Prima Cancer Genetics

MEDGENOME

Comprehensive Leukemia Panel

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COLLEGE of AMERICAN PATHOLOGISTS

Comprehensive Leukemia Panel

The MedGenome 57 Gene risk stratification and prognostication panel comprises of 10 major entities in haematological malignancies (CML, atypical CML, JMML, CMML, AML, MPN, MDS, ALL, CNL, CLL) grossly, and each of them have multiple clinical subtypes. Most of genes included in this panel are related to poor prognosis, with different levels of risk stratification based on co-existing mutations and other clinical presentation.

Genes Covered:

ABL1	CBLB	EZH2	IKZF1	MYD88	RUNX1	U2AF1
ASXL1	CBLC	FBXW7	JAK2	NOTCH1	SETBP1	WT1
ATM	CDKN2A	FLT3	JAK3	NPM1	SF3B1	ZRSR2
ATRX	CEBPA	GATA1	KDM6A	NRAS	SMC1A	
BCOR	CREBBP	GATA2	KIT	PDGFRA	SMC3	
BCORL1	CSF3R	GNAS	KMT2A	PHF6	SRSF2	
BRAF	CUX1	HRAS	KMT2D	PTEN	STAG2	
CALR	DNMT3A	IDH1	KRAS	PTPN11	TET2	
CBL	ETV6	IDH2	MPL	RAD21	TP53	

This is a hybrid capture based target enrichment panel that is designed to screen for mutations in complete coding region of:

- Oncogenes
- Tumor Suppressor Genes
- Genes involved in Chromatin Remodelling
- Genes involved in DNA methylation and epigenetic regulation
- Genes involved in spliceosome machinery
- Genes involved in regulatory molecules known to play an important role in arresting cell maturation in different stages of hematopoietic progenitor cells and thus driving leukemogenesis.

As compared to single gene testing, comprehensive multigene panels provide additional advantage of understanding the clonal drivers at the time of presentation and during the course of disease evolution, thus helping in better treatment planning: right treatment with right choice in first place to achieve good clinical outcomes in most leukemias.

Diagnostic, Therapeutic and Prognostic Markers

All genes covered in comprehensive leukemia (57-gene) panel are of diagnostic, prognostic or therapeutic importance. It helps in the routine clinical management of leukemias from diagnosis to follow-up and tailoring the treatment as the disease evolves. Below tables indicate each marker and its importance.

Genes involved	Leukemia type
JAK2, CALR, MPL	*Myeloproliferative Neoplasms (MPN)*
CSF3R	*Chronic Neutrophilic Leukaemia*
MYD88	*Walderstrom microglobuneria*
BRAF	*Hairy Cell Leukaemia*
TET2, DNMT3A, TP53, SF3B1, SRSF2, U2AF1, ZRSR2, ASXL1, RUNX1, EZH2, NRAS	*Markers of clonal hematopoiesis: to distinguish MDS from other benign causes of cytopenias*

Table 1: Diagnostic markers covered in comprehensive leukemia (57-gene) panel

Genes involved	Particulars
KIT	*Sunitinib/Dasatinib in AML ; Midostaurin in systemic mastocytosis*
FLT3(ITD/TKD)	*Midostaurin[1] (FDA approved for FLT3-ITD/TKD positive), Quizartinib[2] (FDA approved for FLT3-ITD positive), Sorafenib[3,4] (Investigational drug for FLT3-ITD positive), Gilteritinib[5] (Investigational drug for FLT3-ITD positive) , Crenolanib[6] (Investigational drug for FLT3-ITD/TKD positive) in AML*
KRAS	*Good response to high dose Cytrabine[7] (FDA approved) in AML*
NRAS	*Good response to high dose Cytrabine (FDA approved) in AML*
IDH1	*Ivosedenib[8] (FDA approved) in AML*
IDH2	*Enasidenib[9] (FDA approved) in AML*
CALR	*Fedratinib (Investigational drug) in MPN*
JAK2	*AKAFI/Ruxolitinib[10] (FDA approved) in MPN*
TET2	*Good response to hypomethylating agents in MDS*
ABL1	*Imatinib/Dasatinib/Nilotinib/Ponatinib/Bosutinib[11] (FDA approved) in CML*
BRAF	*Vemurafenib (Investigational drug) in HCL*

Table 2: Therapeutic markers covered in comprehensive leukemia (57-gene) panel

Genes involved	References	Prognosis	Comments
CEBPA	*WHO*	*Good*	*Biallelic: higher DFS; single: shorter DFS*
NPM1	*WHO & NCCN*	*Good*	*Significantly higher OS in older patients (> 60 years CN-AML)*
IDH1	*WHO*	*Debatable (Neutral/Poor)*	*No impact on survival except for codon 132 which is associated with poor survival in CN-AML patients (FLT3-ITD negative).*
IDH2	*WHO*	*Debatable (Neutral/Poor)*	*No impact on survival except for codon 172 which is associated with poor survival in CN-AML patients (FLT3-ITD negative).*
KIT (c-KIT)	*WHO & NCCN*	*Poor*	
ASXL1	*WHO & NCCN*	*Poor*	
DNMT3A (non-R882)	*WHO*	*Poor*	*Significantly lower DFS and OS in older patients (> 60 years CN-AML)*
DNMT3A (R882)	*WHO*	*Poor*	*Significantly lower DFS in younger patients (< 60 years CN-AML)*
FLT3-ITD	*WHO & NCCN*	*Poor*	
FLT3-TKD	*NCCN*	*Debatable (Neutral/Favourable)*	
RUNX1	*WHO & NCCN*	*Poor*	
WT1	*WHO*	*Poor*	
TET2	*WHO*	*Debatable (Neutral/Poor DFS and OS)*	
TP53	*WHO*	*Poor*	

Table 3: Prognostic markers covered in comprehensive leukemia (57-gene) panel for Acute Myeloid Leukemia

Genes involved	References	Prognosis	Comments
ASXL1	NCCN & WHO	Bad	
BCOR	WHO	Poor	
ETV6/TEL	NCCN	Poor	
EZH2	NCCN & WHO	Poor	
DNMT3A	WHO	Bad	
TET2	NCCN & WHO	Debatable/Neutral Prognosis	Prognosis in terms of OS is debatable and good marker to predict repsonse to hypomethyalting agents.
IDH1	WHO	Debatable/Neutral Prognosis	Prognosis in terms of OS is debatable and good marker to predict repsonse in clinical trials.
TP53	NCCN & WHO	Poor	
NRAS / KRAS	NCCN & WHO	Poor	
RUNX1	NCCN & WHO	Poor	
SF3B1	NCCN & WHO	Good	
U2AF1	WHO	Poor	
ZRSR2	NCCN & WHO	Poor	
STAG2	WHO	Poor	
SRSF2	NCCN & WHO	Poor	
CBL	WHO	Poor	
IDH2	WHO	Debatable/Neutral Prognosis	Prognosis in terms of OS is debatable and good marker to predict repsonse in clinical trials.

Table 4: Prognostic markers covered in comprehensive leukemia (57-gene) panel for Myelodysplastic Syndromes

Prognostic markers covered in comprehensive leukemia (57-gene) panel for other hematological malignancies

B-ALL	*T-ALL*	*LCH*
IKZF1 (Ph positive ALL), CDKN2A, HRAS (T4,11 positive) , JAK2, ETV6, FLT3, CREBBP, ABL1, KRAS, NRAS, TP53	FBXW7, HRAS, NOTCH1, PHF6, PTEN, RUNX1	BRAF
CLL	***CML***	***Ph negative aCML***
MYD88, NOTCH1, SF3B1, TP53	ABL1, CBL(BC), CBLB(BC), CBLC(BC), PDGFRA (Secondary mutations with Imatinib), IKZF1 , CDKN2A	CBL, CBLB, CSF3R (CNL), PDGFRA
CMML	***JMML***	***MPN (PMF, IDF,PV,ET)***
ASXL1, CBLC, SETBP1, SRSF2, TET2, DNMT3A	CBLC, KRAS, NRAS, HRAS, PTPN11, CBL, SETBP1 , ASXL1, EZH2, DNMT3A	CALR, DNMT3A, JAK2, MPL, ASXL1, SRSF2 , IDH1, IDH2, EZH2, SETBP1

Validation:

This is a thoroughly validated in-house developed assay, with excellent performance characteristics as per the clinical laboratory practice guidelines (CAP, AMP and ASCO) with robust in-house developed bio-informatics pipelines for detection of somatic variants (short InDels and SNVs). This assay includes genes that carries significance for diagnosis, prognosis and therapeutic relevance in various haematological malignancies.

Limit of Detection	Specificity
2.5%	100%

* for all haematological malignancies

NGS for Minimal Residual Disease Monitoring:

Although the majority of AML patients achieve a complete morphological remission (CR) after induction therapy, relapse rates remain high. Molecular Minimal Residual Disease (MRD) detection by PCR-based technologies has been shown to improve relapse prediction but has been restricted to specific genetically-defined subsets of AML only. NGS has the advantage that it allows for the assessment of a broad range of disease-related gene mutations in a single assay. Residual leukemia-specific mutations in bone marrow in morphological CR after induction therapy represents the source of relapse. Other persistent mutations (viz, DNMT3A, TET2, and ASXL1 mutations) may represent clonal haematopoiesis that may contribute to relapse. The superiority of NGS-based approach in detecting MRD has been shown in recent clinical studies[12-14]. NGS-based MRD is widely applicable to AML patients, highly predictive of relapse and survival, and help refining transplant and posttransplant management in AML patients. Hence, this comprehensive leukemia 57-gene panel, which covers the recurrently mutated genes in AML (NPM1, DNMT3A, TET2, ASXL1, FLT3-ITD etc) can be used in MRD-detection in various subsets of AML patients.

Specimen requirement

Bone marrow aspirate

Bone marrow aspirate in EDTA tubes

- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate. In case sample is not adequate an additional 2mL of peripheral blood in sterile EDTA vial is requested.

Peripheral blood

Peripheral blood in EDTA tubes

- 20-25°C handling temperature
- Minimum 3 mL of peripheral blood is required

Isolated DNA

- Minimum 1µg of DNA is required (concentration of 50-100ng/µL).

References

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MEDGENOME

Prima by MedGenome offers a wide range of Oncology and Haematology genetic tests, these include:

Molecular Testing for Hematological Malignancies, Comprehensive Leukemia Panel

Differential Diagnosis

Prognosis

IGHV Gene Mutation Testing for CLL, Comprehensive Leukemia Panel, BCR-ABL1 gene fusion analysis

Hereditary Cancer Panel, BRCA1 and BRCA2 gene test, Thalassemia Mutation Test

Risk Assessment

Therapy Selection

Comprehensive Tumor Gene Panel, Somatic Mutation Panel, Comprehensive Leukemia Panel, Molecular Testing for Lung Cancer

OncoTrack, OncoSelect, OncoFocus (Liquid Biopsy Test)

Surveillance

Therapy Monitoring

NGS based IRMA, BCR- ABL1 gene fusion analysis

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