Performance of the Oncomine Myeloid Research Assay—a comprehensive NGS panel for analysis of myeloid malignancies

Disease background

Hematological or blood cancers are a broad group of distinct cancer subtypes impacting blood-forming tissue such as bone marrow or cells of the immune system. More prevalent subtypes such as acute and chronic myeloid leukemia (AML and CML) together account for close to 2% of new cancer cases and 2% of cancer deaths annually in the United States alone (SEER database) [1]. Molecular biomarkers are increasingly being included in established clinical guidelines for AML, CML, and less prevalent myeloid disease subtypes such as acute promyelocytic leukemia (APL), chronic myelomonocytic leukemia (CMML), myelodysplastic syndrome (MDS), juvenile myelomonocytic leukemia (JMML), and myeloproliferative neoplasms (MPN). Clinical trials are ongoing for multiple markers within each subtype. These malignancies contain a diverse and heterogeneous set of genomic alterations that include recurrent somatic mutations in key driver genes as well as frequent and diagnostically relevant chromosomal rearrangements that generate a wide array of gene fusion products. Current analysis of hematological malignancies involves multiple sequential lab tests and laborious workflows with high sample requirements. To accelerate ongoing translational research into these disorders, we developed a sensitive and robust sample-to-answer workflow for measuring and reporting on key myeloid DNA mutations and RNA fusions using a single next-generation sequencing (NGS) assay.

Detecting and reporting relevant myeloid variants with one NGS assay

Assay content

To identify gene targets that are recurrently altered in myeloid cancers and are relevant for clinical and translational research applications, we performed an extensive survey of investigators at hematology-oncology research labs, followed by a comprehensive review of scientific literature and genomic databases. The assay was designed with an emphasis on potential prognostic or diagnostic markers for myeloid malignancies that are associated with ongoing clinical trials or are listed in clinical guidelines from US (NCCN) or European (ESMO) advisory bodies. Additionally, several of the targets have associated relevant therapies approved by US (FDA) or European (EMA) regulatory bodies. The assay also covers 30 of the 34 genes recommended by the Association for Molecular Pathology for screening for chronic myeloid neoplasms [2].

A targeted Ion AmpliSeq™ panel was generated to support the detection of recurrent single-nucleotide variants (SNVs), insertions/deletions (indels), and gene fusions from blood or bone marrow samples. The resulting Ion Torrent™ Oncomine™ Myeloid Research Assay (Figures 1 and 2) targets 40 DNA genes and 29 RNA fusion driver genes (representing 700 fusion isoforms) associated with major myeloid disorders. This includes full coding sequence coverage of tumor suppressor genes for detection of *de novo* deleterious mutations, and targeted hotspot coverage of genes with activating or resistance mutations. Panel design was also optimized to achieve high coverage of challenging targets such as *CEBPA* and internal tandem duplications of *FLT3* (*FLT3*-internal tandem duplications (ITDs)). The relevancy of the panel content to major myeloid cancers is demonstrated in Figure 3.

Hotspot genes (23)		Full genes (17)		Fusion driver genes (29)			Expression genes (5)	Expression control genes (5)
ABL1	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*HMGA2*	*NUP214*	*BAALC*	*EIF2B1*
BRAF	*MPL*	*BCOR*	*RB1*	*ALK*	*JAK2*	*PDGFRA*	*MECOM*	*FBXW2*
CBL	*MYD88*	*CALR*	*RUNX1*	*BCL2*	*KMT2A (MLL)*	*PDGFRB*	*MYC*	*PSMB2*
CSF3R	*NPM1*	*CEBPA*	*SH2B3*	*BRAF*	*MECOM*	*RARA*	*SMC1A*	*PUM1*
DNMT3A	*NRAS*	*ETV6*	*STAG2*	*CCND1*	*MET*	*RBM15*	*WT1*	*TRIM27*
FLT3	*PTPN11*	*EZH2*	*TET2*	*CREBBP*	*MLLT10*	*RUNX1*		
GATA2	*SETBP1*	*IKZF1*	*TP53*	*EGFR*	*MLLT3*	*TCF3*		
HRAS	*SF3B1*	*NF1*	*ZRSR2*	*ETV6*	*MYBL1*	*TFE3*		
IDH1	*SRSF2*	*PHF6*		*FGFR1*	*MYH11*			
IDH2	*U2AF1*			*FGFR2*	*NTRK3*			
JAK2	*WT1*			*FUS*				
KIT								

Figure 1. Oncomine Myeloid Research Assay gene list.

AML		MDS	MPN	Core myeloid genes	
CEBPA	*BCR-ABL1*	*BCOR*	*CALR*	*ASXL1*	*SETBP1*
FLT3	*CBFB-MYH11*	*ETV6*	*JAK2*	*CBL*	*SF3B1*
IDH1	*DEK-NUP214*	*NF1*	*MPL*	*DNMT3A*	*SRSF2*
IDH2	*KMT2A (MLL)*	*PRPF8*	*CSF3R*	*EZH2*	*TET2*
KIT	*MLLT3*	*PTPN11*	*IDH1*	*KRAS*	*TP53*
NPM1	*PAX5-JAK2*	*STAG2*	*IDH2*	*NRAS*	*U2AF1*
WT1	*PML-RARA*		*SH2B3*	*RUNX1*	*ZRSR2*
	RUNX1-RUNX1T1				

Figure 2. Disease-specific content on the Oncomine Myeloid Research Assay. Content referenced from Acute Myeloid Leukemia, Version 3.2017, Myelodysplastic Syndromes Version 1.2018, and Myeloproliferative Neoplasms Version 2.2018 from NCCN Guidelines™ resource.

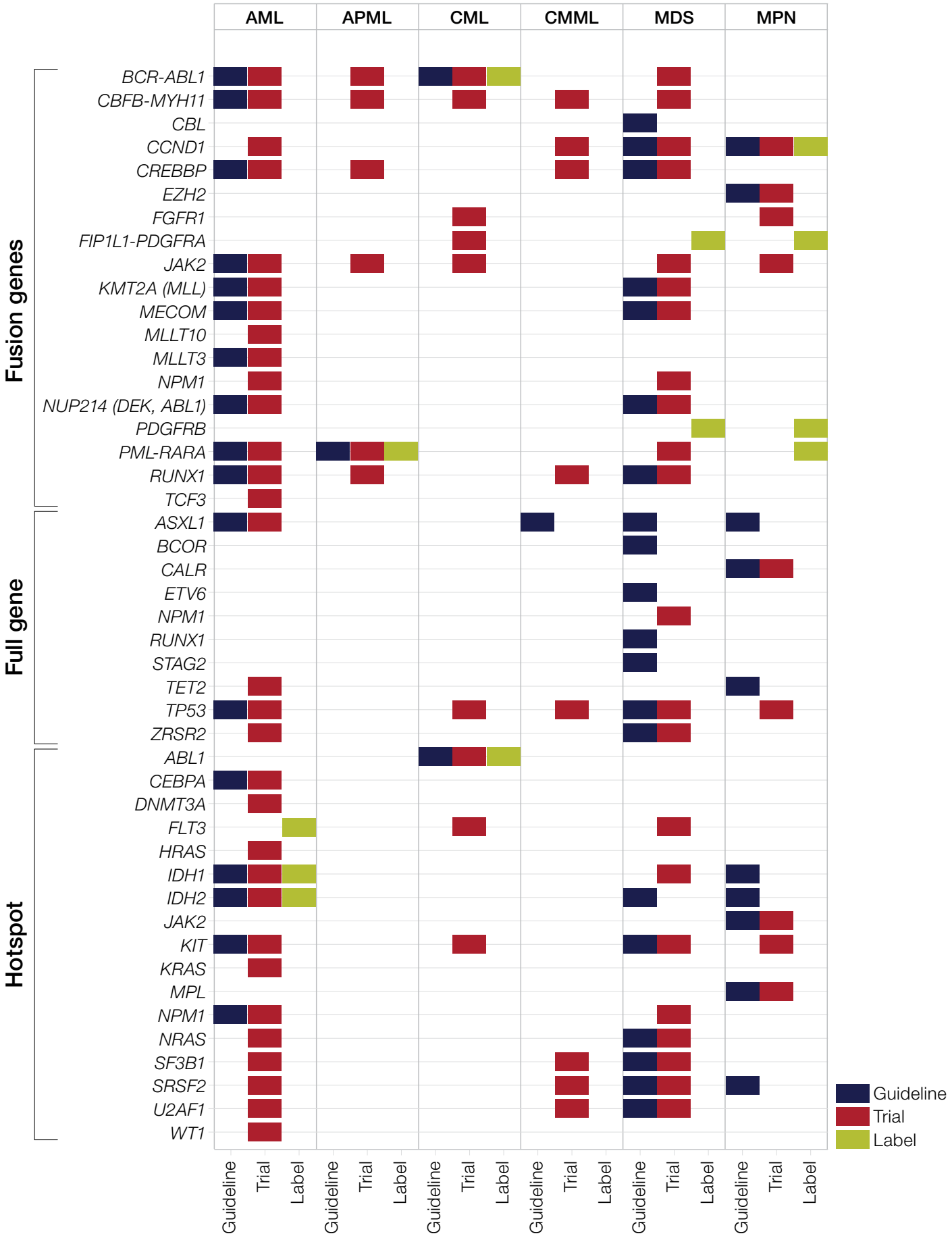


Figure 3. Disease relevancy of Oncomine Myeloid Research Assay content for major myeloid cancers.

Workflows and assay requirements

The Oncomine Myeloid Research Assay was developed using Ion AmpliSeq™ targeted sequencing technology to provide accurate detection of both DNA and RNA variants from as little as 10 ng of input nucleic acid per amplicon pool. This assay has two pools of DNA and one pool of RNA amplicons (526 and 700 amplicons, respectively). As with all Ion Torrent™ Oncomine™ assays, the Oncomine Myeloid Research Assay undergoes enhanced manufacturing QC, checking for presence of every amplicon, uniformity of sequencing, and absence of contamination.

Figure 4 shows the sample-to-answer workflow of the Oncomine Myeloid Research Assay, which can be used with as little as 20 ng DNA and 10 ng RNA extracted from blood or bone marrow samples. The assay supports three workflow configuration options (Figure 5), which offer the flexibility of manual or automated library preparation and sequencing on the Ion PGM™ System or Ion GeneStudio™ S5 System instruments. Results are delivered within 48 hours, starting with library preparation. Sequencing is performed with 400 bp chemistry to optimize detection of difficult variants such as *FLT3* ITD insertions. Data analysis is performed using integrated Ion Reporter™ Software for variant calling, and Ion Torrent™ Oncomine™ Reporter software links variants to relevant labels, guidelines, and clinical trials, as well as offering customized reporting.

Sample prep	Sequencing	Analytics
• Sample type—blood or bone marrow • Library preparation — 2 DNA pools—10 ng of gDNA for each pool – 1 RNA pool—10 ng of RNA for 1 RNA pool • Template preparation (Ion Chef System)	• High-throughput sequencing on Ion Gene Studio S5 Systems or Ion PGM System	• Automated reporting with Oncomine Reporter software

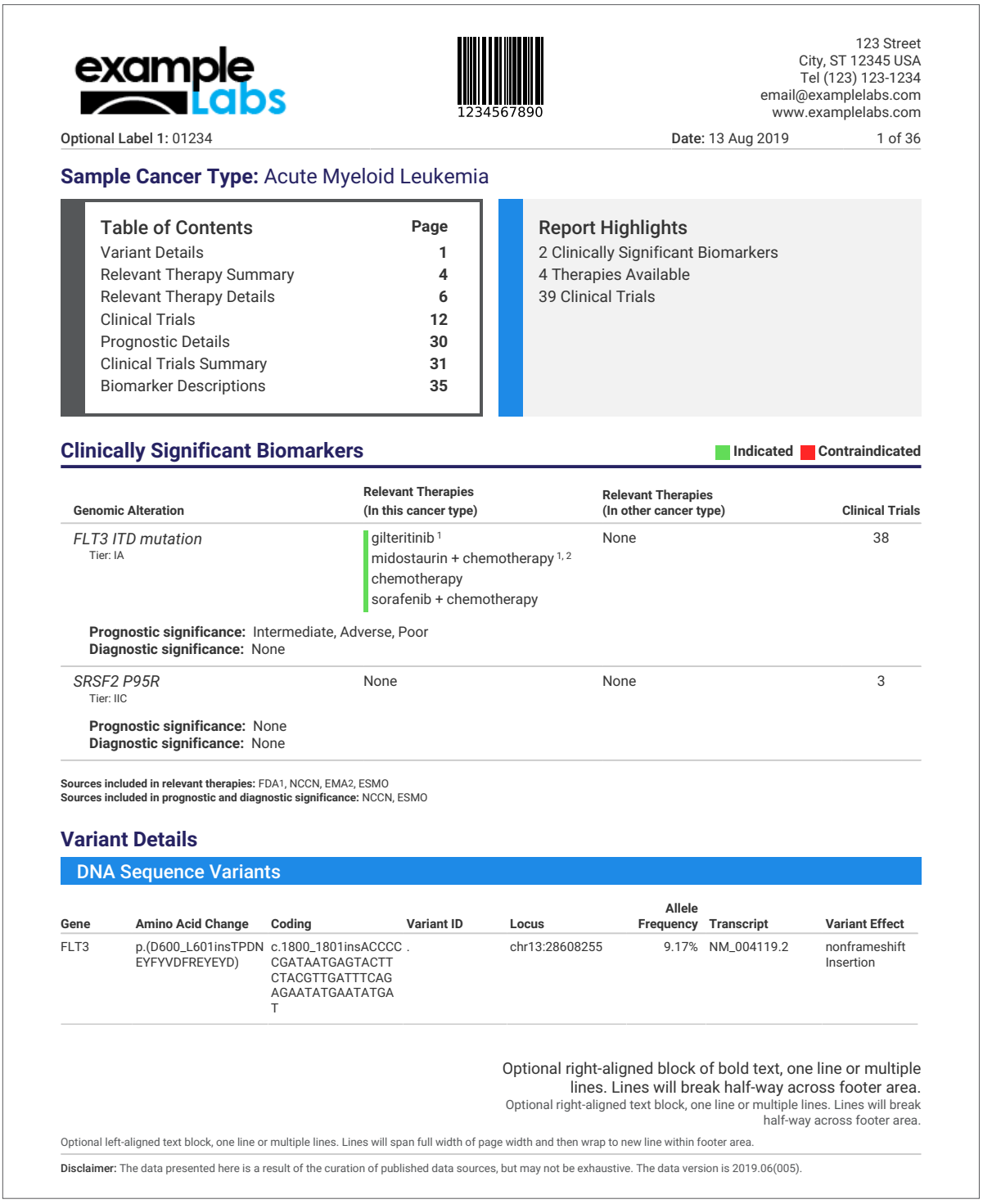
Figure 4. The Oncomine Myeloid Research Assay sample-to-answer workflow enables analysis of blood or bone marrow samples from myeloid malignancies.

	Instrument configuration	Library preparation	Templating	Sequencing	Chip
1	Ion OneTouch 2 System + Ion PGM Sequencer	**Manual** Oncomine Myeloid Research Assay (Cat. No. A36940) 24 libraries	Ion PGM Hi-Q View OT2 Kit (Cat. No. A29900) 8 runs/kit	Ion PGM Hi-Q View OT2 Kit (Cat. No. A29900) 8 runs/kit	Ion 318 Chip Kit v2 BC (Cat. No. 4488150) 8 chips/kit
2	Ion Chef instrument + Ion PGM Sequencer		Ion PGM Hi-Q View Chef 400 Kit (Cat. No. A30798) 8 runs/kit		Ion 318 Chip Kit v2 BC (Cat. No. 4488150)
3	Ion Chef instrument + Ion GeneStudio S5 Systems	**Automated** Oncomine Myeloid Research Assay (Cat. No. A36941) 32 libraries	Ion 510, 520, and 530 Kit - Chef (Cat. No. A34019) 1 run/kit		Ion 530 Chip Kit (Cat. No. A27764)

Figure 5. Three laboratory workflows supported by the Oncomine Myeloid Research Assay.

Data analysis with Oncomine Reporter

Data analysis post-sequencing is supported by Oncomine Reporter software, which enables customizable reporting of evidence for the targets detected by the Oncomine Myeloid Research Assay. The resulting reports (see example in Figure 6) include detailed information about clinical trials, guidelines, and drug labels, as well as gene narratives describing the clinical context for the driver genes with detected variants. The data used to support Oncomine Reporter annotation are meticulously curated from various global sources and updated quarterly. The process has QC steps built in at various stages. After data are collected, a team of expert curation scientists manually reviews all candidate evidence, and two independent reviewers examine each piece of candidate evidence for context and standardization. All approved evidence is then stored in a structured manner in a knowledgebase that serves to support reporting in Oncomine Reporter.



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Optional Label 1: 01234 **Date:** 13 Aug 2019 1 of 36

Sample Cancer Type: Acute Myeloid Leukemia

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Clinically Significant Biomarkers — Indicated, Contraindicated

Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
FLT3 ITD mutation Tier: IA	gilteritinib [1] midostaurin + chemotherapy [1, 2] chemotherapy sorafenib + chemotherapy	None	38
Prognostic significance: Intermediate, Adverse, Poor **Diagnostic significance:** None			
SRSF2 P95R Tier: IIC	None	None	3
Prognostic significance: None **Diagnostic significance:** None			

Sources included in relevant therapies: FDA1, NCCN, EMA2, ESMO
Sources included in prognostic and diagnostic significance: NCCN, ESMO

Variant Details

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect
FLT3	p.(D600_L601insTPDNEYFYVDFREYEYD)	c.1800_1801insACCCCCGATAATGAGTACTTCTACGTTGATTTCAGAGAATATGAATATGAT	.	chr13:28608255	9.17%	NM_004119.2	nonframeshift Insertion

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Optional right-aligned text block, one line or multiple lines. Lines will break half-way across footer area.

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Disclaimer: The data presented here is a result of the curation of published data sources, but may not be exhaustive. The data version is 2019.06(005).

Figure 6. Example of a customizable report generated using Oncomine Reporter software.

Materials and methods

SNV, indel, and *FLT3*-ITD calling performance was assessed using the Thermo Scientific™ AcroMetrix™ Oncology Hotspot Control and a set of orthogonally characterized blood and bone marrow clinical research samples. Additional blood and bone marrow samples, and the Seraseq™ Myeloid RNA control (SeraCare) were used to assess fusion variants representative of myeloid malignancies. Sample extraction was performed using the Invitrogen™ RecoverAll™ kit. A total of 20 ng DNA (10 ng for DNA pool 1 and DNA pool 2) and 10 ng RNA was used as input material for each sample. Library preparation was performed manually using the Oncomine Myeloid Research Assay Kit or automated using the Ion Torrent™ Oncomine™ Myeloid Research Assay—Chef Ready Kit, and templating was performed manually on the Ion OneTouch™ 2 instrument or on the Ion Chef System using Ion Hi-Q™ View or Ion 510™, 520™, and 530™ kits, respectively. DNA and RNA samples were run on the same chip at an 80:20 library ratio, with negative template controls.

Variant calling algorithms for both DNA and RNA were optimized and combined into a single Ion Reporter workflow. To increase the specificity of the assay, systematic errors were suppressed by a position and allele-specific mask trained on workflow-specific runs. For *FLT3*-ITD, a custom algorithm was developed to increase sensitivity of detection. The VCF output is supported by Oncomine Reporter, which contains curated annotations for mutations associated with six myeloid subtypes.

Panel performance

The Oncomine Myeloid Research Assay shows consistent performance across platforms (Table 1), allowing for flexibility in multiplexing while maintaining high average read depth across instruments and workflows.

Table 1. Oncomine Myeloid Research Assay average performance data.

Workflow	Multiplexing (libraries per chip)	Average total reads per run	Average uniformity (%)	Average amplicon-assigned reads	Average read length (bp)	Average total mapped reads (RNA)
Ion PGM System with Ion 318 Chip	4	4,692,087	99	883,190	237	79,567
Ion GeneStudio S5 System with Ion 530 Chip, manual library prep	12	17,618,983	99	1,079,707	232	107,358
Ion GeneStudio S5 System with Ion 530 Chip, library using Ion Chef System	8	14,884,078	97	1,413,317	231	286,257

SNVs, indels, and fusions in analytical controls and patient samples

The Oncomine Myeloid Research Assay showed a high sensitivity and specificity for detecting a range of hotspot and *de novo* SNV and indel mutations in analytical controls. The AcroMetrix Oncology Hotspot Control, which contains variants tiered at allele frequencies between 5–20%, was used to demonstrate reliable detection of hotspot SNV, hotspot indel, and *de novo* SNV and *de novo* indel performance down to frequencies of 5%, 10%, 5%, and 20%, respectively (Figure 7A). The sensitivity for 68 expected AcroMetrix Oncology Hotspot Control variants included in the assay was 99.0%, with a positive predictive value (PPV) of 100% for Ion GeneStudio™ S5 System workflows and 100% sensitivity and PPV for Ion PGM System workflows (Table 2).

Clinical research samples with positive variants in *CEBPA* and *NPM1* genes were also tested. *CEBPA* is a GC-rich gene that's critical for controlling cell differentiation during hematopoiesis, and harbors prevalent driver aberrations in AML [3]. We've shown the ability to detect variants across the length of the gene using blood and bone marrow samples with *CEBPA* variants that were orthogonally characterized using Sanger sequencing (Figure 7B). Some difficulty in detecting the H24fs*84 mutation is attributed to difficult sequence context in the vicinity of the variant. Overall sensitivity of detection of *CEBPA* mutations in clinical samples was 86% (Table 2) with even coverage on both the Ion PGM and Ion GeneStudio S5 platforms (Figure 8).

NPM1 mutations are one of the most prevalent variants in AML, associated with varied clinical research profiles and outcomes [4]. Detection of major families of *NPM1* mutations (types A, B, and D) is demonstrated using blood and bone marrow samples containing variants previously characterized using Sanger sequencing. Overall sensitivity of detection of *NPM1* mutations in clinical research samples was 97% (Table 2).

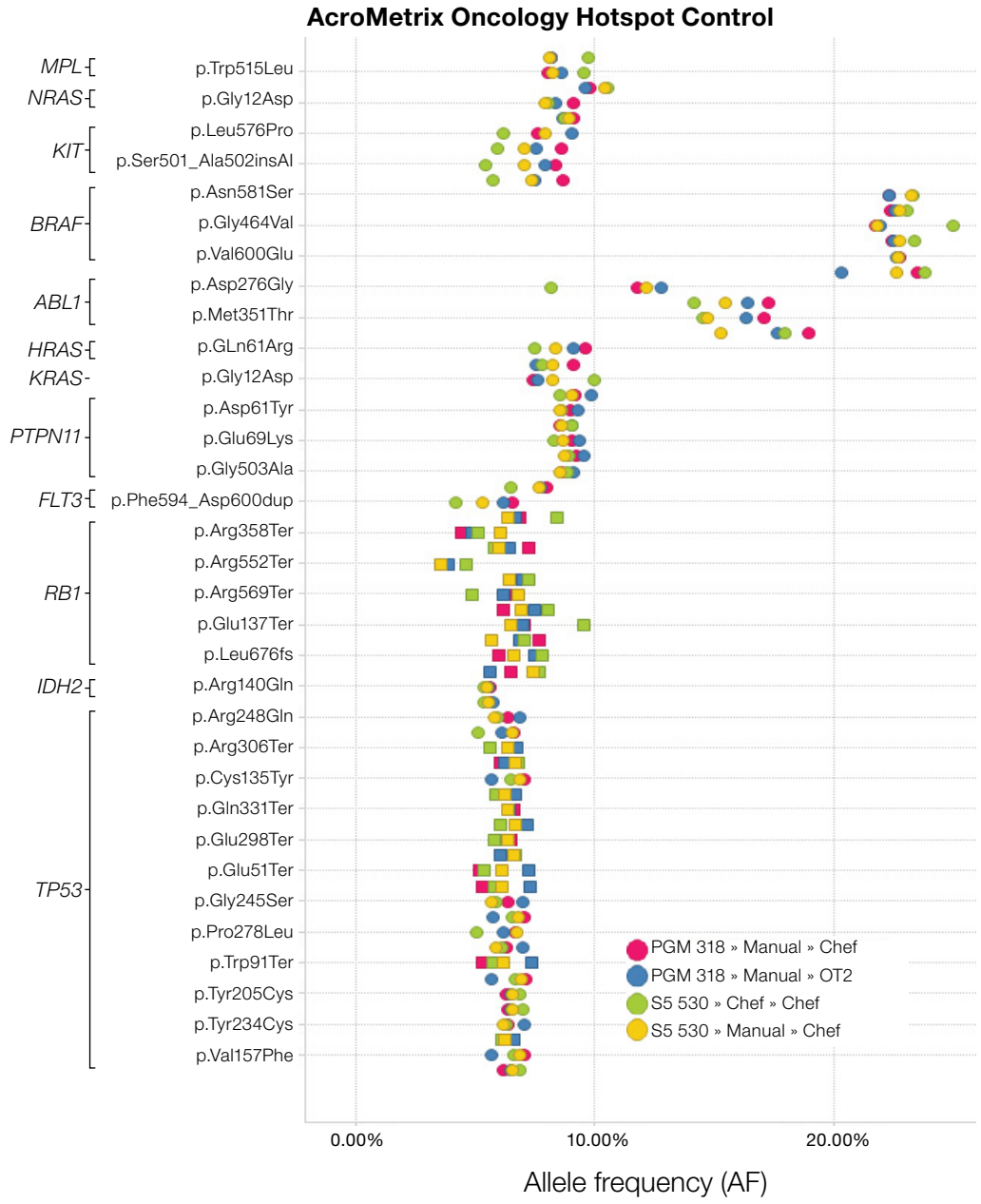
Targeted fusions were detected in bone marrow samples that were orthogonally confirmed to contain fusions relevant to myeloid disorders, including one *ETV6* and several *KMT2A* (*MLL*) fusions (Figure 7D). Sensitivity for detection was 100% on both Ion PGM and Ion GeneStudio S5 instruments. Additionally, several positive fusions were identified in clinical research samples without orthogonal truth, which included several *BCR-ABL1*, *PML-RARA*, *RUNX1-RUNX1T1,* and *KMT2A* isoforms (Figure 7D).

Table 2. Sensitivity and PPV of SNV and indel variants in analytical controls and clinical samples.

Variants evaluated	Platform	Sensitivity	PPV*
AcroMetrix Oncology Hotspot Control samples for Oncomine assay—hotspot (45)	Ion GeneStudio S5 System (library prep with Ion Chef System)	100%	100%
	Ion GeneStudio S5 System (manual library prep)	100%	100%
	Ion PGM System	100%	100%
AcroMetrix Oncology Hotspot Control samples for Oncomine assay—deleterious (22)	Ion GeneStudio S5 System (Library prep with Ion Chef System)	97.5%	100%
	Ion GeneStudio S5 System (manual library prep)	100%	100%
	Ion PGM System	100%	100%
Verified *CEBPA* mutations from clinical research samples	Ion GeneStudio S5 System	86%	NA
Verified *NPM1* mutations from clinical research samples	Ion GeneStudio S5 System	97%	NA
Fusions from clinical research samples	Ion GeneStudio S5 System	100%	NA
	Ion PGM System	100%	NA

* NA: data is not available.

A



B

Detected *CEBPA* mutations

Type	Protein change	AF%	Sample
SNP	p.Gln20Ter	46%	Marrow
Insertion	p.Ala66Valfs*41	30%	Marrow
Deletion	p.A136Sfs*12	15%	Marrow
Insertion	p.Pro187Alafs*135	20%	Marrow
Insertion	p.His195_Pro196dup	32%	Marrow
Deletion	p.Pro235fs	35%	Blood
Insertion	p.Ala274fs	46%	Marrow
Insertion	p.Ala240fs	30%	Marrow
Deletion	p.Ala303dup	38%	Marrow
Insertion	p.K313_V314insK	15%	Control
SNP	p.Asn321Asp	37%	Blood

C

Detected *NPM1* mutations

Type	Sample(s)
c.867_868insGAGAT	Marrow
c.867_868insCGGA	Marrow
insCCGG	Marrow
Type A (InsTCTG)	13 marrow, 6 blood
Type B (InsCATG)	Marrow, 6 blood
Type D (InsCCTG)	Marrow, 6 blood

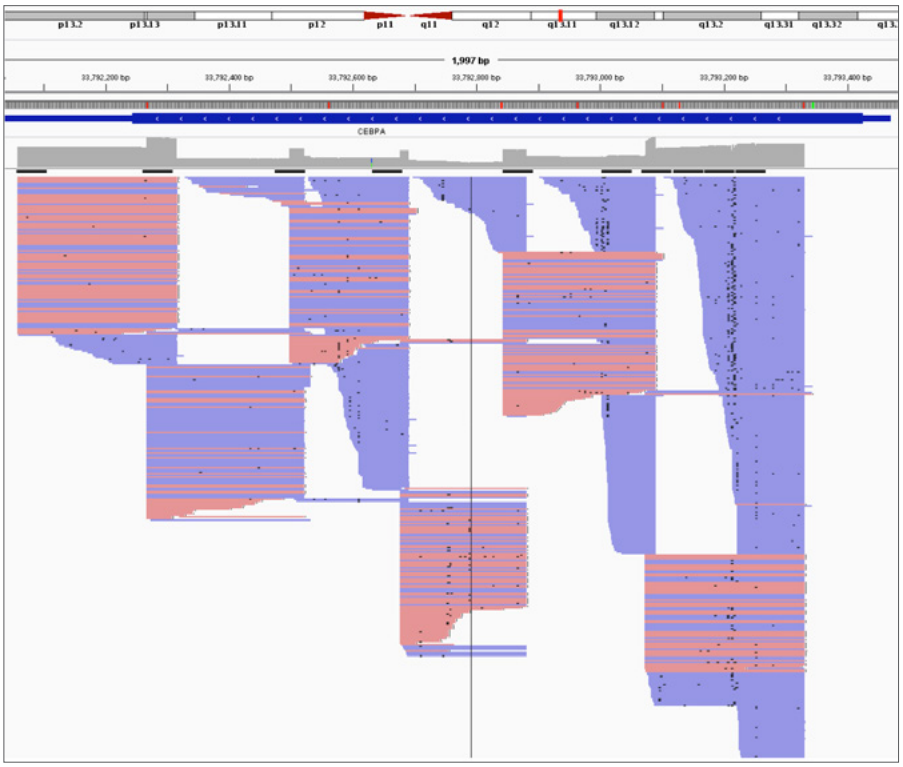
D

Detected fusions:

Fusion	
BCR-ABL1.B14A2	*KMT2A-MLLT3.K10M6*
BCR-ABL1.B19A2	*KMT2A-MLLT3.K8M6*
CBFB-MYH11.C5M33	*KMT2A-MLLT3.K8M9*
DEK-NUP214.D9N18	*KMT2A-MLLT3.K9M6*
ETV6-PDGFRB.E4P11	*PML-RARA.P3R3*
ETV6-RUNX1.E5R4	*PML-RARA.P6R3*
KMT2A-AFF1.K10A5	*PML-RARA.P7R3*
KMT2A-AFF1.K8A4	*RUNX1-MECOM.R4M2*
KMT2A-MLLT1.K10M2	*RUNX1-RUNX1T1.R3R3*
KMT2A-MLLT10.K7M15	

Figure 7. Variant detection in analytical controls and clinical research samples. (A) All 68 expected SNVs and indels ranging from 5–20% allele frequency were detected using multiple workflows compatible with the Oncomine Myeloid Research Assay. **(B)** *CEBPA* mutations detected in 1 control and 10 previously characterized clinical research samples. **(C)** Major families of *NPM1* mutations (type A, B, and D) were successfully detected in 28 previously characterized clinical research samples. **(D)** Multiple gene fusions associated with myeloid disorders were detected in clinical research samples.

A ***CEBPA*** **coverage on Ion GeneStudio S5 instrument**



Ion GeneStudio S5 System with Ion 530 Chip

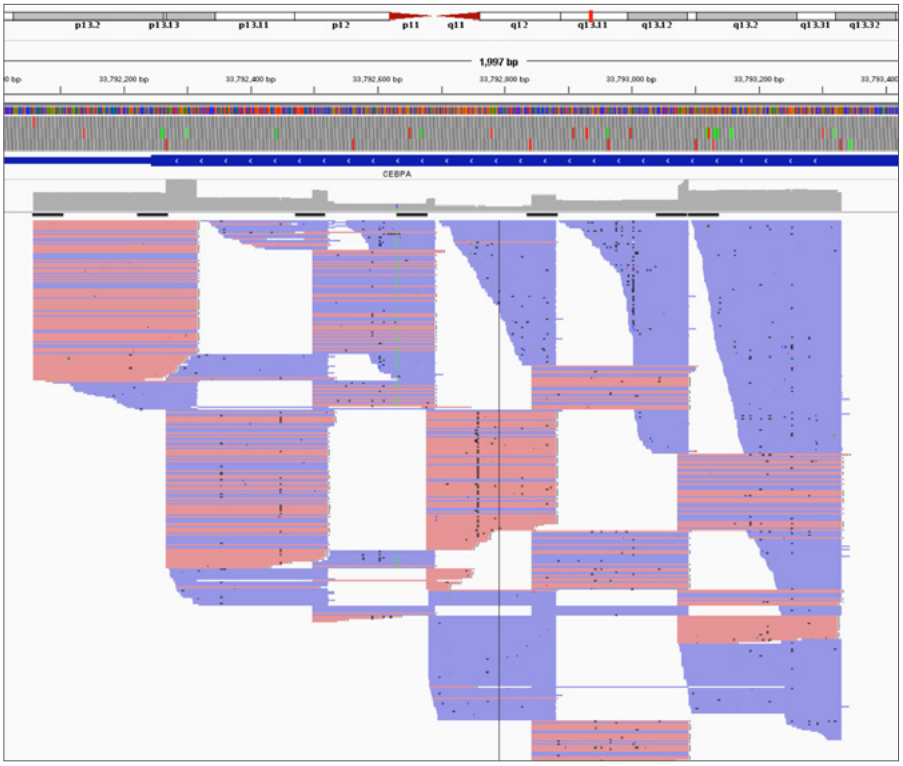
Manual library

Ion Chef template

1,263,574 amplicon-assigned reads

99.05% uniformity of amplicon coverage

B ***CEBPA*** **coverage on Ion PGM instrument**



Ion PGM System with Ion 318 Chip

Manual library

Ion Chef template

1,202,056 amplicon-assigned reads

99.24% uniformity of amplicon coverage

Figure 8. Integrative Genomic Viewer (IGV) visualization of amplicon read pileup shows >99% amplicon coverage uniformity for *CEPBA* on the Ion GeneStudio S5 and Ion PGM sequencing instruments.

FLT3-ITD variants

FMS-like tyrosine kinase-3 (FLT3), a receptor tyrosine kinase, is important for the development of the hematopoietic and immune systems. ITD mutations of *FLT3* are a common molecular abnormality in AML and may play a role in other hematologic malignancies. The poor prognosis of individuals harboring these mutations has made *FLT3* an important target for therapy. The location, size, and sequence of the insertions are different among individual samples. Currently, gel shift and fragment analysis methods that measure the size of a PCR product amplified from this region are standard techniques for identifying *FLT3*-ITD mutations. The limit of detection with these techniques is a 5% allele frequency [5]. Next- generation sequencing (NGS) is also used for *FLT3*-ITD detection. The lowest reported allele frequency of *FLT3*-ITD is 1%, detected by NGS [6].

The Oncomine Myeloid Research Assay covers exons 8, 11–16, 20, 23, and 24 of the *FLT3* gene. *FLT3*-ITDs occur in exons 14 and 15 of the gene and are covered by two amplicons that are anchored in the flanking introns to better accommodate any potential disruption of binding within the coding sequence by an ITD. Several cohorts of orthogonally profiled blood and bone marrow samples with known true positive and true negative status were used to demonstrate the sensitivity and PPV of the *FLT3*-ITD detection algorithm, which were 94% and 100%, respectively (Figure 9).

The Oncomine Myeloid Research Assay detected two initial false-positive observations that were confirmed to be true positives through additional orthogonal testing. The data showed a 0.3% allele frequency of *FLT3*-ITD in one sample, and 1.6% and 2.1% allele frequency of *FLT3*-ITDs in another sample, both of which were reported as *FLT3*-ITD– negative by a collaborator when tested using conventional methods. Subsequent testing with PCR primers designed to specifically amplify the ITD insertion, followed by Sanger sequencing, showed that the three *FLT3*-ITDs were true positives. The false-negative calls reported in Figure 9 were from a single sample with a 126 bp insertion present at a very low allele frequency (<1%). Overall, the Oncomine Myeloid Research Assay detected the lowest allele frequency of *FLT3*-ITD, demonstrating the higher sensitivity of this assay compared to orthogonal methods.

Instrument	Expected positive	Detected positive	False negatives	Expected negative	False positives
Ion PGM System	39	36	3	6	0
Ion GeneStudio S5 System	44	42	2	33	0
Overall	**83**	**78**	**5**	**39**	**0**

Sensitivity 94%

PPV 100%

Samples tested	Positive	Negative
Blood	20	7
Bone marrow	46	15
Overall	**9**	**NA**

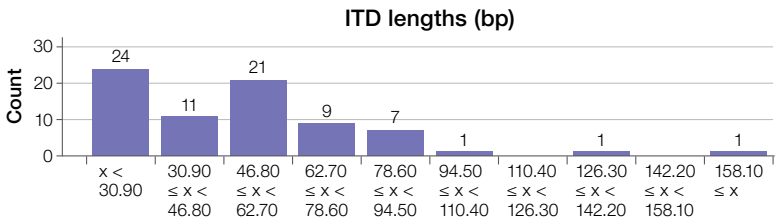


Figure 9. The Oncomine Myeloid Research Assays detected *FLT3*- ITDs in previously tested clinical research samples with 94% sensitivity and 100% PPV.

Support for analytical controls

The assay supports the use of Seraseq™ Myeloid DNA and RNA mixes from SeraCare as recommended controls, which provide a set of 23 DNA and 9 RNA variants relevant to myeloid disorders.

Seraseq Myeloid DNA Mix

Gene ID	Protein	COSMIC ID	Allele frequency	Expected detection	Oncomine variant
ABL1	p.T315I	12560	0.1	Yes	Hotspot
ASXL1	p.E635fs*15	36165	0.1	No	
ASXL1	p.G646fs*12	34210	0.1	No	
BRAF	p.V600E	476	0.1	Yes	Hotspot
CALR	p.L367fs*46	1738055	0.05	Yes	Deleterious
CBL	p.R420Q	34077	0.10	Yes	Hotspot
CBL	p.L380P	34055	0.1	Yes	Hotspot
CEBPA	p.H24fs*84	18922	0.15	No	
CEBPA	p.K313_V314insK	18099	0.15	Yes	Deleterious
CSF3R	p.T618I	1737962	0.05	Yes	Hotspot
FLT3	FLT3-ITD		0.1	Yes	Hotspot
FLT3	p.D835Y	783	0.1	Yes	Hotspot
FLT3	FLT3-ITD		0.05	Yes	Hotspot
IDH1	p.R132C	28747	0.05	Yes	Hotspot
JAK2	p.V617F	12600	0.05	Yes	Hotspot
JAK2	p.N542_E543del	24440	0.1	Yes	Hotspot
MPL	p.W515L	18918	0.05	Yes	Hotspot
MYD88	p.L265P	85940	0.1	Yes	Hotspot
NPM1	p.W288fs*12	17559	0.05	Yes	Hotspot
SF3B1	p.K700E	84677	0.05	Yes	Hotspot
SF3B1	p.K666N	131557	0.05	Yes	Hotspot
SRSF2	p.P95_R102del	146289	0.05	Yes	Hotspot
U2AF1	p.S34F	166866	0.1	Yes	Hotspot

Seraseq Myeloid Fusion RNA Mix

Oncomine Myeloid Research Assay–primary isoform	Oncomine variant
KAT6A(MYST3)-CREBBP.K17C2	Targeted fusion
ETV6-ABL1.E4A2	Targeted fusion
ETV6-ABL1.E5A2	Targeted fusion
PCM1-JAK2.P23J12.COSF1001	Targeted fusion
FIP1L1-PDGFRA.F11P12del45	Targeted fusion
TCF3-PBX1.T16P3.COSF1489	Targeted fusion
BCR-ABL1.B14A2.1	Targeted fusion
RUNX1-RUNX1T1.R3R3	Targeted fusion
PML-RARA.P6del11ins133A3	Targeted fusion

ion torrent

Summary

The Oncomine Myeloid Research Assay is a comprehensive, targeted NGS assay that enables profiling of multiple DNA mutations and RNA fusions found in myeloid malignancies with a single test. This assay targets genomic alterations associated with a range of myeloid diseases, including AML, APL, MDS, MPN, JMML, CML, and CMML. Analytical performance of the assay was evaluated using controls and clinical research samples, and the results demonstrated that the assay generated an average read depth of >2,000 reads per targeted amplicon with an average uniformity of >95%, and a high sensitivity for DNA hotspot and *de novo* variants, as well as RNA fusion transcripts common to myeloid cancers. Important GC-rich targets such as *CEBPA* produced balanced read depth necessary to support variant detection. A cohort of almost 100 clinical research samples positive for *FLT3* alterations was analyzed, and successful detection of *FLT3*-ITD variants was demonstrated with 94% sensitivity and 100% PPV. The Oncomine Myeloid Research Assay offers a sensitive and robust sample-to-answer workflow for supporting clinical and translational research on myeloid cancers.

References

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