VariantPlex® Myeloid NGS panel validation study

Overview

Hematologic malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN) are oligoclonal disorders caused by germline or acquired genetic abnormalities in hematopoietic cells.[1] Testing based on targeted next-generation sequencing (NGS) of the genes associated with these disorders can help identify somatic mutations.[2] This information can be clinically useful in diagnosis, prognostic risk stratification, treatment guidance, and minimal residual disease (MRD) detection and monitoring.[1]

Validated by Genosity, the VariantPlex® Myeloid NGS panel quantitatively detects and characterizes single nucleotide variants (SNVs), copy number variations (CNVs), as well as insertions and deletions (indels) in 73 genes linked to myeloid malignancies. Assessed accuracy via study with an orthogonal method, this panel has greater than 99.99% analytic sensitivity and specificity for detecting SNVs and small indels with allele frequencies equal to or greater than 5%.

Introduction

With NGS, the evolving fields of cancer genomics and biomarker-based therapeutics have led to the development of targeted, disease-specific gene panels to identify mutations. Genosity performed the validation for Invitae's VariantPlex Myeloid NGS panel. Genes covered in this panel are: ABL1, ANKRD26, ASXL1, ATRX, BCOR, BCORL1, BRAF, BTK, CALR, CBL, CBLB, CBLC, CCND2, CDKN2A, CEBPA, CSF3R, CUX1, CXCR4, DCK, DDX41, DHX15, DNMT3A, ETNK1, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KRAS, LUC7L2, MAP2K1, MPL, MYC, MYD88, NF1, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PPM1D, PTEN, PTPN11, RAD21, RBBP6, RUNX1, SETBP1, SF3B1, SH2B3, SLC29A1, SMC1A, SMC3, SRF2, STAG2, STAT3, TET2, TP53, U2AF1, U2AF2, WT1, XPO1, and ZRSR2. **Table 1 (on pages 3–4)** shows prognostic, therapeutic or diagnostic associations of the genes and biomarkers covered in this panel.

Materials and methods

This validation study assessed accuracy, precision and reproducibility, limit of detection, and reportable range. It follows guidelines and recommendations for NGS and somatic testing.[3-7] Automated quality control checks have been reported such as sample accessioning, DNA isolation, library preparation, target capture, and sequencing. Each library preparation also contains one no-template control and at least one fully characterized reference control. This validation study used real-world patient samples (DNA internally or externally extracted from peripheral blood, bone marrow, and buffy coat) and well-characterized reference materials. Archer® Analysis and Genosity's Genome Explorer performed the bioinformatics analyses.[8] **Table 2 (on pages 5–6)** lists targets enriched in this test. Variants are classified according to the standards and guidelines for sequence variant interpretation from the American College of Medical Genetics and Genomics and Association of Molecular Pathology.

Results

Accuracy: Well-characterized reference material and orthogonal method comparison

NIST "Genome in a Bottle" samples (NA12878 and NA24385) were used in this validation study. These samples represent "gold standards," enabling a direct comparison of results from the VariantPlex Myeloid NGS panel to the known high confidence NIST results within regions covered by the panel, a total of 125,945 bases. Results showed greater than 99.99% sensitivity and specificity. Additionally, 30 real world samples processed by an external CAP/CLIA certified laboratory were included in this validation study. A total of 140 variants were reported in 30 samples by the external lab using a 68 genes TruSeq Custom Amplicon panel (Illumina, Inc.). The results of these studies are summarized in **Table 3**. An additional 48 real-world samples were processed by an orthogonal assay, in which all 136 SNVs and indels located in overlapping regions by both panels and with allele frequencies above the assay's limit of detection were confirmed.

Accuracy across sample types

Buffy coat, peripheral blood, bone marrow, and cell line samples were utilized in the accuracy studies. The data demonstrate that DNA quality rather than DNA source is most critical for the panel's performance.

Accuracy across different sample barcodes

Unique barcodes were used across all intra- and inter-batch replicates. Results show the barcode set utilized in this panel does not present a bias or contamination risk.

Precision and reproducibility

The precision of the VariantPlex Myeloid NGS panel was investigated by analyzing intra-batch replicates (3x) of 4 samples (12 samples total), and reproducibility was investigated by analyzing inter-batch replicates (4x) of 6 samples (24 samples total). Highly concordant results were observed across all 36 tested samples for both intra- and inter-batches when comparing the called variants and associated allele frequencies to expected results. Based on these results, this assay showed >99% precision and reproducibility.

Limit of detection (LOD)

In the precision and reproducibility studies, the LOD of the panel was investigated by running a total of 5 samples (Seraseq® Myeloid, and four admixture samples) that had a range of SNVs and indels near the expected lower limit. Across the LOD analysis, 100% sensitivity was achieved down to 5% allele frequency. This corresponds to a neoplastic/dysmorphic content of 10% or greater in the submitted specimen.

Reportable range

Reportable range is defined as the span of all test results that are considered valid.[5] For molecular assays, this includes the target regions, variant types and allele frequency that will be reported. General reportable range includes non-reference SNVs and indels with allele frequency equal to or greater than 5%.

Conclusion

Greater than 99.99% sensitivity and specificity were achieved when comparing results from two NIST "Genome in a Bottle" samples. Additionally, greater than 99% sensitivity was achieved among 78 samples with known results from an external clinical lab and confirmed by an orthogonal method. An investigation of precision and reproducibility at the LOD utilizing 4 admixture samples, 1 NIST reference sample (NA12878), and the biosynthetic sample (Seraseq Myeloid) demonstrated an overall 100% concordance for SNVs, 100% concordance for deletions, and 98.44% concordance for insertions, in inter-/intra-batches, and among the different sample barcodes.

References

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Table 1. Genes and biomarker associations covered by this panel *

The following clinical associations have been established in the literature or are supported by professional guidelines by June 2019

Disease	Mutated gene/Biomarker	Prognostic, therapeutic or diagnostic association
AML	FLT3 (ITD and TKD)	Gilteritinib, Sorafenib, Midostaurin
	IDH1	Ivosidenib
	IDH2	Enasidenib
	Mutated NPM1 without FLT3-ITD or with FLT3-ITDlow*	Favorable risk
	Biallelic mutated CEBPA	Favorable risk
	Mutated NPM1 and FLT3-ITD high	Intermediate risk
	Wild-type NPM1 without FLT3-ITD or with FLT3-ITDlow (without other adverse-risk genetic lesions)	Intermediate risk
	Wild-type NPM1 and FLT3-ITD high	Poor /Adverse risk
	RUNX1	Poor /Adverse risk
	ASXL1	Poor /Adverse risk
	TP53	Poor /Adverse risk
	KIT	Decreased remission duration and decreased OS in patients with t(8;21)
MDS	EZH2, ETV6, RUNX1, and ASXL1	Independently associated with a poor prognosis
	DNMT3A, U2AF1, SRSF2, CBL, SETBP1, and KRAS	Decreased OS
	TP53	Independently associated with a poor prognosis, may predict resistence or replapse to lenalidomide
	SF3B1	Strongly associated with ring sideroblasts and independently associated with a more favorable prognosis
	SRSF2, U2AF1, ZRSR2, STAG2, NRAS, GATA2, IDH2, BCOR, FLT3, WT1	Associated with a poor prognosis
	SETBP1	Associated with disease progression
	PPM1D	Associated with therapy-related MDS, but not associated with adverse prognosis independent of TP53.
MDS/MPN	EZH2	Independently associated with a poor prognosis
CMML	ASXL1	Independently associated with a poor prognosis
	SRSF2	Associated with a poor prognosis
MPN	JAK2, MPL, CALR	One of the major diagnostic criteria of ET and PMF
	JAK2 V617F or exon 12 mutation	One of the major diagnostic criteria of PV
MPN/PMF	JAK2 V617F	Intermediate prognosis and higher risk of thrombosis compared to patients with CALR mutation
	MPL W515L/K	Intermediate prognosis and higher risk of thrombosis compared to patients with CALR mutation
	CALR	Improved survival compared to JAK2 mutation and triple negative PMF. Lower risk of thrombosis compared to JAK2 and MPL mutation.
	CALR Type 1/Type 1 like	Improved overall survival compared to CALR Type 2/type 2 like and JAK2 V617F mutation
	"Triple negative" (non-mutated JAK2, MPL, and CALR)	Inferior leukemia-free survival compared to patients with JAK2 and/or CALR-mutated PMF; Inferior OS compared to patients with CALR mutated PMF
	ASXL1	Independently associated with inferior OS
	IDH1, IDH2	Independently associated with inferior leukemia-free survival

Table 1. Continued *

MPN/PMF	SRSF2	Independently associated with inferior OS and leukemia-free survival
	TP53	Associated with leukemic transformation
	U2AF1 Q157	Inferior OS compared to patients with S34 mutated or U2AF1 unmutated PMF. The effect is most evident in younger patients.
	Combined CALR and ASXL1 status	Intermediate survival for CALR+ ASXL1+ patients, shortest survival for CALR-ASXL1+ patients, longest for CALR+ASXL1- patients
MPN/PV	ASXL1, SRSF2, IDH1 and IDH2	Independently associated with inferior OS. Also affect myelofibrosis-free survival
	JAK2 Exon 12 mutation	Exhibit younger age, increased mean hemoglobin/ hematocrit, and lower mean white blood cell and platelet counts at diagnosis compared to those with JAK2 V617F mutated PV.
MPN/ET	CALR	Lower risk of thrombosis compared to JAK2 mutated ET
	TP53	Associated with inferior leukemia-free survival in multivariate analysis
	SH2B3, IDH2, U2AF1, SF3B1, EZH2, TP53	Independently associated with inferior OS. Also affect myelofibrosis-free survival
MPN/CNL	CSF3R	Diagnostic marker
CML	BCR-ABL mutations Y253H, E255K/V, or F359V/C/I	Dasatinib
	F317L/V/I/C, T315A, or V299L	Nilotinib
	E255K/V, F317L/V/I/C, F359V/C/I, T315A, or Y253H	Bosutinib
	T315I	Ponatinib, Omacetaxine, allogenic HCT, or clinical trial
B-ALL	FLT3, SH2B3, JAK1, JAK3, JAK2	Ph-like ALL, associated with unfavorable prognosis
Relapsed or Refractory BCR-ABL1 positive B-ALL	ABL1 mutations Y253H, E255K/V, or F359V/C/I	Dasatinib
	F317L/V/I/C, T315A, or V299L	Nilotinib
	E255K/V, F317L/V/I/C, F359V/C/I, T315A, or Y253H	Bosutinib
	T315I	Ponatinib
CLL/SLL	BTK C481S	Acalabrutinib should not be used for ibrutinib-refractory CLL with this mutation.
	TP53	Associated with low response rates with chemoimmunotherapy; Associated with unfavorable prognosis when IGHV is <=2% mutated; predictors of poor survival and resistance to fludarabine-based regimens, independent of 17p chromosome status.
	Notch1	Independently associated with Richter's transformation.
HCL	BRAF V600	Distinguish classic HCL from HCL-variant and marginal zone lymphoma; Vemurafenib
Waldenstrom Macroglobulinemia/ Lymphoplasmacytic lymphoma	MYD88 L265	Help differentiate from IgM-secreting B-cell lymphoma, marginal zone lymphoma and IgM plasma cell myeloma; lower overall and absence of major responses observed in MYD88 wild-type patients under treatment with Ibrutinib;
Mantle cell lymphoma	TP53	Associated with poor prognosis in patients treated with conventional therapy, including transplant
T-cell lymphoma	STAT3	Diagnosis of Large Granular Lymphocytic Leukemia (LGLL) and natural killer leukemias

AML: Acute myeloid leukemia
B-ALL: B cell acute lymphoblastic leukemia
MDS: Myelodysplastic syndrome
CML: Chronic myelogenous leukemia
MPN: Myeloproliferative neoplasm
PV: Polycythemia Vera
PMF: Primary Myelofibrosis
ET: Essential Thrombocythemia
CHIP: Clonal hematopoiesis of indeterminate potential
CMML: Chronic myelomonocytic leukemia
CLL: Chronic lymphocytic leukemia
SLL: Small cell lymphocytic lymphoma
HCL: Hairy cell leukemia

Table 2. Targets included in VariantPlex Myeloid Tumor Panel

Gene	Transcript ID	Target exons
ABL1	NM_005157	4,5,6,7,8,9,10
ANKRD26	NM_014915	1(c.-113-c.-134)
ASXL1	NM_015338.5	1,2,3,4,5,6,7,8,9,10,11,12,13
ASXL1	NM_001164603.1	5
ATRX	NM_000489	8,9,10,11,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32
BCOR	NM_017745	2,3,4,5,6,7,9,10,11,12,13,14,15
BCOR	NM_001123385	8
BCORL1	NM_021946	1,2,3,4,5,6,7,8,9,10,11,12
BRAF	NM_004333	3,10,11,12,13,15
BTK	NM_000061	15
CALR	NM_004343	8,9
CBL	NM_005188	2,3,4,5,7,8,9,16
CBLB	NM_170662	3,9,10
CBLC	NM_012116	9,10
CCND2	NM_001759	5
CDKN2A	NM_058197	1
CDKN2A	NM_058195	1
CDKN2A	NM_000077	2,3
CDKN2A	NM_001195132	3
CEBPA	NM_004364	1
CSF3R	NM_156039	17
CSF3R	NM_172313	10,18
CSF3R	NM_000760	14,15,16
CUX1	NM_001202543	15,16,17,18,19,20,21,22,23,24
CUX1	NM_001913	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
CUX1	NM_181552	1
CXCR4	NM_003467	1,2
DCK	NM_000788	2,3
DDX41	NM_016222	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17
DHX15	NM_001358	3
DNMT3A	NM_022552	2,3,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23
DNMT3A	NM_153759	1,2
DNMT3A	NM_175630	4
ETNK1	NM_018638	3
ETV6	NM_001987	1,2,3,4,5,6,7,8
EZH2	NM_004456	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
FBXW7	NM_018315	1,2,3,4,5,6,7,8,9,10,11
FLT3	NM_004119	8,9,10,11,12,13,14,15,16,17,19,20,21
GATA1	NM_002049	2
GATA2	NM_032638	2,3,4,5,6
GNAS	NM_000516	8,9,10,11
HRAS	NM_005343	2,3,4
IDH1	NM_005896	3,4
IDH2	NM_002168	4,6
IKZF1	NM_001220769	5
IKZF1	NM_001220767	2,3,4,5,7
IKZF1	NM_001220771	4
IKZF1	NM_001291845	4
IKZF1	NM_001291847	5
JAK2	NM_004972	12,13,14,15,16,19,20,21,22,23,24,25
JAK3	NM_000215	3,11,13,15,18,19
KDM6A	NM_021140	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29
KDM6A	NM_001291415	14

Table 2. Continued

Gene	Transcript ID	Target exons
KIT	NM_000222	1,2,5,8,9,10,11,12,13,14,15,17,18
KMT2A	NM_005933	1,2,3,4,5,6,7,8,9,10,11,12,13,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36
KMT2A	NM_001197104	14
KRAS	NM_004985	2,3,4
LUC7L2	NM_016019	1,2,3,4,5,6,7,8,9,10
LUC7L2	NM_001244585	2
MAP2K1	NM_002755	2,3
MPL	NM_005373	10,12
MYC	NM_002467	1,2,3
MYD88	NM_002468	4,5
MYD88	NM_001172567	3
NF1	NM_000267	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57
NF1	NM_001128147	15
NF1	NM_001042492	31
NOTCH1	NM_017617	26,27,28,34,c.*370 to c.*380
NPM1	NM_002520	11
NRAS	NM_002524	2,3,4,5
PDGFRA	NM_006206	12,14,15,18
PHF6	NM_032335	2,3,4,5,6,7,8
PHF6	NM_001015877	10
PHF6	NM_032458	9
PPM1D	NM_003620	6
PTEN	NM_000314	1,2,3,4,5,6,7,8,9
PTPN11	NM_002834	3,4,7,8,12,13
PTPN11	NM_080601	11
RAD21	NM_006265	2,3,4,5,6,7,8,9,10,11,12,13,14
RBBP6	NM_006910	p.1444,p.1451,p.1569,p.1654,p.1673
RUNX1	NM_001754	2,3,5,6,7,8,9
RUNX1	NM_001122607	1,5
SETBP1	NM_015559	4 (p.799-p.950)
SF3B1	NM_012433	13,14,15,16,17,18,19,20,21
SH2B3	NM_005475	2,3,4,5,6,7,8
SLC29A1	NM_001078175	4,13
SMC1A	NM_006306	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
SMC1A	NM_001281463	2
SMC3	NM_005445	10,13,19,23,25,28
SRSF2	NM_003016	1,2
STAG2	NM_006603	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33
STAG2	NM_001042749	32
STAT3	NM_003150	20
STAT3	NM_139276	21
TET2	NM_001127208	4,5,6,7,8,9,10,11
TET2	NM_017628	3
TP53	NM_000546	1,2,3,4,5,6,7,8,9,10,11
TP53	NM_001276695	10
TP53	NM_001276696	10
U2AF1	NM_006758	2,6,7
U2AF1	NM_001025204	6
U2AF2	NM_007279	1,2,3,4,5,6,7,8,9,10,11,12
WT1	NM_000378	1,2,3,4,5,6,7,9
WT1	NM_001198552	8
XPO1	NM_003400	15,16,18
ZRSR2	NM_005089	1,2,3,4,5,6,7,8,9,10,11

* Table 1 references

1. NCCN Guidelines v3. May 2019, Acute Myeloid Leukemia
2. NCCN Guidelines v2. May 2019, Acute Lymphoblastic Leukemia
3. NCCN Guidelines v5. May 2019, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma
4. NCCN Guidelines v1. May 2019, Chronic Myeloid Leukemia
5. NCCN Guidelines v3. May 2019, Hairy Cell Leukemia
6. NCCN Guidelines v1. May 2019, Hodgkin Lymphoma
7. NCCN Guidelines v2. May 2019, Multiple Myeloid
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