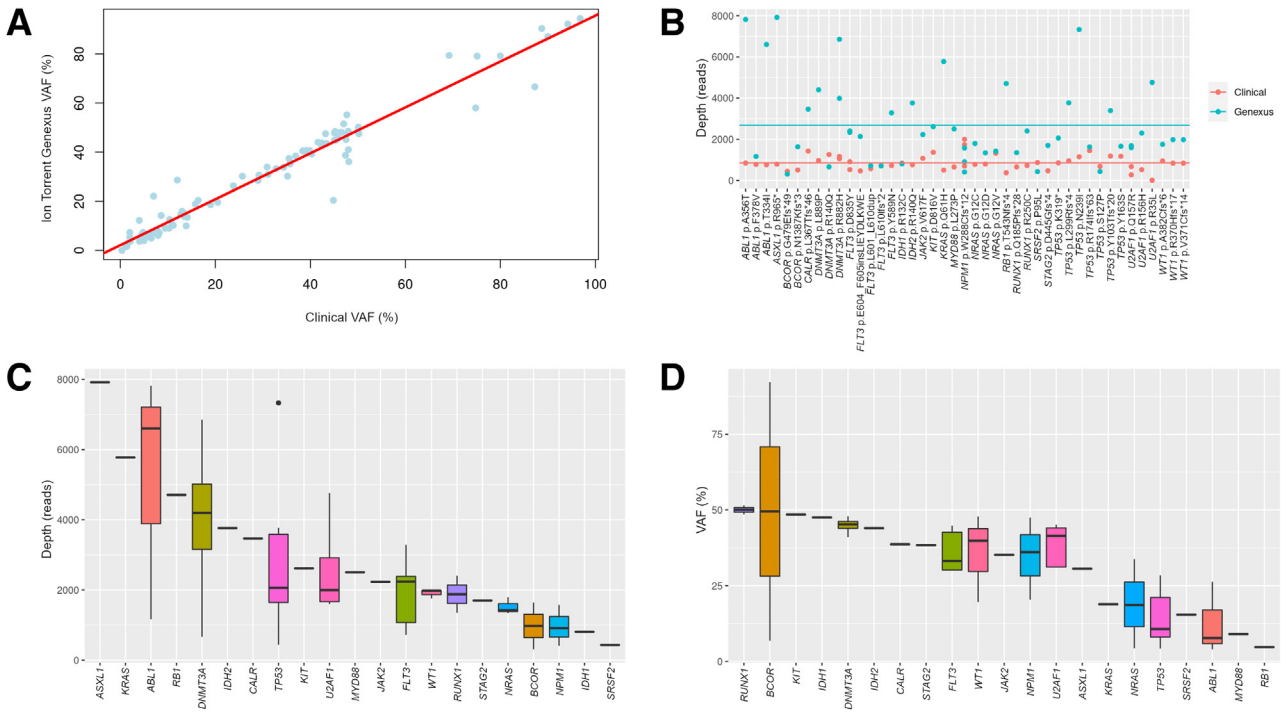

Figure 2 **A:** Comparison of clinical assay variant allele fraction (VAF) and Ion Torrent Genexus VAF showed high correlation ($r^2 = 0.98$) across a broad range of VAFs, including both in-house and external patient samples. All clinically identified DNA variants recovered from Ion Torrent Genexus sequencing data were included in this analysis. **B:** Comparison of depth between the Ion Torrent Genexus and the clinical assay revealed substantial coverage variability in the Ion Torrent Genexus with an overall higher mean depth than the clinical assay. **C** and **D:** When considering Ion Torrent Genexus depth (**C**) and VAF (**D**) on a gene-by-gene basis, relatively greater consistency within a specific gene target was noted, and the overall greater coverage may allow increased sensitivity for low-level variants.

potential to revolutionize the diagnostic workup of neoplastic conditions.[16] The availability of accurate results in clinically relevant timescales will enable deployment of genomic studies in the frontline for diagnostic evaluation of patients. It is anticipated that automated workflows such as these will improve operational efficiency and exert significant economic impact on laboratory expenses given the reduced requirement for human agency for execution of the laboratory tests.

Author Contributions

K.S.J.E.-J. conceptualized and supervised the study; R.W., J.J.D.M., M.S.L., and K.S.J.E.-J. designed the study; P.S. and J.P. coordinated external validation samples; R.T.S. coordinated the study; R.W. performed wet-bench experiments on the Ion Torrent Genexus platform; A.B., C.R., and A.C. performed bioinformatic and statistical analyses; R.T.S. and C.M.S. correlated results of the rapid platform with in-house capture-based platform; C.M.S. compiled and organized the clinical and sequencing data and performed statistical analyses; and C.M.S., G.Y., M.S.L., and K.S.J.E.-J. prepared the manuscript.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2022.11.005*.

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