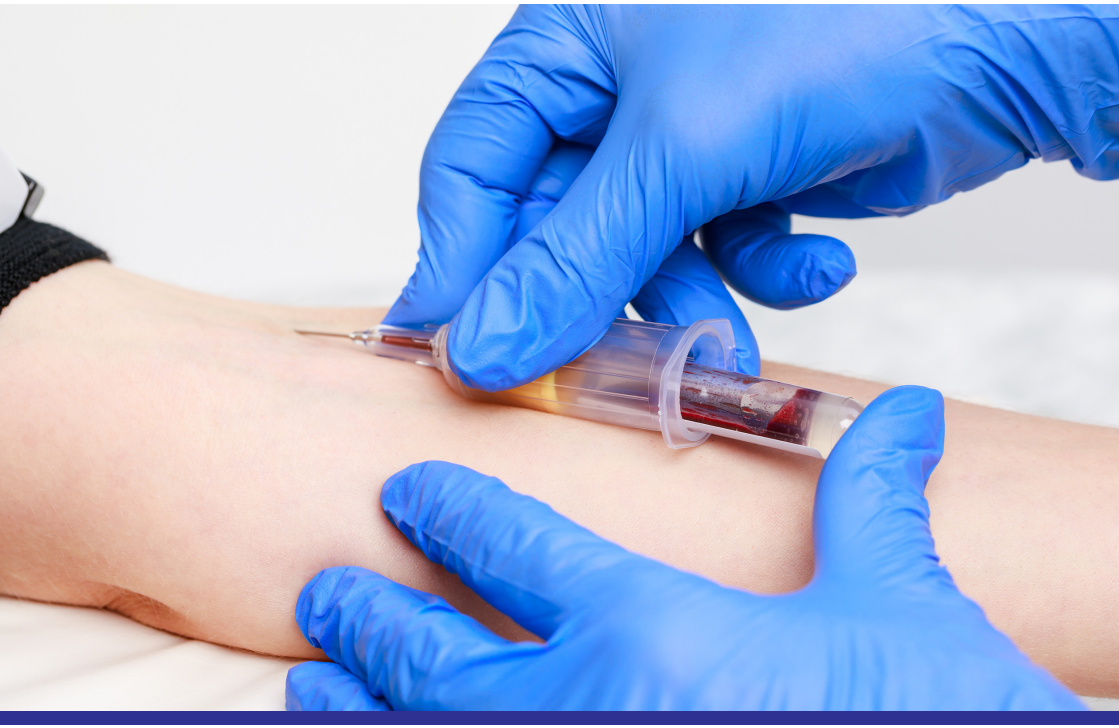
MEDGENOME | Prima Cancer Genetics

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

NGS based Liquid Biopsy Tests

Genomic Profiling of Solid Tumors from Blood

Minimally invasive	High precision multibiomarker	CAP accredited & extensively validated assay



MedGenome Liquid Biopsy NGS Assays

OncoTrack
(*EGFR*, *BRAF*, *KRAS*, *NRAS*)

HRR Track
(15 HRR Gene including *BRCA1* & *BRCA2*)

LungTrack Advance
(SNVs, InDels, Fusions)

OncoTrack Ultima
(Solid Tumors)

Next Generation Sequencing (NGS) based Liquid Biopsy test

NGS based liquid biopsy is a test performed on the blood of the patient to evaluate tumor derived DNA (ctDNA) for molecular biomarker detection.

Cells in the body die continuously in a process known as “apoptosis”, releasing DNA/RNA into the bloodstream. This freely circulating DNA in the blood stream is termed as **Cell free DNA (cfDNA)**.

Tumours cells also shed their contents including DNA into the blood stream. This tumour derived DNA present in the blood stream is called as **Circulating tumour DNA or ctDNA.**

Liquid Biopsy test is advised in the following situations:

Difficulty in obtaining tumor biopsy from cancer patients for practical and clinical reasons

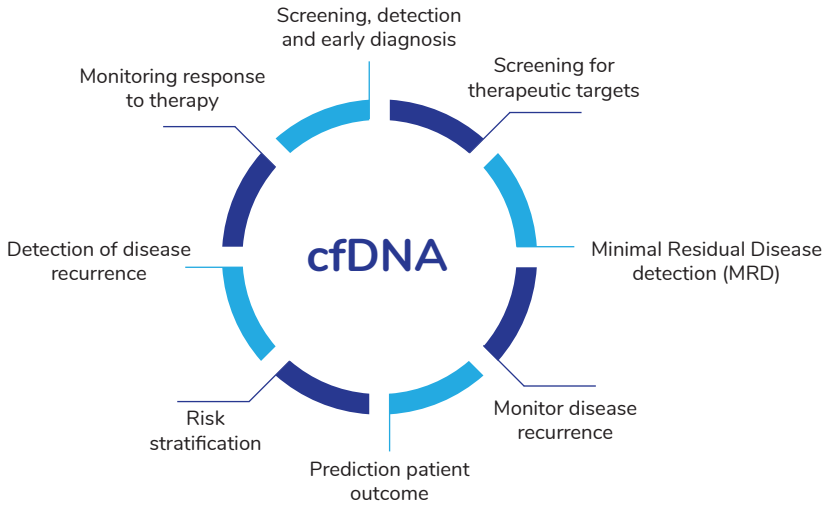
Biopsy material is degraded or damaged or improperly/poorly fixed

Tumour content is insufficient in the existing biopsy material

Treatment monitoring and Relapse in cancer patients (MRD)

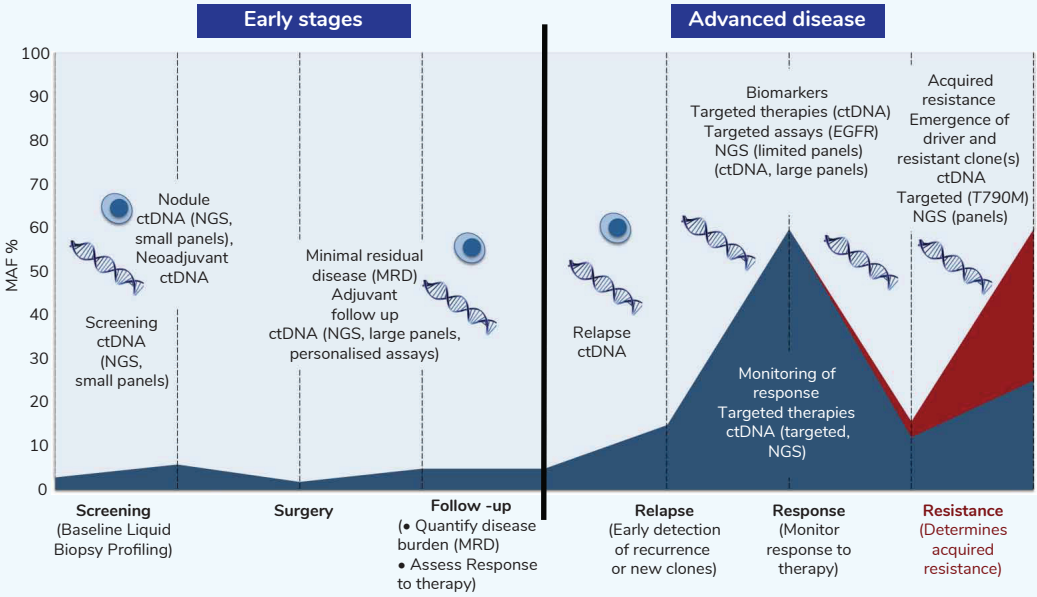
Determination of secondary **acquired resistance** mutations during the course of treatment (example: *EGFR T790M* or *MET* exome skipping mutation in NSCLC)

Clinical Applications of Liquid Biopsy



Liquid Biopsy Test can be used as a **Complementary, Alternative, Reflex** and **Serial** to Tissue Biopsy Test

Liquid Biopsy - Implications in Cancer Management



Ref: https://err.ersjournals.com/content/29/155/190052

MedGenome Liquid Biopsy NGS Assays

OncoTrack

Test code: MGM403 | TAT - 12 working days

- Screens for the presence of oncogenic mutations in four key genes: ***EGFR, KRAS, NRAS*** and ***BRAF***.
- These genes have a **potential role in clinical decision making** for different lines of approved targeted therapy and those in clinical trials.
- This assay also **screens for resistance mutations** that demonstrate the mechanism of acquired secondary/primary resistance to these drugs.

HRR Track

Test code: MGM2455 | TAT - 14 working days

- Indicated for Prostate, Ovarian, Breast and Pancreatic cancer
- Evaluates mutations in cfDNA (ctDNA) shed from dying cancer cells
- Screens for the mutations (SNVs and InDels) in 15 Homologous Recombination Repair(HRR) genes including ***BRCA1 and BRCA2 genes***
- Informs PARPi eligibility (approved targeted therapies and in clinical trials.)
- Complete Coding regions and splice site boundaries (+ /-10 bp) are covered
- Detects both somatic and germline* variants.

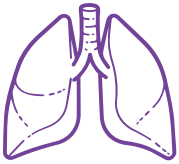
Gene List (15 Genes)

ATM	*BRCA2*	*CHEK1*	*PALB2*	*RAD51C*
BARD1	*BRIP1*	*CHEK2*	*PPP2R2A*	*RAD51D*
BRCA1	*CDK12*	*FANCL*	*RAD51B*	*RAD54L*

**Germline variants to be confirmed on blood DNA*

LungTrack Advance

Test code: MGM2623 | TAT - 14 working days



- NGS based CAP accredited assay to **screen all the NCCN guided actionable biomarkers** in Non-small cell Lung cancer (NSCLC)
- Detects **SNVs, Indels and Fusions**, all known / unknown fusion gene partners are detected
- **Enhanced coverage of intronic region** for key fusion genes and their reported partners
- Variants (SNVs, InDels) can be detected at **0.2% VAF** ; Fusions >3 read support
- Detects **Primary driver mutation** and **Secondary resistance** markers
- Enables **Minimal Residual Disease (MRD)** detection

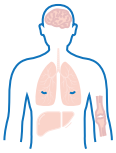
LungTrack Advance - Gene List (24 Genes)

ALK	CTNNB1	ERBB3	**NTRK1**	**FGFR1**	KIT	**MET**	**RET**
BRAF	EGFR	ERBB4	**NTRK2**	**FGFR2**	KRAS	NRAS	**ROS1**
CDKN2A	ERBB2	**NRG1**	**NTRK3**	**FGFR3**	MAP2K1	PIK3CA	TP53

Fusions will be determined in genes highlighted as bold | Novel fusion partners can also be detected

OncoTrack Ultima

Test code: MGM455 | TAT - 14 working days



- Tumor agnostic assay covering **117 Pan cancer** genes as recommended by **guidelines (FDA, NCCN, ASCO, ESMO etc.)**
- Comprehensive coverage of **complete coding regions** of all the genes and **intron/exon boundaries** for **SNVs & InDels** detection
- Highly sensitive and can accurately detect low frequency mutations upto **0.2% Variant Allele Frequency (VAF)** at high confidence
- Analysis and reporting as per **ACMG/AMP/ASCO/CAP** guidelines
- Detects **Primary driver mutation** and **Secondary resistance** markers
- Enables **Minimal Residual Disease (MRD)** detection
- All HRR genes are covered including BRCA1 and BRCA2

Gene List (117 Genes)

ABL1	BRIP1	ERCC2	HRAS	MSH2	PIK3CA	RIT1
ABL2	C11orf65	ESR1	IDH1	MSH6	PMS2	ROS1
AKT1	CCND1	EZH2	IDH2	MTOR	POLD1	SETD2
ALK	CDH1	FANCL	JAK1	MUTYH	POLE	SF3B1
APC	CDK12	FBXW7	JAK2	MYC	PPP2R2A	SMAD4
AR	CDK4	FGFR1	JAK3	MYCN	PTCH1	SMARCB1
ARAF	CDKN2A	FGFR2	KDM5C	MYD88	PTEN	SMO
ARID1A	CDX2	FGFR3	KDM6A	NF1	PTPN11	SPOP
ARID1B	CHEK1	FGFR4	KEAP1	NF2	RAD51B	SRC
ATM	CHEK2	FLT3	KIT	NOTCH1	RAD51C	STK11
ATR	CSF1R	FOXA1	KRAS	NPM1	RAD51D	TERT
ATRX	CTNNB1	FOXL2	MAP2K1	NRAS	RAD54L	TP53
BAP1	DDR2	GATA3	MAP2K2	NTRK1	RAF1	TSC1
BARD1	EGFR	GNA11	MAPK1	NTRK3	RB1	TSC2
BRAF	ERBB2	GNAQ	MET	PALB2	RET	VHL
BRCA1	ERBB3	GNAS	MLH1	PBRM1	RHEB	
BRCA2	ERBB4	HNF1A	MPL	PDGFRA	RHOA	

Validation of Liquid Biopsy for advanced non-small cell lung cancer

Lung Cancer: Targets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Validation of liquid biopsy: plasma cell-free DNA testing in clinical management of advanced non-small cell lung cancer

A total of 163 NSCLC cases were included in the validation, of which 132 patients had paired tissue biopsy and ctDNA. We chose to validate ctDNA using deep sequencing with custom designed bioinformatics methods that could detect somatic mutations at allele frequencies as low as 0.01%. Benchmarking allele specific real time PCR as one of the standard methods for tissue-based *EGFR* mutation testing, the ctDNA NGS test was validated on all the plasma derived cell-free DNA samples. We observed a high concordance (96.96%) between tissue biopsy and ctDNA for oncogenic driver mutations in Exon 19 and Exon 21 of the *EGFR* gene. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of the assay were 91.1%, 100% 100%, 95.6%, and 97%, respectively. A false negative rate of 3% was observed. A subset of mutations was also verified on droplet digital PCR. Sixteen percent EGFR mutation positivity was observed in patients where only liquid biopsy was available, thus creating options for targeted therapy. This is the first and largest study from India, demonstrating successful validation of circulating cell-free DNA as a clinically useful material for molecular testing in NSCLC.

Concordance- 96.96%
Sensitivity: 91.1%
Specificity: 100%
PPV: 100%
NPV: 95.6%
Diagnostic accuracy: 97%

Limit of Detection (LOD) - Liquid Biopsy Assay

Alteration Type	Analytical Sensitivity#	Limit of Detection (LOD)	Analytical Specificity##
		30ng	
SNVs*	≥95%	>0.2 AF%	100%
INDELS*	≥95%	>0.2 AF%	100%
Fusions **	≥95%	≥3 Reads	100%

#Analytical Sensitivity defined as the Detection Rate for variants present at or above the limit of detection (LoD).

##Analytical Specificity defined as 1 minus the per-sample false positive rate

* Tested on cfDNA reference standards
** Tested on Lung track advance

Assay Specifications

Indications	• Advance solid tumers • Before first line therapy or at progression
Specimen Requirement	Peripheral Blood in Streck Tube (10ml X 2)
Shipping condition	Ship same or next day at room temperature. Do not freeze or refrigerate
Assay Type	Next Generation Sequencing (NGS)
Sequencing Platform	Illumina
Average Depth of sequencing (Pre UMI)	≥20,000X
Average Depth of sequencing (Post UMI)	≥1000X

Liquid Biopsy Case Studies

Biopsy material is degraded/damaged/insufficient and difficulty in obtaining biopsy material

60-year-old Female | Adenocarcinoma lung | Tissue Biopsy was not feasible

Test performed - OncoTrack Ultima

Gene	CDS variant details	Amino acid change/ Exon No.	Overall Depth/Mutan Allele Percentage	FDA Approved drugs against variant
ERBB2	c.2313_2324dup (ENST00000269571.10)	p.Tyr772_Ala775dup/Exon20	3248X/1.4%	Trasuzumab Deruxtecan

The OncoTrack Ultima test successfully identified actionable mutation for which an FDA drug is available. This helps the clinician take appropriate treatment decisions.

Tissue material insufficient

47-year-old male | case of NSCLC (ADCC); stage4

Due to limited tissue amount, only EGFR test was performed & found to be negative.

Test performed: LungTrack Advance

Treated with: First line chemotherapy (Carboplatin + Paclitaxel) and progressed after 4 cycles; now started on second line chemotherapy.

LungTrack Advance Test was performed to identify actionable driver mutations

Result - POSITIVE

CLINICALLY RELEVANT VARIANT/S DETECTED

AMP Classification	CDS variant details	Interpretation	Treatment Recommendations	Treatment Response
EZR/ROS1(FUSION) Total Read depth - 76x				
Tier I	NA	Oncogenic	Crizotinib, Entrectinib	Effective
***TP53* p.Ala84ArgfsTer40 (FRAMESHIFT-INS)** Variant Allele Frequency - 3.79%				
Tier II	c.248_249dup (ENST00000269305.9)	Oncogenic	NA	NA

01 **EZR/ROS1**

FDA approved Targeted therapy indicated (crizotinib, entrectinib)

02 **TP53**

Frame shift insertion oncogenic mutation was detected; indicative of poor prognosis.

Therapeutic response monitoring - Detection of resistance

62-year-old male

Diagnosed with Adenocarcinoma lung cancer 3 years ago.

Test performed: LungTrack Advance

Treated using EGFR TKIs & progressed. Test performed to determine resistance mechanism.

Gene	CDS variant details	Amino acid change/ Exon No.	Overall Depth/Mutan Allele Percentage	FDA Approved drugs against variant	Drug response
EGFR	c.2237_2255 delinsT(ENST00 000275493.2)	p.Glu746_Ser752 delinsVal/Exon 19	925904X/16 %	Osimertinib/Gefit inib/Erlotinib/Afat inib/Dacomitinib	Sensitive to EGFR TKIs
EGFR	c.2369C>T(ENST 00000275493.2)	p.Thr790Met /Exon 20	123188X/14 .9%	Osimertinib (Tagrisso):Third generation TKI	Resistance to first /second generation TKIs

LungTrack Advance successfully identified resistance mutation in the cfDNA of the patient

Resistance mutation acquired by patient after treatment

Liquid biopsy test for HRR gene testing

56-year-old Male

Prostatic acinar adenocarcinoma, grade 5

Test Performed: HRR Track and HRR somatic mutation panel

Genomic alteration	Nucleotide change	Depth / VAF	AMP Classification^	Interpretation	Treatment Recommendation
BRCA2 p.Gly3153Al afsTer10	c.9458del (ENST0000038 0152.8)	2720X / 46%	Tier 1	Deleterious	Confers sensitivity to PARP inhibitors

HRR track Liquid Biopsy test identified pathogenic mutation in BRCA2 gene at 46% VAF which can be useful for treatment decisions.

HRR somatic mutation panel on Tissue Biopsy also detected same mutation at 65.6t%.
Germline confirmation is under process

For more information on CAP accredited Liquid Biopsy test portfolio please write to our experts at techsupport@medgenome.com