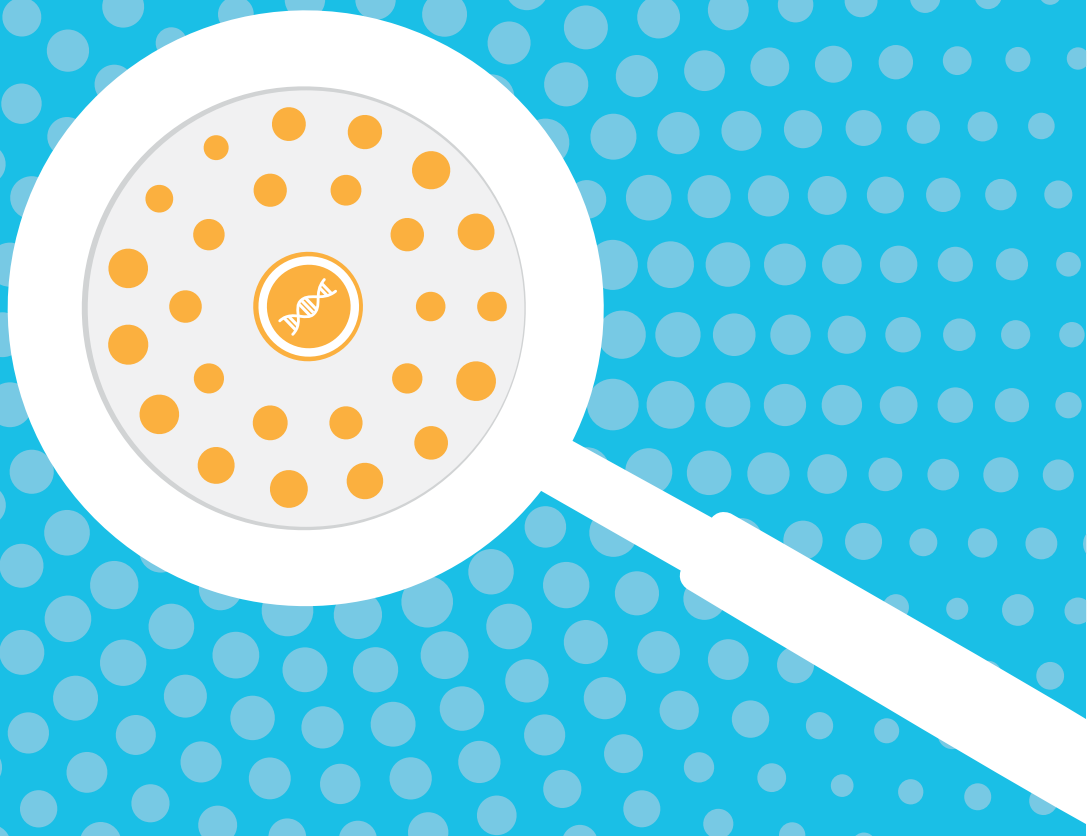
MEDGENOME

Presents

ONCOTRACK

India's first NGS based Liquid Biopsy



Non-invasive, Validated and Accurate

CIRCULATING TUMOR DNA TESTING (LIQUID BIOPSY) USING NEXT-GENERATION SEQUENCING (NGS) IN SOLID TUMORS

What is cell free DNA

Cells in the body die continuously in a process known as "apoptosis", releasing DNA/RNA into the blood-stream. This freely circulating DNA in the blood stream is termed as cell free DNA (cfDNA).

What is circulating tumour DNA (ctDNA)

Tumours are rapidly growing cells and shed their contents including DNA at an accelerated rate into the blood stream due to their high rate of cell death. This tumour derived DNA in the blood stream is called as Circulating cell free Tumour DNA or ctDNA

What is Liquid biopsy

It is a blood test for the evaluation of circulating tumour DNA (ctDNA) in the patient's bloodstream. [1]

Scientific Basis for "Liquid Biopsy"

Dying cancer cells also release their mutated DNA into the bloodstream, enabling determination of mutation type and relative tumour volume. By capturing and sequencing these tumour derived cell free DNA, **tumour genomics can be reconstructed without needing to perform the biopsy of the tumour**, and hence this is also called as **Liquid biopsy.**

Advantages of Liquid Biopsy over Tissue biopsy [1]

- Sample quantity is not limited in contrary to most biopsies (eg; Lung)
- Sample frequency is not limited
- Simple and safe venepuncture
- Doesn't need a medical professional
- Doesn't require any urgent shipping/cold-chain support
- Liquid Biopsy procedure doesn't cost as much as tissue biopsy

Why and When Liquid Biopsy is advised

Liquid biopsy facilitates detection of clinically relevant tumour mutations from blood sample, when there is difficulty in obtaining biopsy from cancer patients for practical and clinical reasons.

It is also applicable in situations where

- The biopsy material is degraded or damaged or improperly/poorly fixed
- The tumour content is insufficient in the existing biopsy material
- No available Tumour Tissue Biopsy
- It has been proven to be a useful molecular tool for treatment monitoring and relapse in cancer patients [2, 3]
- Secondary acquired resistance mutations during the course of treatment (example: T790M mutation in NSCLC)

However, despite the advantages, Liquid biopsy is an investigational test, not for diagnostic purposes and does not replace the conventional biopsy.

Advantages of NGS based Liquid biopsy over other technologies:

- High Sensitivity and ability to detect low frequency mutation [7]
- Unlike other techniques such as Real-Time PCR or Digital PCR where only specific known mutation is screened in individual genes, NGS has the advantage of multi-gene profiling in a single assay providing information on the exon covering the HOTSPOT mutation, with minimal amount of DNA without compromising on the sensitivity and specificity, and can detect unknown mutations too
- Massively Parallel Sequencing, which provide data with high confidence and greater accuracy
- Decreases time-to-answer. (Reduced Turn Around Time)
- Minimizes issues with limited material for sequential testing
- Ability to provide exact mutation, especially the insertion and deletion cases

MedGenome's "ONCOTRACK" Liquid Biopsy test for solid tumors

ONCOTRACK screens for the presence of oncogenic driver mutations in four key genes: EGFR, KRAS, NRAS and BRAF, that has 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.

How does MedGenome's "ONCOTRACK" test work

ONCOTRACK is a testing developed on next-generation sequencing (NGS) platform. The assay is highly sensitive and can accurately detect low frequency mutations at high confidence. By analysing cell-free DNA isolated from a patient's blood, it identifies clinically relevant tumour somatic mutations in ctDNA and match these mutations to targeted therapies and clinical trials.

Technical Details

It is an NGS test, and is designed to identify genetic alterations, which includes missense mutations and short insertions and deletions (<= 25bp)

Details of the genes / HOTSPOT regions covered in MedGenome's "ONCOTRACK"

Gene	NM ID	Exon number	Start codon	End codon	HOTSPOT Covered
	NM_005228.3	EXON 3	V96	L119	NA
	NM_005228.3	EXON 7	P281	R297	NA
	NM_005228.3	EXON 15	G588	C623	NA
	NM_005228.3	EXON 18	P694	G724	G718S, G719A, G719C
EGFR	NM_005228.3	EXON 19	G729	D761	DELETIONS (9 TO 24 bp)
	NM_005228.3	EXON 20	A767	G795	T790M, S768I, INSERTIONS (9 bp)
	NM_005228.3		N808	K823	
	NM_005228.3	EXON 21	T854	K874	L858R AND L861Q
KRAS	NM_004985.3	Ex 2	P34	E4	Codon 12, 13
	NM_004985.3	Ex 3	S65	D38	Codons 59, 61
	NM_004985.3	Ex 4	A146	L114	Codon 117, 146
NRAS	NM_002524.4	Ex 2	H27	M1	Codon 12, 13
	NM_002524.4	Ex 3	E76	I46	Codon 59, 61
	NM_002524.4	Ex 4	Q150	Y137	Codon 146
BRAF	NM_004333.4	Ex 15	H608	N581	Codon 600
	NM_004333.4	Ex 11	G474	R444	NA

It is a highly robust NGS test that could take low inputs of ctDNA (3ng – 50ng) per assay combined with custom designed proprietary computational algorithms that facilitates to detect very low frequency variants at high sequencing depth. The sensitivity of the assay depends of the quality, the yield of circulating tumour DNA, panel coverage and the sequencing depth.

Limit of detection (LOD)

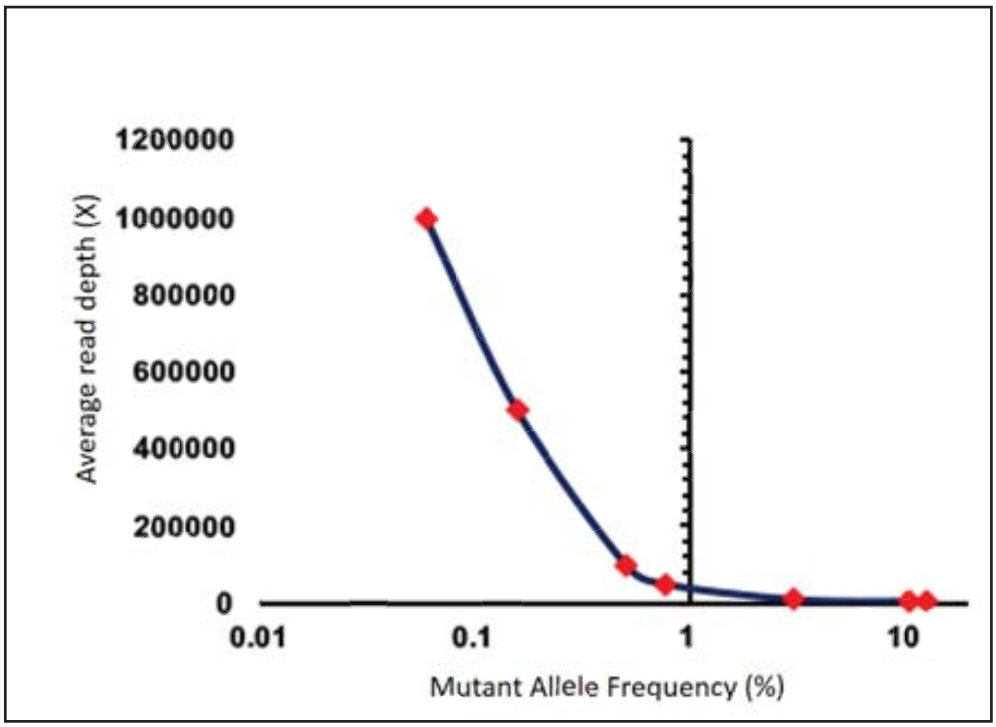


Figure 1. The detection rate depicts that ONCOTRACK can detect mutation at frequency less than 0.1% and sensitivity increases with read depth. The above mentioned LOD is based on validation data of 200 clinical samples

Validation Summary of MedGenome's "ONCOTRACK" Test:

This test has been developed in-house by MedGenome Labs and its Analytical and Clinical performance characteristics have been extensively validated and verified in blood / matched tumour samples from approximately 200 cancer patients.

**Performance Characteristics of the test	Clinical Validation	Validation with ctDNA Reference Standard (HD780)
Mutant Allele Fraction/ Limit of Detection	≥ 0.5% (Insertions/deletions: 1-25bp)** ≥ 1 %(base substitutions)**	≥ 1% (Insertions/deletions: 1-25bp) ≥ 1 %(base substitutions)
Sensitivity	91.1%	100%
Specificity	100%	100%
Accuracy	96.96%	100%
Positive Predictive Value	100%	100%
Negative Predictive Value	95.6%	100%
Overall concordance with tissue testing	96.96%	100%
Turn Around Time (days)	12 days from sample received	
Sample Requirements	2x10ml of peripheral blood collected in Streck® Tubes (please check collection information)	

**Performance characteristics of the test in clinical validation are based on the assessment of Exon 19 (In-Dels) and Exon 21 missense mutations in the EGFR gene from approx. 200 clinical samples.

MedGenome's "ONCOTRACK" test Applications

- 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
- The quantitative assessment for the disease is based on Mutant Allele Frequency
- 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, NRAS and BRAF, which are somatically altered recurrent mutations in solid tumours 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

† As per the current guidelines, PET-CT imaging is recommended to assess the treatment response/detect early recurrence/cancer progression after every 3 months. While, Liquid Biopsy has been shown to detect early recurrence or progression in 30 to 45 days prior to PET-CT [2, 4-8]

WHY ONE SHOULD ASK FOR MedGenome's "ONCOTRACK" TEST?

- Medgenome's ONCOTRACK is NGS based and hence more sensitive, accurate and highly informative as compared to other ctDNA based testing methods
- It is a multi-gene panel, hence could address more than one possible mechanisms of acquired resistance to targeted therapy
- The task has been validated on 200 Non-Small cell Lung Cancer clinical samples to establish the performance characterstics
- Our in-house developed bioinformatics algorithms are trained to detect mutant alleles at frequencies as low as 0.01% in ctDNA
- The annotation of somatic variants is compiled based on OncoMD – Comprehensive Proprietary Somatic Mutation database which provides access to the information on current clinical trials that match these variants/genes in addition to information on clinical response rates for various existing approved drug combinations.[9]

Clinical Reporting of MedGenome's "ONCOTRACK":

Results Summary

Clinically relevant genomic alterations detected by ONCOTRACK are listed with their Mutant Allelic Fraction and depth; and their significance to available targeted therapies and clinical trials is mentioned.

Mutant Allelic Fraction is the frequency of mutant allele identified in the sample for any given missense mutation or In-Dels (Insertions/deletions).

Monitoring the treatment response during the course of therapy on successive samples.

Surveillance monitoring of ctDNA after therapy to detect any events of early relapse/recurrence.

Correlation with Tissue biopsy

The assay also correlates, the molecular profile signature if any available on the tissue biopsy for the patient to that observed in ctDNA results.

Variants of Unknown Clinical Significance (VUS)

Beyond those mutations that have been extensively characterized and recommended by international guidelines, NGS based assay always has a scope to identify new/novel mutations that could be in close proximity to the HOTSPOT mutation, however, may not be very well studied and documented in scientific literature. Such mutations are classified under the category of Variants of Unknown Clinical Significance (VUS). These variants are included in the report, as they could be potential drug targets in future clinical trials.

TARGETED THERAPY INFORMATION

#	Target gene	Generic name of the drug	Trade name of the drug	FDA approved in various solid tumors
1	BRAF	Vemurafenib	Zelboraf	Hairy cell leukemia, Colorectal cancer
2	BRAF	Dabrafenib	Tafinlar	
3	EGFR	Erlotinib	Tarceva	Colorectal cancer
4	EGFR	Cetuximab	Erbitux	Colorectal cancer (only in Wt KRAS+NRAS)
5	EGFR	Lapatinib	Tykerb	Breast cancer
6	EGFR	Vandetanib	Caprelsa	Medullary thyroid cancer
7	EGFR	Gefitinib	Iressa	Colorectal cancer
8	EGFR	Afatinib	Gilotrif	Breast cancer, Gastric cancer
9	EGFR	Panitumumab	Vectibix	Colorectal cancer (only in Wt KRAS+NRAS)

SUMMARY OF VARIANTS WITH APPROVED DRUGS

Gene (function)	Approved drugs	Approved in cancer types
BRAF (Oncogene)	Dabrafenib (Tafinlar)	Brain cancer, Colorectal cancer, Leukemia, Lung cancer, Melanoma, Rare , Rare Gastrointestinal cancer, Thyroid cancer
BRAF (Oncogene)	Dabrafenib + Trametinib (Tafinlar + Mekinist)	Brain cancer, Liver cancer, Melanoma, Sarcoma
BRAF (Oncogene)	Ipilimumab (Yervoy)	Melanoma
BRAF (Oncogene)	Sorafenib (Nexavar)	Melanoma, Thyroid cancer
BRAF (Oncogene)	Sunitinib (Sutent)	Melanoma, Thyroid cancer
BRAF (Oncogene)	Trametinib (Mekinist)	Melanoma, Rare
BRAF (Oncogene)	Vemurafenib (Zelboraf)	Brain cancer, Leukemia, Lung cancer, Melanoma,

SUMMARY OF VARIANTS WITH APPROVED DRUGS

Gene (function)	Approved drugs	Approved in cancer types
EGFR (Oncogene)	Erlotinib (Tarceva)	Myeloma, Ovarian cancer, Rare , Renal cancer, Sarcoma, Thyroid cancer
EGFR (Oncogene)	Gefitinib (Iressa)	Lung cancer
EGFR (Oncogene)	Gefitinib (Iressa)	Lung cancer
EGFR (Oncogene)	Afatinib (Gilotrif)	Lung cancer
EGFR (Oncogene)	Erlotinib (Tarceva)	Lung cancer
EGFR (Oncogene)	Erlotinib + Cetuximab (Tarceva + Erbitux)	Breast cancer, Lung cancer, Thyroid cancer
EGFR (Oncogene)	Gefitinib (Iressa)	Lung cancer
EGFR (Oncogene)	Vandetanib (Caprelsa)	Lung cancer
EGFR (Oncogene)	Afatinib (Gilotrif)	Lung cLung cancer
EGFR (Oncogene)	Erlotinib (Tarceva)	Lung cancer
EGFR (Oncogene)	Gefitinib (Iressa)	Lung cancer
EGFR (Oncogene)	Osimertinib (Tagrisso)	Lung cancer
EGFR (Oncogene)	Gefitinib (Iressa)	Lung cancer

References

[1] McLarty LJ, Yeh CH (2015) Circulating Cell-Free DNA: The Blood Biopsy in Cancer Management. MOJ Cell Sci Rep 2(2): 00021

[2] Nader J, Andrew F, Chen-Hsiung Y. Blood-Drop Liquid Biopsy for Monitoring Mutation Load and Therapeutic Responses. Cancer Therapy & Oncol Int J. 2016; 1(5): 555572

[3] Reck M, Hagiwara K, Han B, Tjulandin S, Grohé C, Yokoi T, Morabito A, Novello S, Arriola E, Molinier O, McCormack R, Ratcliffe M, Normanno N. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. J Thorac Oncol. 2016 Oct;11(10):1682-9.:

[4] Janku F, Huang HJ, Claes B, Falchook GS, Fu S, Hong D, Ramzanali NM, Nitti G, Cabrilo G, Tsimberidou AM, Naing A, Piha-Paul SA, Wheler JJ, Karp DD, Holley VR, Zinner RG, Subbiah V, Luthra R, Kopetz S, Overman MJ, Kee BK, Patel S, Devogelaere B, Sablon E, Maertens G, Mills GB, Kurzrock R, Meric-Bernstam F. BRAF Mutation Testing in Cell-Free DNA from the Plasma of Patients with Advanced Cancers Using a Rapid, Automated Molecular Diagnostics System. Mol Cancer Ther. 2016 Jun;15(6):1397-404.

[5] Bidard FC, Madic J, Mariani P, Piperno-Neumann S, Rampanou A, et al. (2014) Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. Int J Cancer 134(5): 1207-1213.

[6] Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, et al. (2013) Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 368(13): 1199-1209.

[7] Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, et al. (2012) Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Trams Med 4(136): 136-168.

[8] Tie J, Kinde I, Wang Y, Wong HL, Roebert J, et al. (2015) Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. Ann On col 26(8): 1715-1722

[9] Bueno R, Stawiski EW, Goldstein LD, Durinck S, De Rienzo A, Modrusan Z, Gnad F, Nguyen TT, Jaiswal BS, Chirieac LR, Sciaranghella D, Dao N, Gustafson CE, Munir KJ, Hackney JA, Chaudhuri A, Gupta R, Guillory J, Toy K, Ha C, Chen YJ, Stinson J, Chaudhuri S, Zhang N, Wu TD, Sugarbaker DJ, de Sauvage FJ, Richards WG, Seshagiri S. Comprehensive genomic analysis of malignant pleural mesothelioma identifies recurrent mutations, gene fusions and splicing alterations. Nat Genet. 2016 Apr;48(4):407-16.

MEDGENOME

A market leader in Genomics-based Diagnostics and Research

MedGenome is the first commercial Lab in India to offer Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS) and Multi-Gene panels as clinical Genomic Tests

One-stop solution for all your Diagnostics and Research needs

Flow Cytometry | Microarray | Sanger | FISH | NGS | PCR | IHC | Fluidigm

NGS: X10 | HiSeq | MiSeq

BANGALORE | KOCHIN | CALIFORNIA | SINGAPORE

258/A, 3rd Floor, Narayana Nethralaya Building,
Narayana Health City, Hosur Road,
Bommasandra, Bangalore,
Karnataka, India – 560 099

Plot no: SDF 17, First Floor, C Block, CSEZ,
Kakkanad, Cochin, Kerala – 682037

Websites:
www.medgenome.com

E-mail:
sales@medgenome.com
contact@medgenome.com

Phone:
+91 (0)80 67154931
+91 (0)80 67154932