Prima Cancer Genetics

MEDGENOME

Molecular testing for Hematological Malignancies

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

Acute Myeloid Leukemia (AML)

Acute Myeloid Leukemia (AML) is a group of diverse hematologic malignancies characterized by clonal expansion of cells that do not completely mature to carry out their normal functions. AML is the most common type of acute leukemia in adults especially those above 60 years. This type of cancer usually gets worse, if left untreated. The risk of AML increases with age and is more common in men than women.

The most common mutations found in AML include *FLT3*, *NPM1*, *DNMT3A*, *NRAS*, *CEBPA*, *TET2*, *WT1*, *IDH2*, *IDH1* and *KIT*.

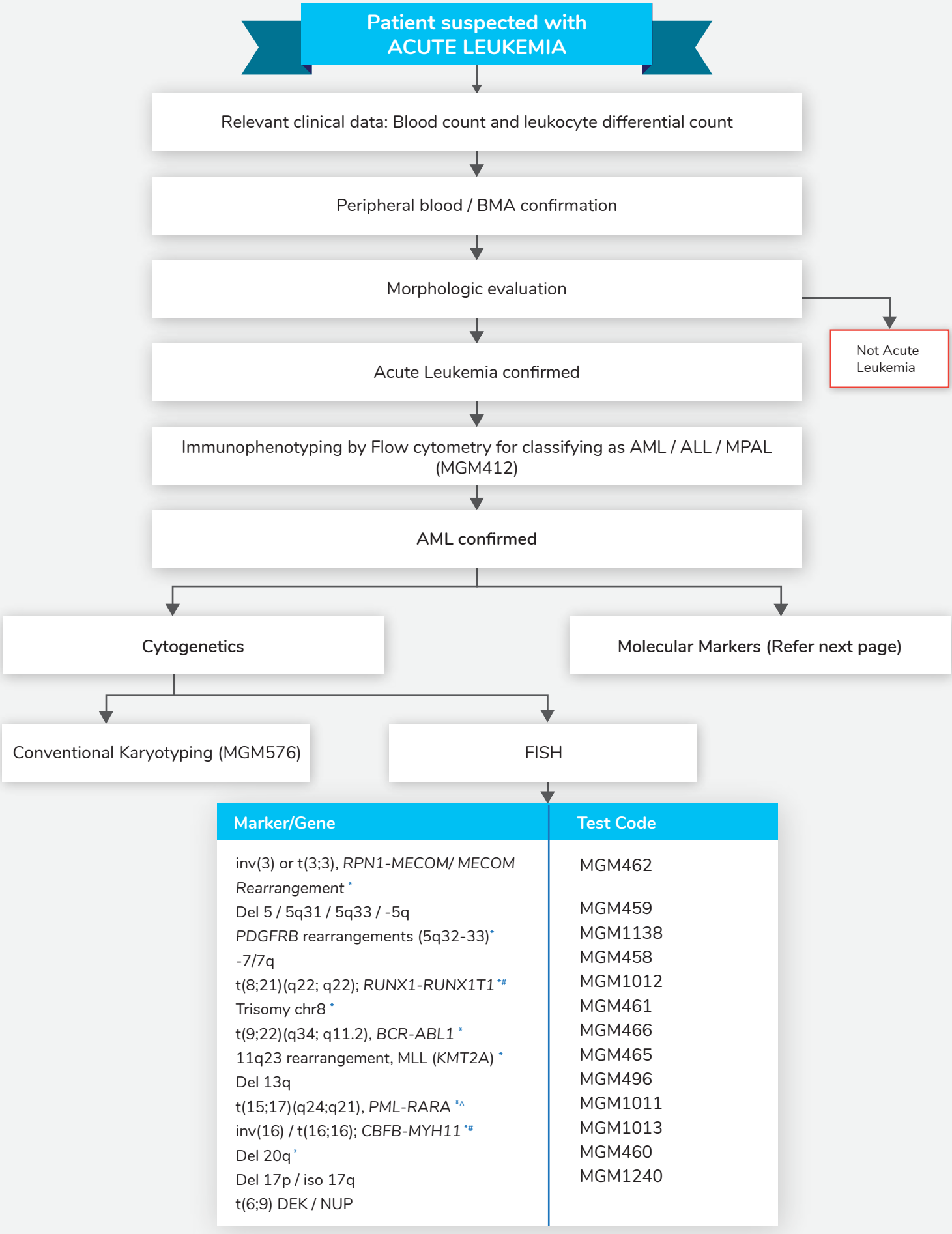
According to the World Health Organization classification, there are increasing number of acute leukemias where categorization is based on molecular genetic abnormalities or cytogenetic abnormalities. These changes form distinct clinical-pathological-genetic entities with diagnostics, prognostic and therapeutic implications.

Prevalence

In India, the annual incidence of AML is 2-3 per 100,000. It is less than 1 per 100,000 under 30 years of age and 17 per 100,000 by 75 years. AML accounts for less than 10% of all leukemias in children less than 10 years of age, and for 25-30% between 10 and 15 years. In adults, AML accounts for 80-90% of cases of acute leukaemia.

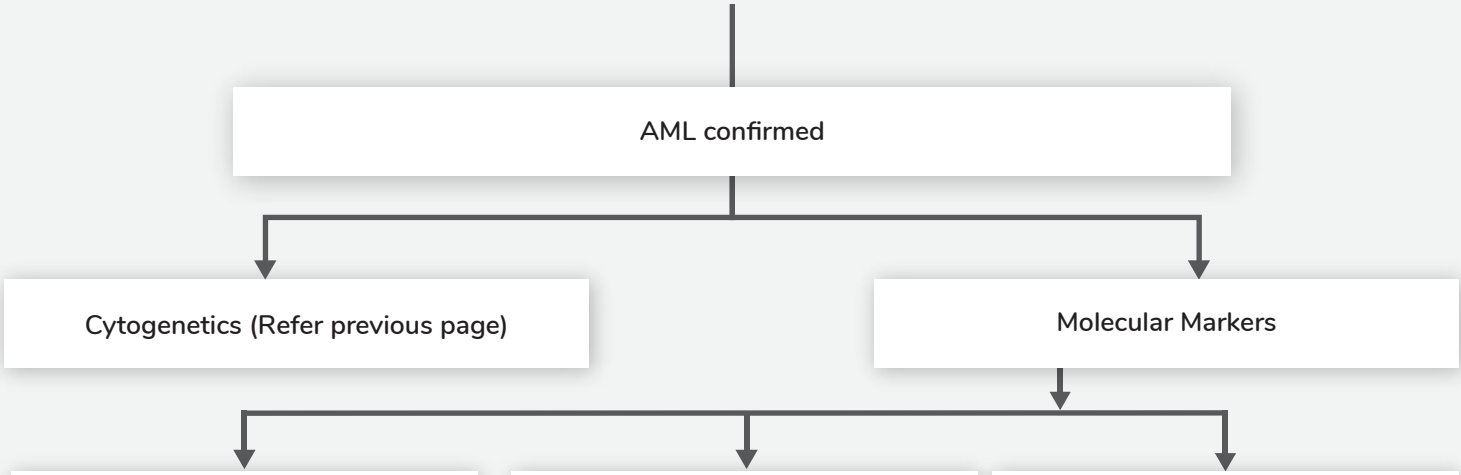
The incidence is higher in males than in females [ICMR, 2018].

The response to treatment and overall survival of patients with AML is heterogeneous. Each subtype has a unique set of biomarkers that classify the subtype for diagnosis, prognosis and therapeutic management. In particular, AML-M3 (APML) is a subtype that harbours unique gene fusion PML-RARA, which is a diagnostic as well as therapeutic marker for APML. In comparison with other clinical subtypes, APML is associated with better clinical outcome, when treated with ATRA+As2O3 as targeted therapy [1,2]. Beyond AML-M3, other subtypes are known to be highly heterogeneous in terms of their molecular genetics. To address the heterogeneity in AML, and to arrive at unique molecular subtype specific to each individual patient, which could be driver mutations or secondary markers associated with prognosis, MedGenome offers a comprehensive spectrum of gene tests adapting to different platforms and a comprehensive Acute Myeloid Leukemia panel by Next-Generation Sequencing, that includes 30 commonly mutated genes in AML with clinical relevance.



Marker/Gene	Test Code
inv(3) or t(3;3), *RPN1-MECOM/ MECOM Rearrangement* *	MGM462
Del 5 / 5q31 / 5q33 / -5q	MGM459
PDGFRB rearrangements (5q32-33)*	MGM1138
-7/7q	MGM458
t(8;21)(q22; q22); *RUNX1-RUNX1T1* *#	MGM1012
Trisomy chr8 *	MGM461
t(9;22)(q34; q11.2), *BCR-ABL1* *	MGM466
11q23 rearrangement, MLL (*KMT2A*) *	MGM465
Del 13q	MGM496
t(15;17)(q24;q21), *PML-RARA* *^	MGM1011
inv(16) / t(16;16); *CBFB-MYH11* *#	MGM1013
Del 20q *	MGM460
Del 17p / iso 17q	MGM1240
t(6;9) DEK / NUP	

* Recommended by World Health Organisation
# Recommended by American Society for Hematology
^ Strongly recommended by American Society for Hematology



Marker/Gene	Test Code
ABL1, *ASXL1**, *ATM*, *ATRX*, *BCOR*, *BCORL1*, *BRAF*, *CALR*, *CBL**, *CBLB*, *CBLC*, *CDKN2A*, *CEBPA**^, *CREBBP*, *CSF3R*, *CUX1*, *DNMT3A**^, *ETV6*, *EZH2*, *FBXW7*, *FLT3* (ITD - D835)*, *GATA1*, *GATA2**, *GNAS*, *HRAS*, *IDH1**^, *IDH2**^, *IKZF1*, *JAK2*, *JAK3*, *KDM6A*, *KIT**^, *KMT2A**, *KMT2D*, *KRAS**, *MPL*, *MYD88*, *NOTCH1*, *NPM1**^, *NRAS**, *PDGFRA*, *PHF6*, *PTEN*, *PTPN11**, *RAD21*, *RUNX1**^, *SETBP1*, *SF3B1**, *SMC1A*, *SMC3*, *SRSF2*, *STAG2*, *TET2*^, *TP53*^, *U2AF1*, *WT1*^, *ZRSR2*	MGM499, MGM170

Marker/Gene	Test Code
t(3;5); *NPM1-MLF1**	MGM1060
t(3;21); *RUNX1-MECOM**	MGM1060
t(5;12); *ETV6-PDGFRB**	MGM1060
t(5;17); *NPM1-RARA**	MGM1060
t(6;9); *DEK-NUP214**	MGM1060
t(6;11); *MLL-AFDN**	MGM1060
t(8;21); *RUNX1-RUNX1T1**	MGM1060
t(9;11); *MLL-MLLT3**	MGM1060
t(10;11); *MLL-MLLT10**	MGM1060
t(15;17); *PML/RARA**^	MGM219, MGM477
t(16;16)/inv(16); - *CBFB-MYH11**	MGM1060

Marker/Gene	Test Code
FLT3 gene internal tandem duplication (*FLT3* - ITD) analysis^	MGM1018, MGM357
NPM1 Gene Analysis (hotspot exon 12)	MGM216
CEBPA gene sequencing^	MGM406
FLT3 D835 Point mutation analysis*	MGM193

* Recommended by World Health Organisation
# Recommended by American Society for Hematology
^ Strongly recommended by American Society for Hematology

Tests offered at MedGenome for AML

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Flowcytometry	Acute Leukemia Classifier Panel	MGM412	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	**2** working days
Karyotyping	Cytogenetic markers	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	inv(3) or t(3;3), *RPN1/MECOM* -5/5q-, RPS14/EGR1/SP15 *PDGFRB* rearrangements t(6;9) DEK / NUP -7/7q-, MLL5/MET/D7ZZ1 t(8;21), *RUNX1T1/RUNX1* +8, D8Z2/MYC t(9;22), BCR/*ABL1* 11q23 rearrangement, MLL (KMT2A) Del 13q-, DLEU/LAMP t(15;17), *PML/RARA* inv(16), *MYH11/CBFB* *Del 17p / iso 17q* Del 20q-, D20S108/20qter t(8;21)(q22;q22), t(15;17)(q24;21), inv(16) or t(16;16), inv 3, Del 5q, Del 7q, MLL amplification, BCR/ABL t(9;22) MDS/AML (6 markers- Del 5/5q, Del 7/7q, Trisomy 8, RUNX-RUNX1 t(8;21), PML-RARA t(15;17), Inv(16)	MGM462 MGM459 MGM1138 MGM458 MGM1012 MGM461 MGM466 MGM465 MGM496 MGM1011 MGM1013 MGM1240 MGM460 MGM1014 (3 Markers) MGM1015 (5 Markers) MGM1016 (8 Markers) MGM1273 (6 Markers)	1 vial of BMA in Sodium-Heparin tube Peripheral blood if blasts is >70% on peripheral smear	**8** working days
RT-PCR	t(3;5)(q25.3;q35.1) - *NPM1-MLF1* t(3;21)(q26.2;q22.1) - *RUNX1-MECOM* t(5;12)(q32;p13.2) - *ETV6-PDGFRB* t(5;17)(q32;p13.2) - *NPM1-RARA* t(6;9)(p23;q34) - *DEK-NUP214* t(6;11)(q27;q23.3) - *MLL-AFDN* t(8;21)(q22;q22.1) - *RUNX1-RUNX1T1* t(9;11)(p22;q23) - *MLL-MLLT3* t(10;11)(p12;q23) - *MLL-MLLT10* t(15;17), *PML/RARA* t(16;16)(p13.1;q22)/inv(16)(p13.1q22) - *CBFB-MYH11*	MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM1060 MGM219, MGM477 MGM1060	1 vial of BMA in EDTA tube	**5** working days
Fragment Analysis	*FLT3* gene internal tandem duplication (*FLT3* - ITD) analysis NPM1 Gene Analysis (hotspot exon 12)	MGM1018, MGM357 MGM216	1 vial of BMA in EDTA tube	**7 & 14** working days
NGS	*ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2* *ASXL1, BRAF, CEBPA, CUX1, DNMT3A, ETV6/TEL, EZH2, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2, JAK2, KIT (c-KIT), KRAS, MLL, NPM1, NRAS, PDGFRA, PHF6, PTPN11, RUNX1, SETBP1, SRSF2, STAG2, TET2, TP53, WT1, ZRSR2*	MGM499 MGM170	1 vial of BMA in EDTA tube 1 vial of BMA in EDTA tube	**21** working days **21** working days
Sanger Sequencing	*CEBPA* gene sequencing *FLT3* D835 Point mutation analysis	MGM406 MGM193	1 vial of BMA in EDTA tube	**10** working days **14** working days
Fragment Analysis & Sanger Sequencing	*FLT3* (ITD, D835), *NPM1*, *CEBPA* Gene analysis	MGM557	1 vial of BMA in EDTA tube	**14** working days

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

References

1. Tallman, Martin S., et al. "All-trans-retinoic acid in acute promyelocytic leukemia." New England Journal of Medicine 337.15 (1997): 1021-1028.
2. Shen, Zhi-Xiang, et al. "All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia." Proceedings of the National Academy of Sciences 101.15 (2004): 5328-5335.

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MEDGENOME

Molecular testing for Acute Lymphoblastic Leukemia (ALL)

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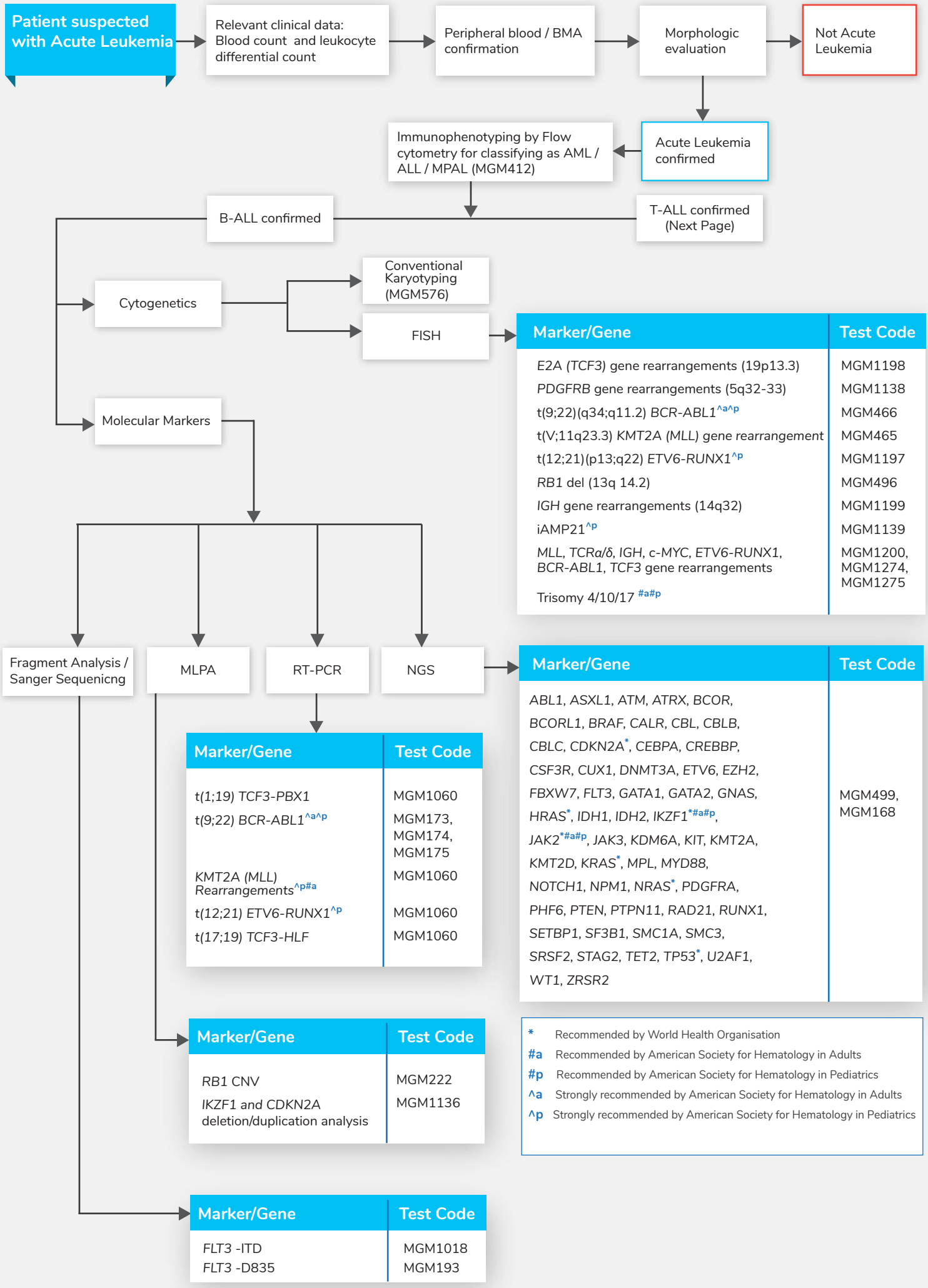
Acute Lymphoblastic Leukemia (ALL)

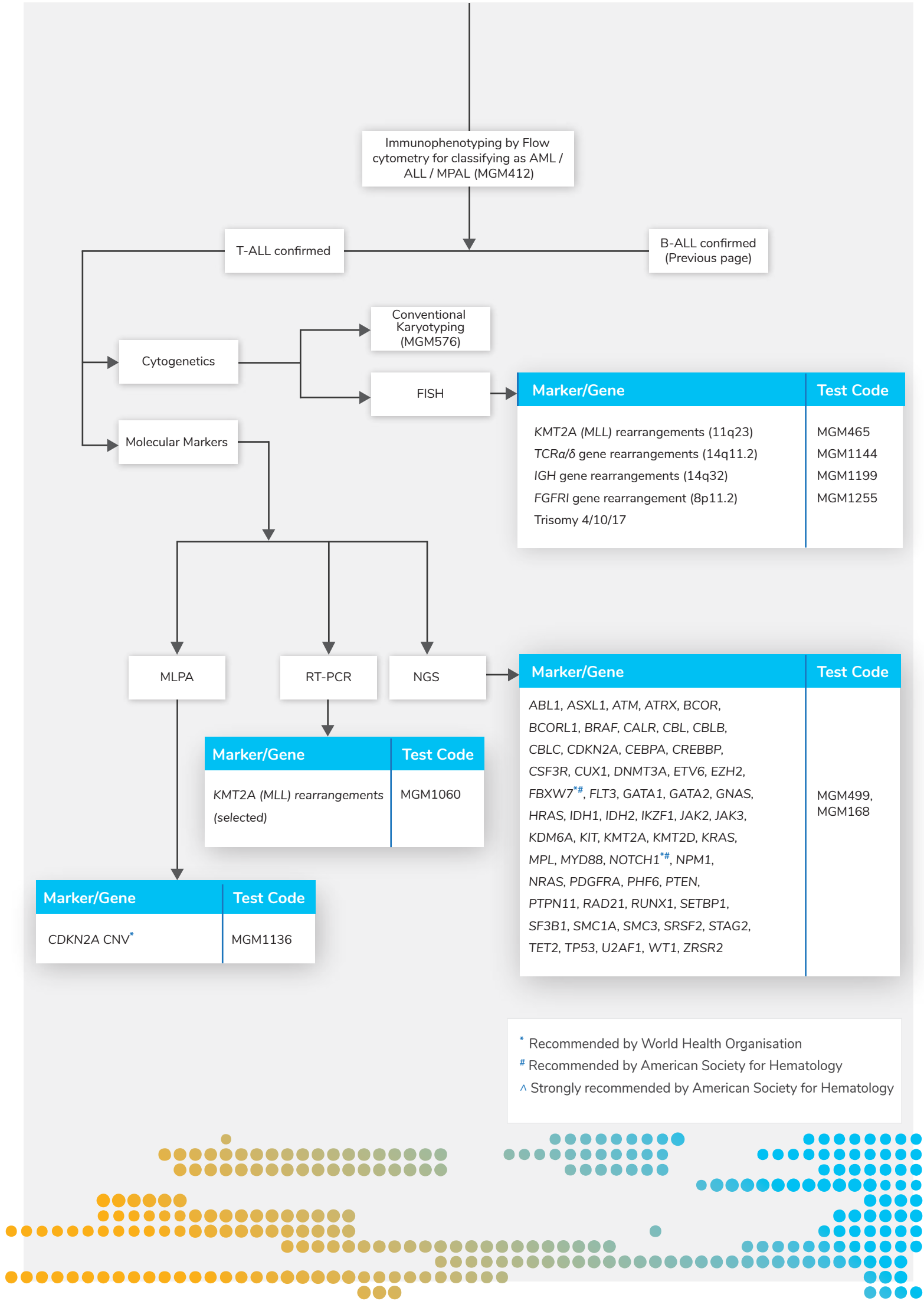
Acute lymphoblastic leukemia is characterized by presence of immature white blood cells ultimately developing into lymphocytes in blood and bone marrow. It is an acute, rapidly progressing form of leukemia. It can be fatal within a few months without treatment. ALL is considered as the second most common acute leukemia in adults and the most common leukemia in children.

Prevalence

In 2015, ALL affected about 8,76,000 people and resulted in 1,11,000 deaths globally [1, 2]. ALL is the most common malignancy in children [3]. It is estimated to affect 1 in 1500 children [4]. It accounts for 25% of all childhood cancers and approximately 75% of all cases of childhood leukemia [5]. Overall survival in ALL ranged from 45% to 81% (commonly >60%) and event-free survival ranged from 41% to 70% (commonly >50%).

The comprehensive genetic testing of patients suspected of having ALL can confirm the diagnosis of ALL and identify important prognostic and predictive biomarkers which can be used to tailor therapy. Mutations involved in various key pathways are found in different subtypes of B-ALL. Genes related to various pathways including *IKZF1*, *TP53*, *CDKN2A*, *ABL1*, *FLT3*, *JAK2*, *NRAS*, *KRAS*, *HRAS* and *CREBBP* are commonly known to be mutated in B-ALL [6]. Among them, specific genetic alterations are found to be associated with adverse clinical outcome and increased risk for relapse. Analysis of these genetic lesions enable the clinician to accurately assess the patient considering the age of the patient for precision medicine, predict the outcomes and need for allogeneic Stem Cell Transplantation (Allo-SCT). Furthermore, mutational analysis may further aid in diagnosis, evaluating response to treatment using Minimal Residual Disease (MRD) and risk assessment. To address the heterogeneity in ALL and to arrive at unique molecular subtype specific to each individual patient, which could be driver mutations or secondary markers associated with prognosis, MedGenome offers a comprehensive spectrum of single gene tests adapting to different platforms and a comprehensive Acute Lymphoblastic Leukemia panel by Next-Generation Sequencing, that includes most commonly mutated genes in ALL.


Patient suspected with Acute Leukemia
Relevant clinical data: Blood count and leukocyte differential count
Peripheral blood / BMA confirmation
Morphologic evaluation
Not Acute Leukemia
Acute Leukemia confirmed
Immunophenotyping by Flow cytometry for classifying as AML / ALL / MPAL (MGM412)
B-ALL confirmed
T-ALL confirmed (Next Page)
Cytogenetics
Conventional Karyotyping (MGM576)
FISH
Molecular Markers
Fragment Analysis / Sanger Sequenicng
MLPA
RT-PCR
NGS
Marker/Gene
Test Code
E2A (TCF3) gene rearrangements (19p13.3) MGM1198
PDGFRB gene rearrangements (5q32-33) MGM1138
t(9;22)(q34;q11.2) BCR-ABL1^a^p MGM466
t(V;11q23.3) KMT2A (MLL) gene rearrangement MGM465
t(12;21)(p13;q22) ETV6-RUNX1^p MGM1197
RB1 del (13q 14.2) MGM496
IGH gene rearrangements (14q32) MGM1199
iAMP21^p MGM1139
MLL, TCRα/δ, IGH, c-MYC, ETV6-RUNX1, BCR-ABL1, TCF3 gene rearrangements MGM1200, MGM1274, MGM1275
Trisomy 4/10/17 #a#p
Marker/Gene
Test Code
t(1;19) TCF3-PBX1 MGM1060
t(9;22) BCR-ABL1^a^p MGM173, MGM174, MGM175
KMT2A (MLL) Rearrangements^p#a MGM1060
t(12;21) ETV6-RUNX1^p MGM1060
t(17;19) TCF3-HLF MGM1060
Marker/Gene
Test Code
ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A*, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS*, IDH1, IDH2, IKZF1*#a#p, JAK2*#a#p, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS*, MPL, MYD88, NOTCH1, NPM1, NRAS*, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53*, U2AF1, WT1, ZRSR2
MGM499, MGM168
Marker/Gene
Test Code
RB1 CNV MGM222
IKZF1 and CDKN2A deletion/duplication analysis MGM1136
Marker/Gene
Test Code
FLT3 -ITD MGM1018
FLT3 -D835 MGM193
* Recommended by World Health Organisation
#a Recommended by American Society for Hematology in Adults
#p Recommended by American Society for Hematology in Pediatrics
^a Strongly recommended by American Society for Hematology in Adults
^p Strongly recommended by American Society for Hematology in Pediatrics



Marker/Gene	Test Code
KMT2A (MLL) rearrangements (11q23)	MGM465
TCRα/δ gene rearrangements (14q11.2)	MGM1144
IGH gene rearrangements (14q32)	MGM1199
FGFRI gene rearrangement (8p11.2)	MGM1255
Trisomy 4/10/17	

Marker/Gene	Test Code
KMT2A (MLL) rearrangements (selected)	MGM1060

Marker/Gene	Test Code
CDKN2A CNV*	MGM1136

Marker/Gene	Test Code
ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7#, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1*#, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*	MGM499, MGM168

* Recommended by World Health Organisation
\# Recommended by American Society for Hematology
^ Strongly recommended by American Society for Hematology

Tests offered at MedGenome for ALL

Clinical Diagnosis	Methodology	Markers	MGM Test code	Sample Requirement	TAT
B-ALL	Flowcytometry	Acute Leukemia Classifier Panel	MGM412	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	2 working days
		B-MRD	MGM1230	2 vials of BMA in EDTA tube (3mL each)	2 working days
	Karyotyping	Cytogenetic markers	MGM576	1 vial of BMA in Sodium-Heparin tube	8 working days
	FISH	*E2A (TCF3)* gene rearrangements	MGM1198	1 vial of BMA in Sodium-Heparin tube Peripheral blood if blasts is >40% on peripheral smear	8 working days
		PDGFRB gene rearrangements	MGM1138		
		t(9;22)(q34.1;q11.2) *BCR-ABL1*	MGM466		
		t(V;11q23.3) *KMT2A (MLL)*	MGM465		
		t(12;21)(p13.2;q22.1) *ETV6-RUNX1*	MGM1197		
		RB1 del (13q 14.2)	MGM496		
		IGH gene rearrangements	MGM1199		
		iAMP21,	MGM1139		
		MLL, TCRα/δ, IGH, c-MYC, ETV6-RUNX1, BCR-ABL1, TCF3 gene rearrangements	MGM1200 (6 Markers) MGM1274 (4 Markers) MGM1275 (7 Markers)		
		TCRA α/δ gene rearrangement	MGM1144		
		FGFR1 gene rearrangement (8p11.2)	MGM1255		
		C-MYC gene rearrangement (8q32)	MGM1201		
		Trisomy 4/10/17	MGM1417		
	RT-PCR	t(9;22)(q34.1;q11.2) *BCR-ABL1*	MGM173, MGM174, MGM175	1 vial of BMA in EDTA tube	5 working days
		t(12;21)(p13.2;q22.1) *ETV6-RUNX1*	MGM1060		
		t(1;19)(q23;p13.2) *TCF3-PBX1*	MGM1060		
		t(17;19)(q22;p13.3) *TCF3-HLF*	MGM1060		
		KMT2A (MLL) Rearrangements	MGM1060		
	MLPA	*RB1 CNV*	MGM222	1 vial of BMA in EDTA tube	14 working days
		IKZF1 and *CDKN2A* deletion/duplication analysis	MGM1136		
	Fragment Analysis	*FLT3-ITD*	MGM1018	1 vial of BMA in EDTA tube	7 working days
	NGS	*ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A*, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS*, IDH1, IDH2, IKZF1*, JAK2*, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS*, MPL, MYD88, NOTCH1, NPM1, NRAS*, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53*, U2AF1, WT1, ZRSR2*	MGM499	1 vial of BMA in EDTA tube	21 working days
		ABL1, CDKN2A, CREBBP, ETV6, FLT3, IKZF1, JAK2, KDM6A, KRAS, MLL2, NRAS, PTEN, TP53	MGM168	1 vial of BMA in EDTA tube	21 working days

Tests offered at MedGenome for ALL

Clinical Diagnosis	Methodology	Markers	MGM Test code	Sample Requirement	TAT
T-ALL	Flowcytometry	Acute Leukemia Classifier Panel	MGM412	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	**2** working days
	Karyotyping	Cytogenetic markers	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
	FISH	t(V;11q23.3) KMT2A (MLL) rearrangements TCRα/δ gene rearrangements IGH gene rearrangements FGFRI gene rearrangement (8p11.2) Trisomy 4/10/17	MGM465 MGM1144 MGM1199 MGM1255	1 vial of BMA in Sodium-Heparin tube	**6** working days
	MLPA	CDKN2A CNV	MGM1136	1 vial of Peripheral blood in EDTA tube	**14** working days
	NGS	*ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7*, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1*, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*	MGM499	1 vial of BMA in EDTA tube	**21** working days
		DNMT3A, FBXW7, HRAS, KRAS, NOTCH1, NRAS, PHF6, PTEN, RUNX1	MGM169	1 vial of BMA in EDTA tube	**21** working days
	RT-PCR	t(V;11q23.3) *KMT2A (MLL)* rearrangements (selected)	MGM1060	1 vial of BMA in EDTA tube	**5** working days

Clinical Diagnosis	Methodology	Markers	MGM Test code	Sample Requirement	TAT
ALL	Comprehensive ALL panel (Karyotyping, FISH, MLPA, NGS, RT-PCR)	Karyotyping- bone marrow BCR-ABL quantitative (International Scale) gene fusion analysis by RT-PCR Multiplex RT-PCR panel for Leukemia by RT-PCR C-MYC rearrangements by FISH iAMP21 by FISH TCR-alpha and TCR-delta gene rearrangement by FISH IKZF1 and CDKN2A deletion/duplication analysis by MLPA Comprehensive leukemia gene panel (57 genes) by NGS	MGM1140	1 vial of BMA in EDTA tube and 2 vial of BMA in Sodium-Heparin tube	**21** working days

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

References

1. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 388 (10053): 1545–1602.
2. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 388 (10053): 1459–1544.
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Prima Cancer Genetics

MEDGENOME

Molecular testing for Chronic Lymphocytic Leukemia (CLL)

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Chronic Lymphocytic Leukemia (CLL)

It becomes difficult for the physicians to identify the patients who may benefit from an early treatment strategy and to provide the patients with relevant information on disease prognosis due to heterogeneous clinical behavior of patients with CLL. The B-cell malignancies share a common cell lineage but a wide range of clinical, laboratory, molecular, and genetic features exists. For decades, “watchful waiting” has been the standard of care for patients with early stage CLL. This approach has focused on minimizing toxicity for the minority of patients whose disease may never evoke clinical symptoms. Considering the clinical heterogeneity of CLL, genetic markers have been established as routinely used prognostic factors in addition to the traditional staging systems.

Single assay for 4 significant genes involved in CLL prognosis (*MYD88*, *NOTCH1*, *TP53*, *SF3B1*) are tested for presence or absence of somatic mutations in the tumor sample with 100% coverage for all genes, except *TP53* (95% gene coverage).

The presence and pattern of gene mutations in CLL can provide critical prognostic information and may help in guiding therapeutic management decisions including the usefulness of hematopoietic stem cell transplant by physicians.

High sensitivity at low input amounts from bone marrow samples.

External quality assessment (EQA/proficiency testing) done and the same accredited by College of American Pathologists.

Cytogenetic and Molecular Markers

Various cytogenetic and molecular markers used in CLL prognostication and risk stratification include

TP53 mutations	*NOTCH1* mutations	*SF3B1* mutations
Increase in progressive stages and accumulate in chemo- refractory patients and is an indicator of very poor prognosis	Unmutated IGHV, trisomy 12 are associated with shorter overall survival	11q deletion and ATM deletion associated with shorter survival
MYD88	Del 13q	Del 11q
Associated with favorable outcome with potential targeted therapy options against Toll-like receptors	Good prognosis	Short overall survival
Del 17p		
Poor outcome		

Expected Survival of CLL Patients

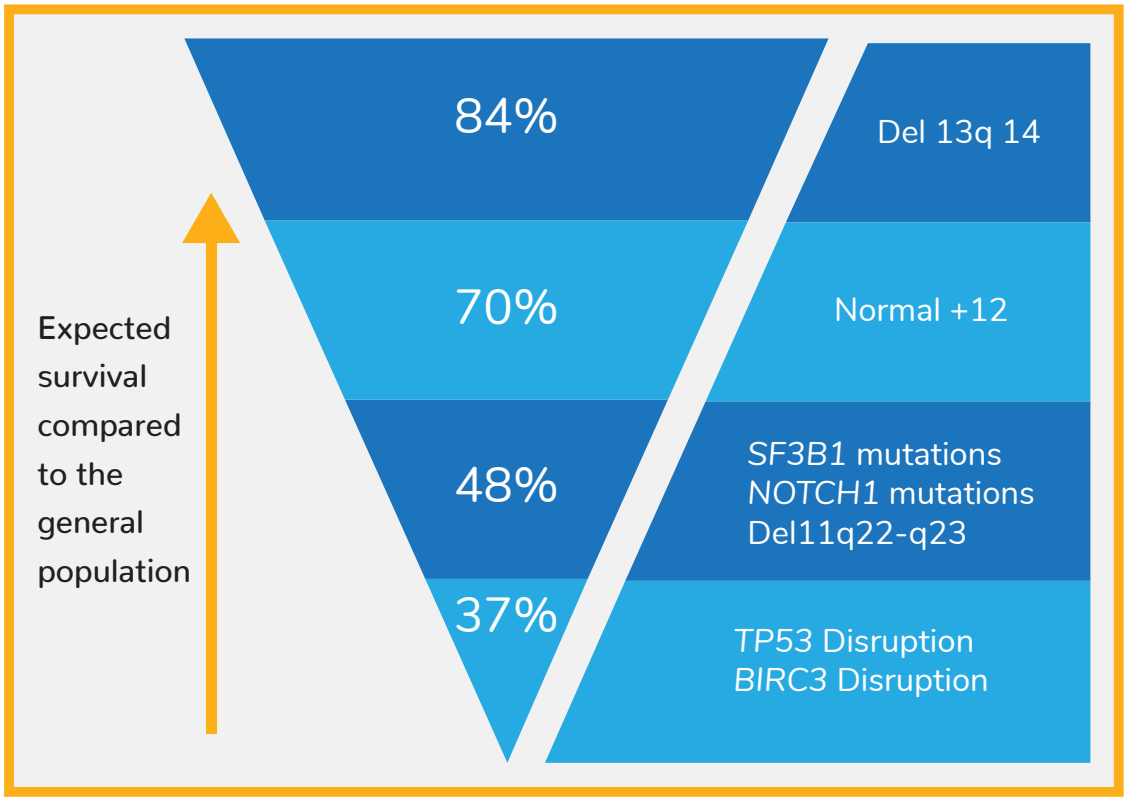

Fig 1:

Expected survival of CLL patients stratified according to the integrated mutational and cytogenetic model and compared to the matched general population.

Expected survival is calculated at ten years. (Adapted from Foà et al., 2013)

Tests offered at MedGenome for CLL

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Flowcytometry	Chronic Lymphoid Leukemia panel, Chronic Lymphoproliferative disorder panel	MGM415 MGM416	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	**2** working days
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	c-MYC gene rearrangements	MGM1201	1 vial of BMA in Sodium-Heparin tube	**6** working days
	IGH rearrangements	MGM1199		
	Del 6q, Del 11q, Trisomy 12, Del 13q, 17p13 (TP53 gene) deletion	MGM1205 (4 markers) MGM1264 (5 markers)		
	Del 6q, Del 17p, Del 13q, ATM gene deletion / trisomy 11, Trisomy 12, IGH gene rearrangement	MGM1276		**7** working days
	Del6q	MGM1204		
	Del17p(TP53)	MGM497		
	Del13q(RBI)	MGM496		
	Del11q (ATM Deletion)	MGM1202		
	Trisomy 12	MGM1203		
MLPA	TP53 gene deletion*	MGM374	1 vial of Peripheral blood in EDTA tube	**14** working days
NGS	ABL1, ASXL1, ATM*, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88*, NOTCH1*, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1*, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53*, U2AF1, WT1, ZRSR2	MGM499	1 vial of BMA in Sodium-Heparin tube	**21** working days
	NOTCH1*, SF3B1, TP53*, ATM, MYD88	MGM185		

* - Gene mentioned in WHO guidelines for the specific disease entity

Tests offered at MedGenome for B-PLL

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Flowcytometry	Chronic Lymphoid Leukemia panel, Chronic Lymphoproliferative disorder panel	MGM415	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	**2** working days
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	c-MYC gene rearrangements IGH rearrangements 17p13 (TP53 gene) deletion IGH-CCNDI t(11;14)	MGM1201 MGM1199 MGM497 MGM1159	1 vial of BMA in Sodium-Heparin tube	**5** working days
MLPA	17p13 (TP53 gene) deletion	MGM374	1 vial of Peripheral blood in EDTA tube	**14** working days

IGHV gene mutation testing in Chronic Lymphocytic Leukemia (CLL)

IGHV mutation

Prediction of the clinical course of the disease based on the level of SHM (somatic hyper mutation) defines 2 subsets of CLL (based on the cut-offvalue of 98% identity with the closest germ line *IGHV* genes)

- *Mutated CLL (M-CLL)- Sequence differs by more than 2% from their corresponding germline sequences*
- *Unmutated CLL (U-CLL)- Sequence differs by lesser than 2% from their corresponding germline sequences*

IGHV mutation in disease prognostication [3-8]

Presence of somatic hypermutation (SHM) of the IGHV region is strongly predictive of a good prognosis, while lack of SHM predicts a poorer prognosis. Patients with an unmutated IGHV gene usually have a more aggressive disease and shorter overall survival. Patients with a mutated IGHV gene usually have a less aggressive disease, with longer overall survival.

IGHV mutation in response prediction [9,10]

Patients with mutated IGHV and without Deletion 17p or TP53 mutations, have shown better response and longer survival with the use of frontline FCR (Fludarabine, Cyclosporine, Retuximab) regimen as compared with that seen in patients with unmutated IGHV. However, CLL patients with unmutated IGHV could be considered for therapy with a BTK inhibitor.

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

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MEDGENOME

Molecular testing for Chronic Myeloid Leukemia (CML)

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Chronic Myeloid Leukemia (CML)

Chronic myeloid leukemia (CML) is a type of myeloproliferative neoplasm that accounts for 15% - 20% diagnosed leukemia cases in adults. CML is characterised by acquisition of Philadelphia (Ph) chromosome due to reciprocal translocation t(9;22)(q34;q11). Expression of BCR-ABL1 fusion protein is the hallmark of CML that causes the uncontrolled myeloid cell proliferation. Quantitative reverse transcription polymerase chain reaction (RT-PCR) is used for quantitation of BCR-ABL1 transcript to determine molecular response (MR) to tyrosine kinase inhibitors (TKIs).

Prevalence

CML is one of the most common adult leukemia in India. It accounts for 30% to 60% of all adult leukemia. The median age varies from 32 years to 42 years in India. The median age at presentation is 10 years younger compared to European and American data

The most common symptom is splenomegaly followed by hepatomegaly, fatigue, weakness, dragging pain, pallor or sometimes asymptomatic seen in 30% cases. Failure in treatment of CML often is associated with poor compliance due to BCR-ABL1 mutation (resulting in altered drug binding) and acquisition of other gene mutations eventually leading to TKIs resistance as disease progresses in CML patient. Mutations in genes such as IKZF1 and CDKN2A are classical mutations implicated in >50% of BP-CML patients. Other molecular markers that aids in risk stratification in CML patients are shown in the table 1. Therefore, analysis of the network of genes will aid in improving the prognosis and therapeutic relevance by accessing for risk-associated therapy and prevention of progression [1].

Moreover, during treatment BCR-ABL1 transcript levels may go undetectable, that may lead the patient to eventually stop the therapy. Discontinuing TKIs was found being risky as rapid recurrence has been observed in 60% of CML patients [2]. Therefore, molecular testing also enables detection of minimum residual disease (MRD) for clinical evaluation of therapy.

Tests offered at MedGenome for CML

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	8 working days
FISH	Trisomy 8 BCR-ABL t(9;22) (q34.1; q11.2) BCR-ABL, iso 17q, Del 7, Trisomy 8 ISO(17q)	MGM461 MGM466 MGM1241 MGM1240	1 vial of BMA in Sodium-Heparin tube	5 working days
RT-PCR	BCR-ABL t(9;22) (q34.1; q11.2); Qualitative BCR-ABL t(9;22) (q34.1; q11.2); Quantitative (International scale)	MGM173 MGM174, MGM175	1 vial of BMA in EDTA tube	4 working days
NGS	ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3 KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2	MGM499	1 vial of BMA in EDTA tube	21 working days
	BCR-ABL1 mutations – Imatinib Resistance (ABL1 kinase) gene analysis (IRMA by NGS)	MGM198		10 working days

TKI therapy in CML

Risk Status	Cytogenetic abnormalities
Favorable	-Y Trisomy 8 extra copy of Philadelphia chromosome
Adverse	i(17)(q10) -7/del(7q) 3q26

Risk stratification by additional chromosomal abnormalities (ACAs) for CML TKI therapy [3]

Mutation	Action
T315I	Consider Ponatinib
T315A, F317L/V/I/C	Consider Nilotinib or Bosutinib rather than Dasatinib
Y253H, F359V/C/I	Consider Dasatinib or Bosutinib rather than Nilotinib
V299L	Consider Nilotinib rather than Bosutinib or Dasatinib
E255K/V	Consider Dasatinib rather than Bosutinib or Nilotinib

TKI therapy according to BCR-ABL1 mutation status [4]

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

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MEDGENOME

Molecular testing for Myelodysplastic Syndromes (MDS)

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Myelodysplastic Syndromes (MDS)

Myelodysplastic Syndromes (MDS) are a group of diverse clonal bone-marrow neoplasm, that is characterized by dysplasia, cytopenia in one or more of myeloid lineages with increased risk of progression to Acute Myelogenous Leukemia (AML) with decreased overall survival. Chromosomal abnormalities and somatic mutations leads to dysplastic changes, and as a result MDS presents with (transfusion dependent) anemia, infections due to neutropenia and/or bleeding due to thrombocytopenia [1]. MDS is diagnosed and classified according to the WHO 2008 classification system, which is based on the microscopic examination of peripheral blood and bone marrow smear findings. Other essential investigations include flow cytometry, genetic profiling and chromosomal analysis. Various prognostic scoring system have been developed which help guide the treatment. The most commonly mutated genes in MDS include *DNMT3A*, *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *CBL*, *IDH1*, *IDH2*, *EZH2*, *TET2*, *ASXL1*, *NRAS*, *RUNX1*, *BCOR*, *STAG2* and *TP53*.

MDS is a disease of the elderly with a median age of 70 years, and an incidence of 3 – 5/100 000 persons rising to >20/100 000 among those over 70 years. [1]. In India, incidence of MDS is unknown, however, the median age of diagnosis is <60 years, which is lower than what is seen in Western population [2, 3]. In US, according to SEER, the age- adjusted incidence of MDS was 4.9 per 100,000 for the year 2007-2011[4].

Some of the subtypes in MDS are associated with clinical biomarkers that aid in disease diagnosis and overall survival [5]. *ETV6*, *DNMT3A*, *EZH2*, *CUX1*, *RUNX1*, *U2AF1* and *STAG2* mutations independently predict poor prognosis in MDS patients [6]. *DNMT3A* mutations were particularly found to be associated with RARS and lowest with RA subtype. Furthermore, MDS patients with *DNMT3A* mutation displayed higher risk of leukemia evolution and shorter overall survival [6, 7].

TET2 is seen at frequency of 20% in MDS and is a marker of favourable prognosis [6]. *SF3B1* mutation were found to be highest in RARS and lowest in RA subtype and has favourable prognosis in MDS [8]. With a frequency of 5-15% in MDS, mutant RAS has high likelihood of transformation to AML and shows an overall poor prognosis. To address the heterogeneity in MDS, and to arrive at unique molecular subtype specific to each individual patient, which could be driver mutations or secondary markers associated with prognosis, MedGenome offers, a comprehensive spectrum of genetic testing adapting different platforms and MDS prognostication and risk stratification gene panel by Next-Generation Sequencing, that includes 23 commonly mutated genes.

Patient with Pancytopenia/Bicytopenia

Relevant clinical data: Blood count and leukocyte differential count

Peripheral blood / BMA confirmation

Morphology - Suspicious of MDS

Immunophenotyping - Suggestive of MDS (MGM417)

Cytogenetics

Molecular Markers (Refer next page)

Conventional Karyotyping (MGM576)

FISH

Marker/Gene	Test Code
MECOM rearrangements inv(3)/ t(3;3)*	MGM462
-5/5q-*	MGM459
-7/7q-*	MGM458
Trisomy 8*	MGM461
MLL rearrangement*	MGM465
Del 13q*	MGM496
Del 17p*	MGM497
Del 20q*	MGM460
-5/5q-, -7/7q-, Del 20q, trisomy 8, MECOM rearrangement, MLL rearrangement, del 17p, del 13q	MGM1017

* Recommended by World Health Organisation

Immunophenotyping - Suggestive of MDS
(MGM417)

Cytogenetics (Refer previous page)

Molecular Markers

NGS

RT-PCR

Marker/Gene	Test Code
ABL1, *ASXL1**, *ATM*, *ATRX*, *BCOR**, *BCORL1*, *BRAF*, *CALR*, *CBL**, *CBLB*, *CBLC*, *CDKN2A*, *CEBPA*, *CREBBP*, *CSF3R*, *CUX1*, *DNMT3A**, *ETV6*, *EZH2**, *FBXW7*, *FLT3*, *GATA1*, *GATA2*, *GNAS*, *HRAS*, *IDH1**, *IDH2**, *IKZF1*, *JAK2*, *JAK3*, *KDM6A*, *KIT*, *KMT2A*, *KMT2D*, *KRAS*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS**, *PDGFRA*, *PHF6*, *PTEN*, *PTPN11*, *RAD21*, *RUNX1**, *SETBP1**, *SF3B1**, *SMC1A*, *SMC3*, *SRSF2**, *STAG2**, *TET2**, *TP53**, *U2AF1**, *WT1*, *ZRSR2**	MGM499, MGM206

Marker/Gene	Test Code
*MECOM fusion**	MGM1060

* Recommended by World Health Organisation

Table 1: Tests offered at MedGenome for MDS

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Flowcytometry	Immunophenotypic markers by Myelodysplasia Panel	MGM417	1 vial of BMA in EDTA tube and 2 freshly prepared air-dried smears	**2** working days
Karyotyping	Cytogenetic markers	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	MECOM rearrangements inv(3)(q21.3q26.2) / t(3;3)(q21.3;q26.2) -5/5q- -7/7q- Trisomy 8 MLL rearrangement Del 13q (RB1) Del 17p (TP53) Del 20q Multiple Markers -5/5q-, -7/7q-, Del 20q, trisomy 8, MECOM rearrangement, MLL rearrangement, del 17p, del 13q	MGM462 MGM459 MGM458 MGM461 MGM465 MGM496 MGM497 MGM460 MGM463, MGM464 MGM1017	1 vial of BMA in Sodium-Heparin tube	**8** working days
NGS	*ATRX, ASXL1, BCOR, CALR, CUX1, BCORL1, ETV6/TEL, EZH2, DNMT3A, GATA1, TET2, IDH1, TP53, NRAS, KRAS, RUNX1, SF3B1, U2AF1, ZRSR2, STAG2, SRSF2, SETBP1, IDH2*	MGM206	1 vial of BMA in EDTA tube	**21** working days
NGS	*ABL1, ASXL1*, ATM, ATRX, BCOR*, BCORL1, BRAF, CALR, CBL*, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A*, ETV6, EZH2*, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1*, IDH2*, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS*, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1*, SETBP1*, SF3B1*, SMC1A, SMC3, SRSF2*, STAG2*, TET2*, TP53*, U2AF1*, WT1, ZRSR2**	MGM499	1 vial of BMA in EDTA tube	**21** working days
RT-PCR	*MECOM fusion*	MGM1060	1 vial of BMA in EDTA tube	**5** working days

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

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MEDGENOME

Molecular testing for Myelo- proliferative Neoplasms (MPN)

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Myeloproliferative Neoplasms (MPN)

The myeloproliferative neoplasms (MPNs) are characterized by proliferation of one or more hematopoietic cell lines. The proliferation is clonal in nature and predominantly in the bone marrow. The myeloid cell expansion extends to peripheral blood in contrast to MDS. MPNs are chronic in nature and remain stable for many years progressing gradually in most cases

Prevalence

MPN is more commonly diagnosed in people over the age of 50 although it can rarely occur in younger people, even very rarely in children.

The entities that are included under MPNs are:

- Chronic myelogenous leukemia (CML)
- Primary myelofibrosis (PMF)
- Unclassifiable MPNs.
- Chronic neutrophilic leukemia
- Essential thrombocythemia (ET)
- Polycythemia vera (PV)
- Chronic eosinophilic leukemia

The overall incidence of myelofibrosis (MF) is approximately the same in men and women. The prevalence of PV is slightly higher in men than women (male-to-female ratio, 1.8:1), and ET is more prevalent in women than men (male-to-female ratio, 1:2). Many people have no symptoms when they are first diagnosed with an MPN and the disease is picked up accidentally during a routine blood test or physical examination.

Recent years have witnessed major advances in the understanding of the molecular pathophysiology of these rare subgroups of chronic myeloproliferative disorders. Identification of somatic mutations in genes associated with pathogenesis and evolution of these myeloproliferative conditions (Janus Kinase 2; myeloproliferative leukemia virus gene; calreticulin) led to substantial changes in the international guidelines for diagnosis and treatment of Ph-negative MPN during the last few years.

These entities can be difficult to distinguish on morphologic bone marrow exam, and diagnosis can be complicated by changing disease patterns: PV and ET can evolve into PMF or undergo leukemic transformation. With a unique pathogenesis and treatment, CML is often considered separately from the rest of the MPNs. The most commonly recognized mutation in the remainder of the Philadelphia chromosome-negative MPNs is Janus kinase 2 (JAK2) V617F, which is present in more than 90% of patients with PV and approximately half of those with PMF or ET.

Mutations within the thrombopoietin receptor gene (MPL) also have been identified in ET and PMF. calreticulin (CALR), have been identified in a large proportion of patients with MPN who did not have a JAK2 or MPL mutation. These three "driver mutations" are often mutually exclusive, meaning that if one is present the others are absent. Nonetheless, roughly 10% of patients with ET or PMF lack JAK2, CALR, or MPL gene mutations and have been referred to as being "triple-negative." Chromosomal abnormalities in patients with PV is less than 20%. The most common abnormalities include partial duplication of chromosome 1, trisomy 8 or 9, or deletion of 13q or 20q. In ET, chromosomal abnormalities are uncommon (< 5% to 10% of cases).

Concurrent mutations, common to myeloid neoplasms and their precursor states, most frequently in SETBP1 and ASXL1, are frequent and appear to be of prognostic significance in CNL. Tests for chronic eosinophilic leukemia include checking for rearrangements with PDGFRA, PDGFRB, FGFR1 and JAK2 genes.

Tests offered at MedGenome for CNL

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	8 working days
FISH	Trisomy 8 Del 7q Del 20q Del 11q	MGM461 MGM458 MGM460 MGM465	1 vial of BMA in Sodium-Heparin tube	5 working days
NGS	*ABL1, ASXL1*, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R*, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2*, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1*, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2* *CSF3R, SETBP1, ASXL1, JAK2*	MGM499 MGM186	1 vial of BMA in EDTA tube	21 working days

Tests offered at MedGenome for ATYPICAL CML

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	8 working days
FISH	Trisomy 8 Del 20q *PDGFRA* gene rearrangement *PDGFRB* gene rearrangement JAK2 gene rearrangement *FGFR1* gene rearrangement	MGM461 MGM460 MGM1137 MGM1138 MGM1254 MGM1255	1 vial of BMA in Sodium-Heparin tube	5 working days
NGS	*ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R*, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1*, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2* *CSF3R, SETBP1, ASXL1, JAK2*	MGM499 MGM186	1 vial of BMA in EDTA tube	21 working days

Tests offered at MedGenome for CEL, NOS

Methodology	Markers	MGM Test code	Sample Requirement	TAT
NGS	*PDGFRA and KIT* *ABL1, ASXL1*, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R*, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2*, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1*, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*	MGM330 MGM499	1 vial of BMA in EDTA tube	**21** working days

Tests offered at MedGenome for MYELOID and LYMPHOID NEOPLASMS WITH EOSINOPHILIA

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Karyotyping	Cytogenetic markers	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	BCR-ABL t(9;22) FGFR1 gene rearrangement PDGFRA gene rearrangement PDGFRB gene rearrangement JAK2 gene rearrangement PDGFRA, PDGFRB, FGFR1, JAK2 gene rearrangements PDGFRA, PDGFRB, JAK2, FGFR1, BCR-ABL gene rearrangements	MGM466 MGM1255 MGM1137 MGM1138 MGM1254 MGM1256 MGM1278	1 vial of BMA in Sodium-Heparin tube	**5** working days
NGS	*ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2*, JAK3, KDM6A, KIT*, KMT2A, KMT2D, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA*, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*	MGM499	1 vial of BMA in EDTA tube	**21** working days
RT-PCR	FIP1L1-PDGFRA ETV6-PDGFRB	MGM520 MGM1060	1 vial of BMA in EDTA tube	**5** working days

Tests offered at MedGenome for MPN COMBO PANEL BY FISH

Methodology	Markers	MGM Test code	Sample Requirement	TAT
FISH	*BCR/ABL, PDGFRA, PDGFRB, JAK2 and FGFR1 gene rearrangements*	MGM1277 MGM1278	1 vial of BMA in Sodium-Heparin tube	**5** working days

Tests offered at MedGenome for PV, MF and ET

Methodology	Markers	MGM Test code	Sample Requirement	TAT
Karyotyping	Cytogenetic Abnormalities	MGM576	1 vial of BMA in Sodium-Heparin tube	**8** working days
FISH	Trisomy 8 Del 20q Del 13q Del 7q BCR-ABL Fusion	MGM461 MGM460 MGM1090 MGM458 MGM466	1 vial of BMA in Sodium-Heparin tube	**5** working days
NGS	*ASXL1, CALR, JAK2, MPL, DNMT3A, TET2* *ABL1, ASXL1, ATM, ATRX, BCOR, BCORL1, BRAF, CALR*, CBL, CBLB, CBLC, CDKN2A, CEBPA, CREBBP, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2*, JAK3, KDM6A, KIT, KMT2A, KMT2D, KRAS, MPL*, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2*	MGM211 MGM499	1 vial of BMA in EDTA tube	**21** working days
Fragment Analysis	CALR (Exon 9)	MGM208	1 vial of BMA in EDTA tube	**7** working days
Sanger Sequencing	JAK2 V617F MPL (Exon 10) JAK2 (Exons 12 & 14)	MGM209 MGM210 MGM199	1 vial of BMA in EDTA tube	**7** working days
Sanger Sequencing and Fragment Analysis	MPN Reflex Testing	MGM1010	1 vial of BMA in EDTA tube	**8** working days
RT-PCR, Sanger Sequencing and Fragment Analysis	*BCR-ABL1, JAK2, MPL, CALR*	MGM574	1 vial of BMA in EDTA tube	**10** working days
MLPA	*CALR, JAK2, KIT , MPL* mutation analysis	MGM538	1 vial of peripheral blood in EDTA tube	**5** working days

Specimen requirement

Bone marrow aspirate

- For all tests except FISH and karyotype: Bone marrow aspirate in EDTA
- For FISH & Karyotype: Bone marrow aspirate in Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 1-2 mL of bone marrow aspirate
- For FISH & Karyotype, in case sample is not adequate, an additional 2mL of peripheral blood in sterile sodium heparin vial is requested

Peripheral blood

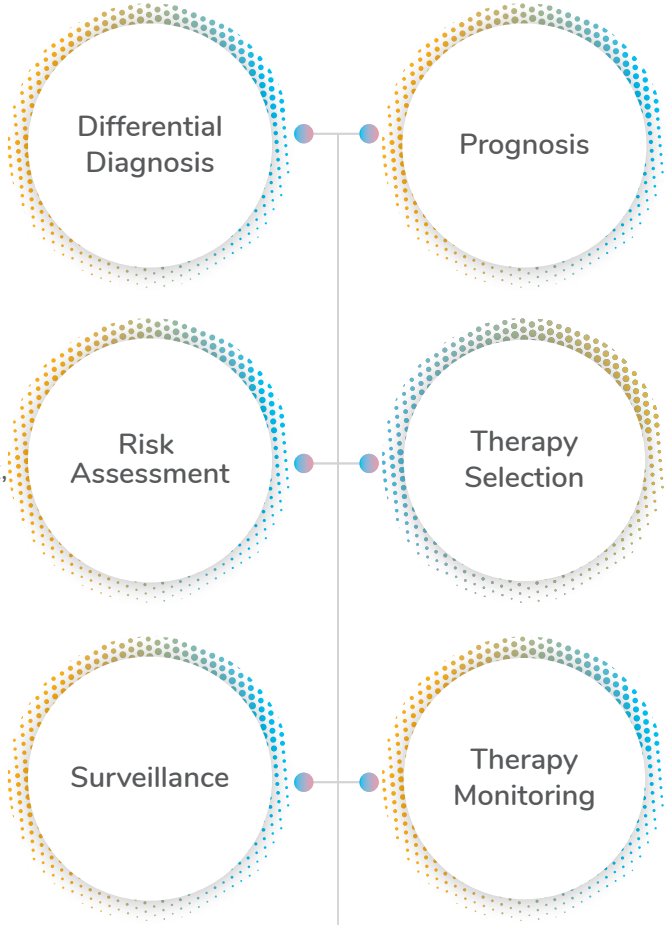
- For all tests except FISH and karyotype: EDTA anticoagulated peripheral blood
- For FISH and karyotype: Sodium heparin vacutainer (green top)
- 20-25°C handling temperature
- Minimum 3ml of peripheral blood is required

Sample handling, storage and cautions (If any)

- Peripheral blood or Bone Marrow Aspirates collected in EDTA vaccutainer tubes should be sent within 48 hours of collection with a cool pack or (if stored at -80^{0}C) with dry ice
- Peripheral blood or Bone Marrow Aspirates collected in Sodium heparin vaccutainer (green top) tubes should be sent within 48 hours of collection in ambient temparature and not to be freezed
- Please ensure that isolation of DNA that is to be sent as a specimen for clinical testing at Medgenome occurs in an accredited laboratory for DNA-based tests

MEDGENOME

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



Molecular Testing for Hematological Malignancies, Comprehensive Leukemia Panel

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

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

OncoTrack, OncoSelect, OncoFocus (Liquid Biopsy Test)

NGS based IRMA, BCR- ABL1 gene fusion analysis

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