FOCUS::MYELOID™

Next-Generation Sequencing Panel

Acute Myeloid Leukemia (AML)
Myelodysplastic Syndrome (MDS)
Myeloproliferative Neoplasms (MPN)

Targeted NGS Panel for Myeloid Malignancies

Designed for acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN), Focus::Myeloid™ is a unique NGS panel with 54 biomarkers that provides actionable information for improved diagnosis, prognosis, and risk stratification. Based on the Focus::Myeloid™ result, each patient can receive the most suitable treatment tailored to their unique cancer. By personalizing diagnosis and improving risk stratification, Focus::Myeloid™ delivers on the promise of precision medicine.

An Actionable Genomic Assessment of Your Patient's Cancer

With a 5% analytical sensitivity, Focus::Myeloid™ surpasses other sequencing methodologies and offers robust specificity (>99%).

Acute Myeloid Leukemia (AML)

- Delivers faster results on the four well-established biomarkers (NPM1, FLT3, CEBPA, KIT) as part of the 54 gene panel.
- Expands therapy options for patients with appropriate enrollment in clinical trials.

Myelodysplastic Syndrome (MDS)

- Identifies patients classified as very low to intermediate risk by IPSS that could benefit from more aggressive therapies.
- Includes all biomarkers listed in current diagnostic and treatment guidelines.

Myeloproliferative Neoplasms (MPN)

- Faster results for multiple biomarkers in panel allow patients to start appropriate therapy sooner.
- Complete genomic assessment in a single assay provides accurate risk stratification.

Comprehensive, Targeted Panel of Genes

Focus::Myeloid™ is an actionable tool to help predict disease progression and guide patient management.

Focus::Myeloid™ NGS Panel [54 genes]

ABL1	BRAF	CDKN2A	ETV6	GATA2	IKZF1	KMT2A	NPM1	PTPN11	SMC1A	TP53
ASXL1	CALR	CEBPA	EZH2	GNAS	JAK2	KRAS	NRAS	RAD21	SMC3	U2AF1
ATRX	CBL	CSF3R	FBXW7	HRAS	JAK3	MPL	PDGFRA	RUNX1	SRSF2	WT1
BCOR	CBLB	CUX1	FLT3	IDH1	KDM6A	MYD88	PHF6	SETBP1	STAG2	ZRSR2
BCORL1	CBLC	DNMT3A	GATA1	IDH2	KIT	NOTCH1	PTEN	SF3B1	TET2	

Methodology and Results

After extraction, regions of interest relative to the 54 target genes are amplified using specific primers. Multiplexed sequencing by synthesis is performed using the MiSeq System (Illumina©). Sequencing reads are aligned and annotated variants identified in specimens are confirmed by repetition or Sanger sequencing with pre-designed primers to cover the respective region. Confirmed variants are reported with the functional significance of the variant (pathogenic or uncertain) on the respective gene product with the respective nucleotide change.

Specimen Requirements

One Lavender (EDTA) tube of peripheral blood or bone marrow aspirate. Minimum: 2-3 mL. Shipped at room temperature.

TAT 10-14 days

CPT Codes 81455

CGI Laboratory Licensure

CAP (Laboratory #: 7191582, AU-ID: 1434060), CLIA (Certificate#: 31D1038733), New Jersey (CLIS ID #: 0002299), New York State (PFI: 8192), Pennsylvania (031978), Florida (800018142), Maryland (1395), California (COS 00800558).

Focus::Myeloid™ AML Sample Report

Results: **Pathogenic mutations are detected in the FLT3, NPM1, and WT1 genes. A mutation of uncertain significance is detected in the STAG2 gene.**

GENE	REFERENCE SEQUENCE	EXONS TESTED	MUTATION(S) DETECTED	FUNCTIONAL IMPACT
FLT3	NM_004119.2	14, 15, 20	c.2503G>T; p.Asp835Tyr	Pathogenic
NPM1	NM_002520.6	12	c.860_861insCTGC; p.Trp288CysfsTer12	Pathogenic
STAG2	NM_001042749.1	full	c.2197G>A; p.Ala733Thr	Uncertain
WT1	NM_024426.4	7, 9	c.1406A>G; p.His469ARG	Pathogenic

Negative for mutations in:
ABL1 (ex4-6), ASXL1 (ex12), ATRX (ex8-10,17-31), BCOR, BCORL1, BRAF (ex15), CALR (ex9), CBL (ex8-9), CBLB (ex9-10), CBLC (ex9-10), CDKN2A, CEBPA, CSF3R (ex14-17), CUX1, DNMT3A, ETV6, EZH2, FBXW7 (ex9-11), GATA1 (ex2), GATA2 (ex2-6), GNAS (ex8-9), HRAS (ex2-3), IDH1 (ex4), IDH2 (ex4), IKZF1, JAK2 (ex12,14), JAK3 (ex13), KDM6A, KIT (ex2,8-11,13,17), KMT2A (MLL) (ex5-8), KRAS (ex2-3), MPL (ex10), MYD88 (ex3-5), NOTCH1 (ex26-28,34), NRAS (ex2-3), PDGFRA (ex12,14,18), PHF6, PTEN (ex5,7), PTPN11 (ex3,13), RAD21, RUNX1, SETBP1 (ex4, partial), SF3B1 (ex13-16), SMC1A (ex2,11,16-17), SMC3 (ex10,13,19,23,25,28), SRSF2 (ex1), TET2 (ex3-11), TP53 (ex2-11), U2AF1 (ex2,6), ZRSR2

In addition to those listed below in Methodology, the following targets with reduced coverage were assessed at 20% sensitivity in this specimen [NRAS (chr1:115256391-115256650), NOTCH1 (chr9:139390488-139390712)].

Interpretation: **A single nucleotide variant in the FLT3 gene was detected. This missense mutation is expected to impact the function of the protein.**

A 4 nucleotide insertion in the NPM1 gene was detected. This frameshift mutation is expected to impact the function of the protein.

A single nucleotide variant in the STAG2 gene was detected. The impact of this missense mutation on the function of the protein is uncertain.

A single nucleotide variant in the WT1 gene was detected. This missense mutation is expected to impact the function of the protein.

Description: Acute myeloid leukemia (AML) is characterized by a clonal expansion of myeloid blasts in the bone marrow, peripheral blood, and/or other tissues. It is the most common form of acute leukemia among adults and displays great heterogeneity both clinically and genetically. Approximately 5-20% of AML are therapy-related and generally have overall poorer outcome than de novo AML. As part of a diagnostic work-up, bone marrow analysis with cytogenetics (karyotype with/without FISH) is routinely performed, to not only confirm diagnosis but is also important for predicting remission rates, relapse risks, and overall survival outcomes according to current guidelines.[1] Molecular markers such as mutations and small insertions/deletions also exhibit clinical relevance by helping to refine prognostic groups, in particular those in the intermediate-risk cytogenetic group with a normal karyotype (NK-AML).[1,2] Ongoing studies continue to define the clinical utility of such markers in other distinct cytogenetic sub-groups such as those with monosomal karyotype. Importantly, the clinical impact of the various mutations must be considered in the context of the full clinical and cytogenetic characteristics of each case.

Overall in AML, recurrently altered genes have been detected in different functional pathways involved in the pathogenesis of the disease: spliceosome (in ~13% AML), activated signaling (FLT3, KIT, KRAS, NRAS in ~59%), chromatin modifiers (ASXL1, EZH2, MLL-PTD, and MLL fusions in ~30%), DNA methylation (TET1, TET2, IDH1, IDH2, DNMT3A in ~46%), cohesin complex (in ~13%), tumor suppressors (TP53, WT1, PHF6 in ~13%), transcription factor fusions (PML-RARA, MYH11-CBFB, other in ~18%), NPM1 in ~27%, and myeloid transcription factors (CEBPA, RUNX1, others in ~22%).[2,3] The FLT3 and NPM1 genes exhibit the most frequent abnormalities with prognostic relevance.[1-3] In NK-AML, NPM1 mutations occur in about 50% of cases and confer better responses and improved outcome in the absence of FLT3-ITD mutation as compared with NK-AML NPM1-negative cases.[1,4] FLT3 mutations occur predominantly as internal tandem duplications (ITD) and it has a well-recognized negative prognostic influence.[1,5,6] The clinical relevance of the less frequent tyrosine kinase domain (TKD) point mutations in FLT3 (mostly at p.D835) is less consistent across studies.[1,6,7] Mutations in the CEBPA gene are observed in about 10% of AML cases, and are generally associated with a favorable outcome in NK-AML, more so in those displaying double mutations, than single variants.[8,9] KIT mutations are found in about 20% of AML patients with inv(16) or t(16;16) or t(8;21), and mark increased risk of relapse and decreased overall survival in this subgroup.[10,11]

Another frequently mutated gene is DNMT3A, where the most common mutation is found at p.R882 and is often found together with NPM1 and FLT3 mutations.[6,12] The clinical relevance of DNMT3A mutations is less well understood.[1,2] Mutations have also been reported frequently in AML in the IDH1 and IDH2 genes, the former portending worse outcome in favorable-risk NK-AML (with NPM1 mutation without FLT3-ITD) and intermediate risk (without NPM1 mutation without FLT3-ITD).[1,2,13] Mutations of these two genes are generally mutually exclusive, and for IDH2, several hot spots have been identified: p.R172, p.R140, though the clinical relevance have as yet to be fully investigated. Mutations in the ASXL1, WT1, PHF6, TET2, and RUNX1 genes in NK-AML (and other cytogenetically-defined intermediate-risk AML) have been reported to be associated with poor prognosis in several studies, mostly in those cases that are FLT3-ITD negative.[1-3,14] In other studies, mutations in ASXL1 and U2AF1 associate with myelodysplastic-related changes, while those in TP53 are observed in higher incidence in cases with more complex karyotypes.[2,3] Thus, interpretation of the clinical relevance of mutations in AML must be considered in the context of cytogenetic-risk categories but also with respect to other mutations such as FLT3-ITD and NPM1.

References:

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SAMPLE

Focus::Myeloid™ MDS Sample Report

Results: **Pathogenic mutations are detected in the BCOR and TET2 genes. A mutation of uncertain significance is detected in the EZH2 gene.**

GENE	REFERENCE SEQUENCE	EXONS TESTED	MUTATION(S) DETECTED	FUNCTIONAL IMPACT
BCOR	NM_001123385.1	full	**c.572G>A; p.Trp191Ter**	**Pathogenic**
EZH2	NM_004456.4	full	**c.397T>A; p.Tyr133Asn**	**Uncertain**
TET2	NM_001127208.2	3-11	**c.5476G>T; p.Glu1826Ter**	**Pathogenic**

Negative for mutations in:
ABL1 (ex4-6), ASXL1 (ex12), ATRX (ex8-10,17-31), BCORL1, BRAF (ex15), CALR (ex9), CBL (ex8-9), CBLB (ex9-10), CBLC (ex9-10), CDKN2A, CEBPA, CSF3R (ex14-17), CUX1, DNMT3A, ETV6, FBXW7 (ex9-11), FLT3 (ex14,15,20), GATA1 (ex2), GATA2 (ex2-6), GNAS (ex8-9), HRAS (ex2-3), IDH1 (ex4), IDH2 (ex4), IKZF1, JAK2 (ex12,14), JAK3 (ex13), KDM6A, KIT (ex2,8-11,13,17), KMT2A (MLL) (ex5-8), KRAS (ex2-3), MPL (ex10), MYD88 (ex3-5), NOTCH1 (ex26-28,34), NPM1 (ex12), NRAS (ex2-3), PDGFRA (ex12,14,18), PHF6, PTEN (ex5,7), PTPN11 (ex3,13), RAD21, RUNX1, SETBP1 (ex4, partial), SF3B1 (ex13-16), SMC1A (ex2,11,16-17), SMC3 (ex10,13,19,23,25,28), SRSF2 (ex1), STAG2, TP53 (2-11), U2AF1 (ex2,6), WT1 (ex7,9), ZRSR2

Interpretation: **A single nucleotide variant in the BCOR gene was detected. This nonsense mutation is expected to impact the function of the protein.**

A single nucleotide variant in the EZH2 gene was detected. The impact of this missense mutation on the function of the protein is uncertain.

A single nucleotide variant in the TET2 gene was detected. This nonsense mutation is expected to impact the function of the protein.

Description: Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell malignancies characterized by ineffective hematopoiesis and peripheral blood cytopenias. The bone marrow of MDS patients is often hypercellular, but may be hypocellular. The major clinical concerns are morbidities caused by the cytopenias and evolution to acute myeloid leukemia (AML).[1] The median age at diagnosis is 70-75 years. Diagnostic evaluation of MDS involves assessment of peripheral blood smears, bone marrow morphology, and abnormal cell counts where dysplastic changes in hematopoietic lineages are used for classification purposes and along with clinical features assists to distinguish MDS and AML for intent to treat decisions.[1] Other diagnostic evaluations of serum factor levels and flow cytometry assist in classification, and cytogenetic/FISH analyses and more recently gene sequencing identifying clonal mutations and small insertions/deletions are of both diagnostic and prognostic value according to current guidelines.[1] In cases with equivocal histology and flow cytometry or with normal karyotype but with clinical data supportive of a diagnosis of MDS, mutation analysis can assist to establish diagnosis. MDS patients are risk-stratified according to the International Prognostic Scoring System (IPSS) which combines cytogenetic, morphologic, and clinical data and in the most recent revision, classifies patients into one of five risk groups: very low, low, intermediate, high, and very high.[1,2] Mutation analysis can help to better define risk-stratification of MDS patients, in particular those in the intermediate risk group.

About 80% of MDS patients will have a mutation in one of the over 40 recurrently mutated genes reported to date for large MDS sample datasets with potential underlying functional relevance, and importantly no one gene mutation is diagnostic of MDS.[1,3-7] Current guidelines give lists of gene mutations that are likely to be somatic and indicative of clonal hematopoiesis.[1] The most frequently mutated genes include: splicing factor genes (SF3B1, SRSF2, U2AF1, ZRSR2, PRPF8), DNA methylation (TET2, IDH1, IDH2, DNMT3A), histone modification (ASXL1, EZH2), signal transduction and transcription factors (RUNX1, TP53, NRAS, KRAS, ETV6, EVI1, JAK2, FLT3) cohesion complex (STAG2, RAD21, SMC3), and others (CBL, SETBP1, BCOR, and CSNK1A1).[3,4,7] Mutations in SF3B1, SRSF2, and U2AF1 are seen in about 40% of MDS patients. Mutations in SF3B1 are associated with the presence of ring sideroblasts and occurs with high frequency in MDS or MDS/myeloproliferative neoplasm subgroups of RARS or RARS-T, and are associated with a lower risk of leukemic transformation.[8,9] JAK2 mutations are also found commonly in RARS-T. While the presence of mutations in other genes is not specifically associated with a specific subtype, there is data supporting their clinical relevance.[3,4,6,10,11] For example, mutations in RUNX1, NRAS, and TP53 are associated with clinical adverse features including with excess bone marrow blast proportion and severe thrombocytopenia.[1] The independent prognostic value of TP53, EZH2, ETV6, RUNX1, and ASXL1 mutations to predict decreased overall survival (OS) within IPSS(-R) risk groups has been documented, where one mutation in any other the five genes identified patients with a survival risk more consistent with that of the next highest IPSS(-R) risk group.[10,11] Mutations in DNMT3A, U2AF1, SRSF2, CBL, PRPF8, SETBP1, and KRAS have also variously been reported to be associated with shorter OS.[3,4,6,12] On the other hand, mutations in SF3B1 reportedly are associated with a more favorable outcome.[9] TP53 mutations are associated with MDS bearing complex and monosomal karyotypes.[13] In the absence of TP53 mutation, patients with complex karyotypes exhibit an OS comparable to those with non-complex karyotypes.[14] Also, patients with del(5q) exhibit a higher frequency of TP53 mutations, associated with reduced response to lenalidomide.[15] Therapy-related MDS with overall increased clinical aggressiveness than de novo MDS, has recently been reported to have a mutational profile distinct from de novo MDS, with the most highly mutated gene being TP53 in about 35% of cases.[16]

References:

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SAMPLE

Focus::Myeloid™ MPN Sample Report

Results: **Pathogenic mutations are detected in the DNMT3A and JAK2 genes.**

GENE	REFERENCE SEQUENCE	EXONS TESTED	MUTATION(S) DETECTED	FUNCTIONAL IMPACT
DNMT3A	NM_022552.4	full	**c.2711C>T; p.Pro904Leu**	**Pathogenic**
JAK2	NM_004972.3	12,14	**c.1849G>T; p.Val617Phe**	**Pathogenic**

Negative for mutations in:
ABL1 (ex4-6), ASXL1 (ex12), ATRX (ex8-10,17-31), BCOR, BCORL1, BRAF (ex15), CALR (ex9), CBL (ex8-9), CBLB (ex9-10), CBLC (ex9-10), CDKN2A, CEBPA, CSF3R (ex14-17), CUX1, ETV6, EZH2, FBXW7 (ex9-11), FLT3 (ex14,15,20), GATA1 (ex2), GATA2 (ex2-6), GNAS (ex8-9), HRAS (ex2-3), IDH1 (ex4), IDH2 (ex4), IKZF1, JAK3 (ex13), KDM6A, KIT (ex2,8-11,13,17), KMT2A (MLL) (ex5-8), KRAS (ex2-3), MPL (ex10), MYD88 (ex3-5), NOTCH1 (ex26-28,34), NPM1 (ex12), NRAS (ex2-3), PDGFRA (ex12,14,18), PHF6, PTEN (ex5,7), PTPN11 (ex3,13), RAD21, RUNX1, SETBP1 (ex4, partial), SF3B1 (ex13-16), SMC1A (ex2,11,16-17), SMC3 (ex10,13,19,23,25,28), SRSF2 (ex1), STAG2, TET2 (ex3-11), TP53 (ex2-11), U2AF1 (ex2,6), WT1 (ex7,9), ZRSR2

In addition to those listed below in Methodology, the following targets with reduced coverage were not assessed in this specimen [RUNX1 (chr21:36164340-36164578), KDM6A (chrX:44732770-44733003)].

Interpretation: **A single nucleotide variant in the DNMT3A gene was detected. This missense mutation is expected to impact the function of the protein.**

A single nucleotide variant in the JAK2 gene was detected. This missense mutation is expected to impact the function of the protein.

Description: The myeloproliferative neoplasms (MPN) comprise a group of clonal hematopoietic stem cell disorders characterized by overproduction of one or several myeloid lineages in peripheral blood, and additionally manifested as a hypercellular bone marrow.[1] The major clinical concerns for patients with chronic MPNs are the risk of vascular events)thrombosis) and a long-term risk of transformation to acute myeloid leukemia (AML). MPNs are generally diagnosed based on peripheral blood smears and counts, bone marrow morphology, karyotype/FISH and molecular genetic tests.[1] They constitute two main groups: BCR-ABL1-defined chronic myeloid leukemia (CML) and the BCR-ABL1-negative MPN. The latter group encompasses essential thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF), post-ET and post-PV MF, and unclassifiable MPN, and are the target diseases for the current assay.

It has been well documented that about 95% of patients with PV bear the JAK2V617F mutation, as does about 60% of patients with ET and 40-50% of PMF, underscoring the importance of dysregulated growth factor signaling in these neoplasms, in particular the JAK-STAT pathway.[2,3] Other JAK2 mutations were evident in JAK2V617F-negative PV, in particular in exon 12, but not so for the remainder of ET and PMF not bearing JAK2V617F mutation.[4] In these JAK2-unmutated cases, about 10-20% displayed activating mutations in the MPL gene, predominantly p.W515L.[5] In approximately 70-85% of JAK2/MPL wild-type ET and PMF, frameshift mutations were evident in the CALR gene in exon 9 as insertions or deletions.[6] Of the CALR mutations, about 80% could be accounted for by either a 52bp deletion (p.L367fs*46) more frequent in PMF, or a 5bp TTGTC insertion (p.K385fs*47). Mutations in the JAK2, MPL, and CALR genes occur in a mutually exclusive manner. For the most part CALR-mutated ET/PMF patients are younger, have lower leucocyte count and higher platelet count. Other gene members of the JAK-STAT pathway also exhibit mutation in MPNs but at reduced frequencies and include LNK and CBL.[7,8]

Mutation profiling of BCR-ABL1-negative MPNs has revealed the presence of somatic mutations in other genes, including the epigenetic-regulating genes: TET2, DNMT3A, IDH1, and IDH2.[9] TET2 mutations occur in about 7-17% of MPNs, with a higher relative frequency in MF than ET, and is the most common co-occurring mutation with JAK2V617F.[10,11] DNMT3A mutations are evident in up to 5% of MPNs (frequently R882) but upon transformation to AML, the frequency increases up to 20%.[9] Mutations in IDH1/IDH2 are similarly enriched in MPN-derived AML and in JAK2V617F positive PMF cases, the presence of IDH mutations are associated with patients more likely to develop leukemic transformation.[12]

ASXL1, EZH2, SF3B1, SRSF2, and U2AF1 mutations are found in PMF and reportedly with higher frequency in patients with normal karyotype.[13] High-risk PMF disease has been reported to be defined by CALR-negative/ASXL1-positive mutation status.[14] Similarly, mutations in these genes have been reported in PV and ET, but their clinical relevance is less well understood.[15]

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SAMPLE

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