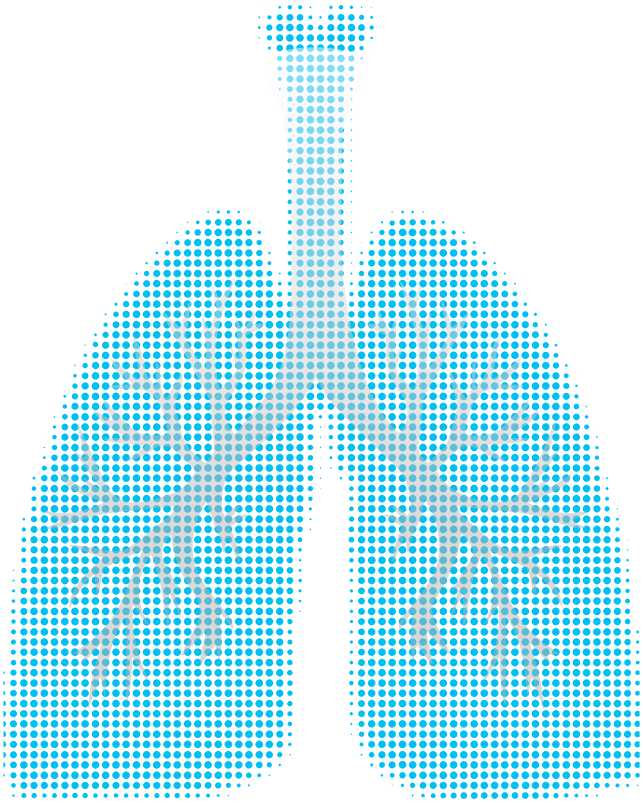
Prima
Cancer Genetics

MEDGENOME

Molecular Testing For
Lung Cancer

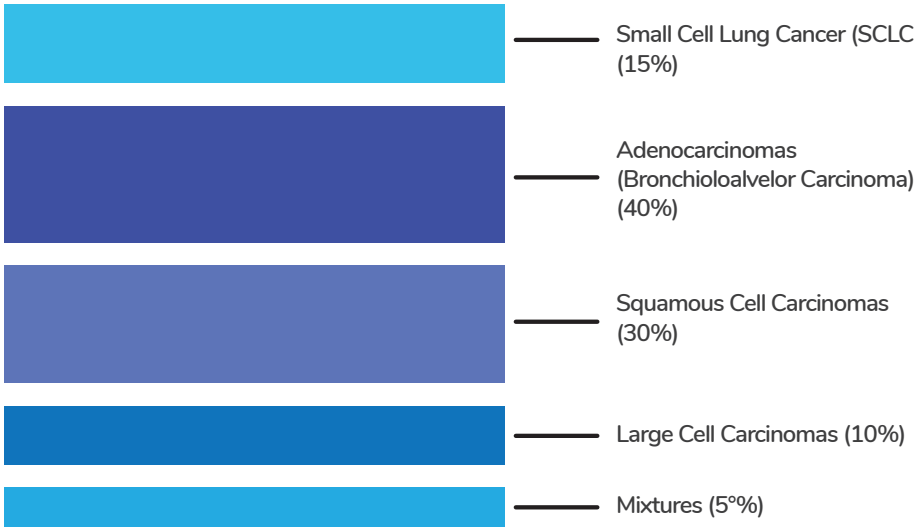


Alarming scenario of Cancer in India*

- In 2016 the total number of new cancer cases was expected to be around 14.5 lakh and the figure is likely to reach nearly 17.3 lakh new cases in 2020.
- Over 7.36 lakh people were expected to succumb to the disease in 2016 while the figure is estimated to shoot up to 8.8 lakh by 2020.

Lung Cancer in India is rising*

- Lung Cancer continues to be one of the commonest cancer in Indian Males
- There is an alarming rise in the incidence of Lung Cancer in Women
- The Cancer incidence in India is increasing rapidly, specifically lung cancer with an estimated 0.1 million new cases during 2016 which was expected to increase to 0.14 million cases by 2020, accounting for 23% increase in incidence rate
- Lung cancer is the most common cause of death from cancer, with 1.6 million deaths (19.4% of all cancer-related deaths)



MedGenome's comprehensive offering of the most advanced Genetic and Molecular Tests for Cancer

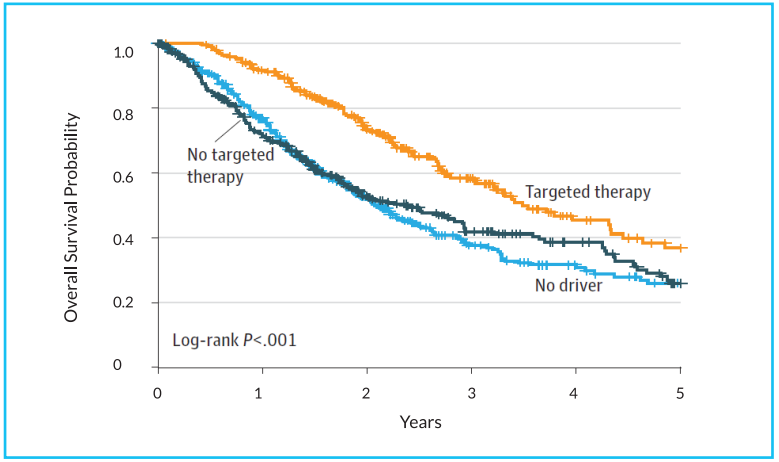
Advanced testing techniques in Prima

1. Next Generation Sequencing (NGS)
2. Fluorescence In Situ Hybridization
3. Sanger Sequencing & Fragment Analysis
4. Real-Time qPCR
5. Droplet-Digital PCR
6. Immunohistochemistry
7. Flow Cytometry

How Molecular and Genetic Tests Help

- Ability to sub-classify the type of cancer and accurate diagnosis
- Disease prognostication (assessment of survival and response to treatment)
- Theranostic value (Finding the best fit targeted drugs and minimum adverse effect related to chemo/radiation therapy)
- Hereditary cancer risk assessment

Effect of Molecular Testing for Non-Small Cell Lung Cancer patients



No. at risk	Patients with oncogenic driver					
No. targeted therapy	318	205	110	64	43	20
Targeted therapy	260	225	143	72	36	23
Patients with no driver	360	250	122	59	36	23

Survival Comparisons: ALK indicates anaplastic lymphoma kinase gene; EGFR(s), the epidermal growth factor receptor gene (sensitizing); EGFR(o), epidermal growth factor receptor gene (other); KRAS, Kirsten rat sarcoma; NA, not applicable. Median survival (95% CI): oncogenicdriver + no targeted therapy, 2.38 (I.81 ·2.93); oncogenic driver+ targeted therapy, 3.49 (3.02-4.33); no oncogenic driver, 2.08 (1.84-2.46).

Reference: Kris MG, Johnson BE, Berry LO, et al: Using roottipfexed assays of oncogenic drivers In lungc.ancers to select targeted drugs. JAMA311:1998·2006, 2014.

Molecular Testing is the first step to suitable treatment of Lung Cancer

Currently, the targeted therapies guided by the molecular diagnostics became the standard of treatment for lung cancer patients. The presence of the genetic alterations in the cancer eel Is which drives the growth of the tumor (Driver alterations) allows the selection of treatment regime for individual lung cancer patients.

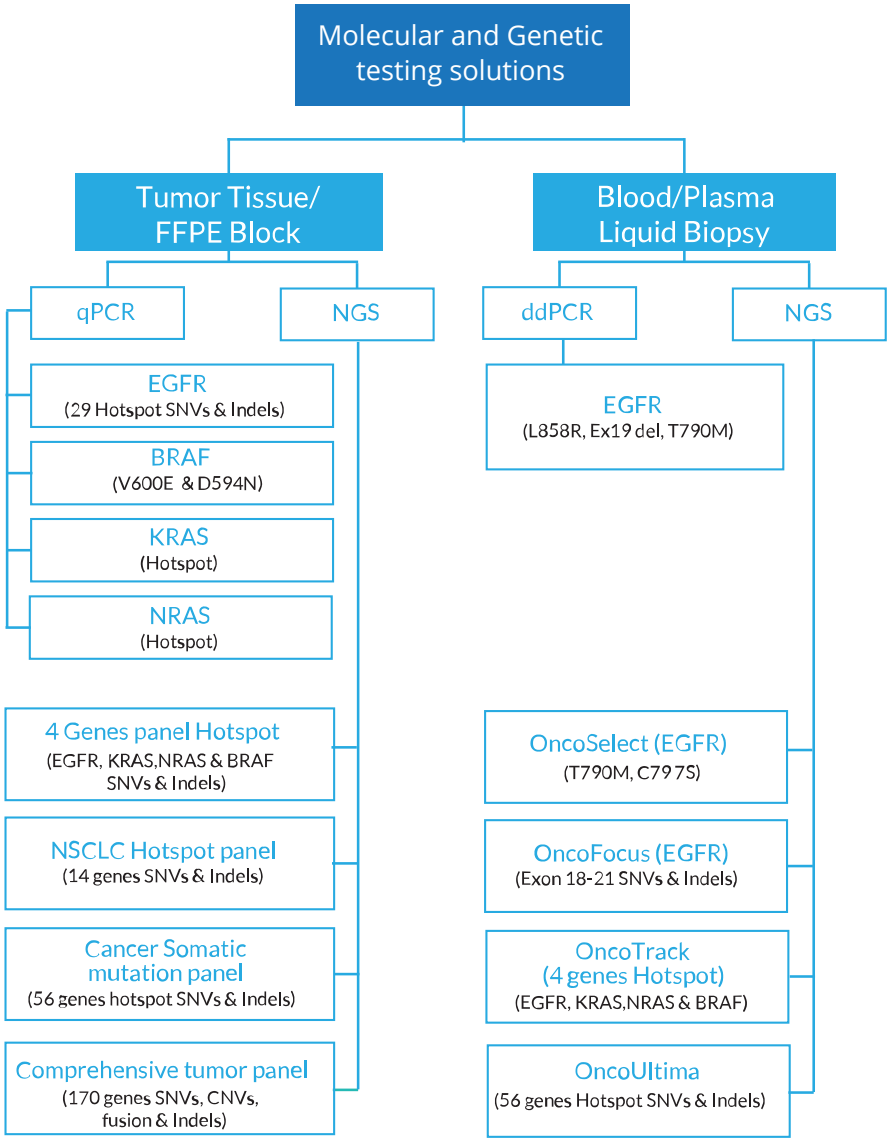
Most Commonly occurring Genomic alterations in Lung Cancer

Frequency in NSCLC	Gene	Variant Type	Inhibitors
1%	RET	Rearrangement	Cabozantinib, Vandetanib, or Alectinib
1%	ROS1	Rearrangement	Crizotinib, Cabozantinib
1%	NRAS	SNVs	Trametinib, Selumetinib
1%	AKT1	SNVs	Buparlisib
1%	MEK1	SNVs	Rearrangement
2%	BRAF	SNVs	Rearrangement
2%	HER2	SNVs & Indels	Rearrangement
2%	PIK3CA	SNVs	Rearrangement
3%	MET	Amplification	Rearrangement
4%	DDR2	SNVs	Rearrangement
5%	ALK	Rearrangement & SNVs	Rearrangement
6%	PTEN	SNVs & Indels	Rearrangement
20%	KRAS	SNVs	Rearrangement
20-35%	EGFR	SNVs & Indels	Rearrangement
35%	Unknown alterations	Rearrangement	Rearrangement

Actionable Genes/Mutations covered in MedGenome Lung hotspot tumor panel

Gene's	Testing Types	Hotspot covered	SNV	Indel	Fusion	CNV	NCCN/ ESMO	FDA approved Inhibitor
ALK	Somatic	YES	●	●	●	●	YES	Crizotinib,Ceriti nib, Alectinib
BRAF	Somatic	YES	●	●	●	●	YES	Vemurafenib, Dabrafenib plus Trametinib;
EGFR	Somatic	YES	●	●	●	●	YES	Erlotinib, Gefitinib, Afatinib and Osimertinib
ERBB2	Somatic	YES	●	●	●	●	YES	Aafatinib,Neratinib
MET	Somatic	YES	●	●	●	●	YES	Crizotinib, Cabozantinib
PIK3CA	Somatic	YES	●	●	●	●	NO	PI3K inhibitors
RET	Somatic	YES	●	●	●	●	YES	Cabozantin i b, Vandetani b, or Alectinib
ROSI	Somatic	YES	●	●	●		YES	Crizotinib, cabozantinib
KRAS	Somatic	YES	●	●		●	YES	Trametinib, Selumetinib
NRAS	Somatic	YES	●	●		●	NO	Trametinib, Selumetinib
PTEN	Somatic	YES	●	●		●	NO	PI3K-AKT inhibitors
AKTI	Somatic	YES	●	●			NO	DZBuparlisib
DDR2	Somatic	YES	●	●			NO	Dasatinib + Erlotinib
HRAS	Somatic	YES	●	●			NO	Trametinib, Selumetinib
MAP2K1	Somatic	YES	●	●			NO	Trametinib, Selumetinibz
RICTOR	Somatic	YES				●	NO	TORC1/2 inhibitors
NTRK1	Somatic	YES			●		NO	TRKA/8/C inhibitor

MedGenome's Prima Testing solution for Molecular and Genetic evaluation of Lung Cancer



Therapeutics using Liquid Biopsy with Onco Track

- It is a minimally invasive blood test to monitor patients during the initiation of therapy (baseline) and at regular intervals (follow-up) for assessment of clinical response to the treatment
- It facilitates early detection of emergent genetic alterations that can be associated with resistance to therapy during cancer progression
- This test is designed to sequence regions of oncogenes: EGFR, KRAS, NRAS and BRAF, which are somatically altered recurrent mutations in solid tumors with role in targeted therapy
- This test also screens for novel mutations that has relevance in targeted therapy; and are in pipeline for approval as well as in clinical trials

OncoTrack is based on NGS Technology

- High sensitivity and ability to detect low frequency mutations
- Unlike other techniques, NGS has the advantage of multi-gene profiling in a single assay providing information on the exon covering the HOTSPOT mutation
- Massively parallel sequencing, which provides data with high and greater accuracy
- High throughput enables a faster turn around time
- Enables sequential testing over long periods of time without any limited material (tumour tissue) constraints
- Ability to identify the exact mutation, especially for insertion and deletion cases
- Extensively validated (CAP accredated) publication given below

Validation of Liquid Biopsy: plasma cell-free DNA testing in clinical management of advanced non-small cell lung cancer. Lung Cancer: Targets and Therapy, 2017(1n press). Vidya H Veldore, A. C., Tejaswi Routhu, Nitin Mandloi, Vanita Noronha, Amit Joshi, Amit Dutt, Ravi Gupta, Ramprasad L Vedam, Kumar Prabhash.

Benefits of Liquid Biopsy to a Lung Cancer Patient

1. Can be performed on the patients with the known molecular signature of the tumor (either EGFR, ROS1, KRAS, BRAF, NRAS or ALK1 translocations and resistance mutations)
2. Being Non-invasive, easier to perform multiple times during the course of the disease
3. rovides an overall indication of tumor burden of the patient. however, still requires pathological analysis of tumor specimen that cannot be achieved through Liquid Biopsy
    - Tumor Biopsy and histopathology remains the standard of care for diagnosis of Lung Cancer
4. Tumor heterogeneityore Biopsy can miss sections of the tumor
    - The advantage of a Liquid Biopsy is that analysis of the plasma may better capture the spectrum of clones present within a patient
5. Can be used periodically to monitor disease progression, response to therapy, and development of treatment resistance

ctDNA based Liquid Biopsy Application in Oncology

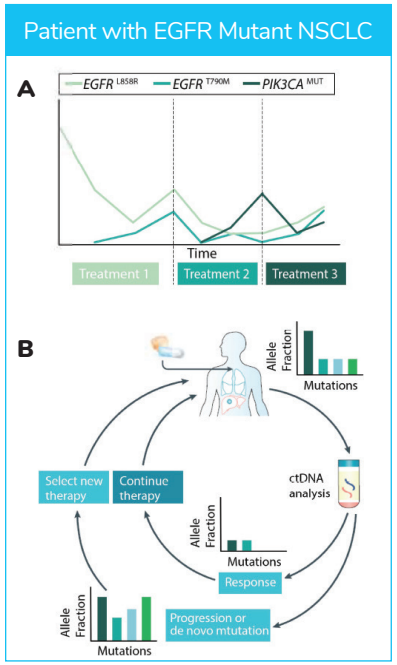
1. The first line of targeted therapy given based on the tumor tissue EGFR testing
2. First relapse and emergence of resistance mutation (T790M) and then receive the second line of targeted therapy
3. Second relapse due to the emergence of another mutation in PI K3CA and treatment of the third line of targeted therapy

Applications of circulating tumour DNA analysis during the course of disease management.

(Reproduced from Wan, J., Massie, C. E., Garcia-Corbacho, J., Mouliere, F., Brenton, J., Caldas, C. M., Pacey, S. C., et al. Liquid biopsies come of age: towards implementation of circulating tumor DNA. Nature Reviews Cancer, 17 223-238)

(a) A schematic time course for a hypothetical patient who undergoes surgery (or other initial treatment), has a disease relapse and then receives systemic therapy. The potential applications of liquid biopsies during this patient's care are indicated. The patient starts with one single disease focus, but multiple metastases and distinct clones (depicted in different colors) emerge following treatment.

(b) The information extracted from circulating tumor DNA (ctDNA) may be classified, broadly, as quantitative information (that is, relating to tumor burden) or genomic information. Quantification of ctDNA at a single time point may allow disease staging and prognostication, and genomic analysis can inform the selection of targeted therapies. Therefore, the longitudinal analysis allows the quantitative tracking of tumor burden to monitor treatment response, for example, and by comparing genomic profiles over time, clonal evolution may be monitored



OncoTrack Test Applications

A One-shot blood test to monitor drug response status for NSCLC and CRC patients

- It is a minimally invasive blood test to monitor patients during the initiation of therapy (baseline) and at regular intervals (follow-up) for assessment of development of 'drug resistant' mutations
- It facilitates early detection of emergent genetic alterations that can be associated with resistance to therapy during cancer progression. (Example: T790M in NSCLC)
- This test is designed to sequence regions of oncogenes: EGFR, KRAS, N RAS and BRAF
- These genetic alterations help in designing personalised therapy for cancer patients

KRAS
Exons 2,3 & 4

BRAF
Exons 11 & 15

NRAS
Exons 2,3, & 4

EGFR
Exons 18, 19, 20, & 21
Includes T790M & C7975 Mutation which indicated resistance to EGFR TKI treatment's

HRAS
Exons 2, 3

Droplet Digital PCR based detection of EGFR mutation in Lung Cancer Patients

Droplet Digital polymerase chain reaction (ddPCR) measures absolute quantities by counting nucleic acid molecules encapsulated in discrete, volumetrically defined

- It provides a greater sensitivity of the targeted set of known mutations
- It is cost effective for the rapid genotyping and serial monitoring
- Absolute quantification of wild-type and mutant copies could be determined
- MedGenome test for EGFR (L858R, Exon19del and T790M) using ddPCR

The Liquid Biopsy - Test process

1.
Order Liquid Biopsy kit from MedGenome Labs 48 hours before Liquid Biopsy. Kit will be dispatched to you at room temperature.

2.
Carry out the Liquid Biopsy as shown, in the Liquid Biopsy kit and collect the samples using 21 gauge Vacutainer provided with kit

3.
Contact the local MedGenome representative to arrange for shipping to the laboratory

4.
Leave the filled tubes in the box at room temperature till collection for transport
(Temp: 6° C to 36° C)

Do not store in a refrigerator or freezer

Checklist Details

(Must send the following along with the Liquid Biopsy specimen)

- Completely filled and signed TRF
- Previous tissue Biopsy report (Histopathology Report)
- Molecular testing report on Tissue Biopsy, if any
- Treatment history: Treatment naïve/on treatment/completed treatment
- Please mention in the TRF if it is first time evaluation/follow-up evaluation
- If it's a follow-up evaluation, please enclose the previous ctDNA report

Sample types for Molecular Testing

Peripheral Blood

- Liquid Biopsy: 20ml (2x10ml) in streck tubes or Liquid Biopsy kit ordered from MedGenome
- FFPE Tissue Block (Formalin Fixed Paraffin Embeded Tissue Block)

FFPE tumor tissue

- 3-4mm section containing up to 10% of the tumor content as verified by inhouse pathologis

Sample Shipments

- FFPE Tissue Blocks in card wood box Ambient (20-26°C)
- Liquid Biopsy samples: From Blood collectionto shipment in Liquid Biopsy kit with streck tubes 2x10ml blood in gel pack. Do not store in refrigerator or freezer, Ambient (20-26°C)

Molecular Testing In Lung Cancer - MedGenome Test Menu

Test Code	Test Name	Methodology
MGM481	OncoFocus - ctDNA for EGFR	NGS
MGM482	OncoFocus Express ctDNA for EGFR	NGS
MGM420	OncoSelect ctDNA for EGFR T790M and C797S	NGS
MGM403	OncoTrack -ct DNA for Hot Spot mutations in 4 genes (EGFR, KRAS, NRAS, BRAF)	NGS
MGM455	Oncotrack - Ultima [Liquid biopsy for 56 theranostic genes]	NGS
MGM331	Non Small cell Lung Cancer (NSCLC) NGS Panel (Hot Spot)	NGS
MGM547	EGFR (T790M, L858R, exon 19 deletion) screening by ddPCR	Droplet Digital PCR
MGM548	EGFR T790M mutation screening by ddPCR	Droplet Digital PCR
MGM190	EGFR gene analysis (Hot Spot) - 4 exons (18, 19, 20, 21)	RT-PCR
MGM1085	ALKD5F3 by FISH	FISH
MGM573	Lung combo panel - IHC (ALK D5F3, ROS1) & RT-PCR (BRAF V600E, EGFR[Hot Spot] exons 18, 19, 20, 21)	IHC, RT-PCR, NGS
MGM1084	ROS1 by FISH	FISH
MGM236	ALK D5F3	IHC
MGM539	ALK D5F3 & ROS1 IHC analysis	IHC
MGM238	c-MET IHC analysis	IHC
MGM506	Lung tumor panel I (ALK D5F3, ROS1, c-MET)	IHC
MGM505	Lung tumor panel II (ANY TWO OF - ALK D5F3, ROS1, c-MET)	IHC

Test Code	Test Name	Methodology
MGM243	ROS1 IHC analysis	IHC
MGM525	PDL1 IHC	IHC
MGM1495	Lung Advanced Panel by NGS & IHC [EGFR, ALK, ROS1, BRAF,MET (SNVs,Indels,Skipping mutations), RET, Her2 by NGS & PDL1 by IHC]	Next Generation Sequencing & IHC
MGM1494	Lung Advanced Panel by NGS [EGFR, ALK, ROS1, BRAF,MET (SNVs,Indels,Skipping mutations), RET, Her2]	Next Generation Sequencing
MGM1493	Lung Basic Panel by NGS & IHC [EGFR, ALK, ROS1, BRAF,MET (SNVs & Indels) by NGS & PDL1 by IHC]	Next Generation Sequencing & IHC
MGM1492	Lung Basic Panel by NGS [EGFR, ALK, ROS1, BRAF,MET (SNVs & Indels)]	Next Generation Sequencing
MGM1496	Lung Comprehensive Panel [EGFR, ALK, ROS1, BRAF, MET (SNVs,Indels,Skipping mutations), RET, Her2 by NGS, PDL1 by IHC & MSI by Fragment Analysis]	Next Generation Sequencing , IHC & Fragment Analysis

Sample Report

MEDGENOME

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DNA TEST REPORT – MEDGENOME LABORATORIES

Patient Name	ABCD	Gender / Age	M / 55 Y
Hospital Name	EFGH Hospital, Bangalore	Unique Identification Number (UID)	FGHI
Physician Name	Dr. XYZ	Sample Type	Blood in Streck® tubes
Test requested	Liquid biopsy panel fusion gene panel	Collection date	10-02-2018,10:00:00
		Received date	13-02-2018,12:53:32
MG Samples ID/ Order ID	2222 / 333333	Report Date	21-02-2018, 11:00:00
Sample Acceptance Criteria	PASSED. The plasma DNA from the patient yielded ≥30 ng, which is sufficient to proceed further with the test.		

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Non-Small Cell Lung Carcinoma.

RESULTS

Gene fusion associated with *ALK* gene was detected in this subject

No clinically relevant actionable mutations were detected in *EGFR* gene of this subject

TABLE 1: GENOMIC ALTERATIONS THAT CAN BE TARGETED WITH APPROVED DRUGS IN THE SUBJECT'S TUMOR TYPE

Gene	CDS variant#	Amino acid variant / Exon No.	Overall Depth / Mutant Allele (%)	FDA Approved drugs	Drug response	Hot spot Mutation	Function of the gene in cancer
None							

TABLE 2: NON-DRUGGABLE/DRUGGABLE CLINICALLY SIGNIFICANT GENOMIC ALTERATIONS INDICATED IN OTHER TUMORS

Sl. No.	Gene	CDS variant#	Amino acid variant / Exon No.	Overall Depth/ Mutant Allele (%)	Impact on Protein Function	Function of the gene in cancer	Pathway in which the gene functions
None							

TABLE 3: VARIANTS OF UNKNOWN SIGNIFICANCE

Sl. No.	Gene	CDS variant#	Amino variant / Exon No.	Overall Depth/ Mutant Allele (%)	Function of the gene in cancer
None					

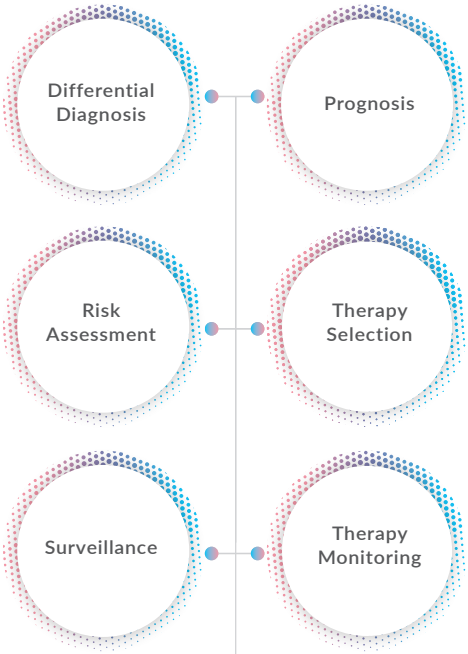
TABLE 4: FUSIONS GENES SCREENED

Sl. No.	Gene	Paired reads spanning breakpoint	Fusions status
1.	***ALK***	**10**	**Detected**
2.	*RET*	None	Not detected
3.	*ROS1*	None	Not detected

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

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

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