Utility of a targeted NGS oncology assay for circulating tumor DNA in a multi-histology clinical setting

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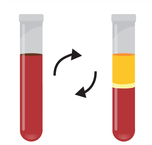
OVERVIEW

Profiling of circulating-tumor DNA (ctDNA) found in peripheral blood of cancer patients is an attractive alternative to invasive tissue biopsy for tumor genotyping and screening into clinical trials.

The Resolution Bioscience ctDx-Lung v1.0 assay targets actionable, somatic **SNVs**, **indels**, **fusions** and **copy number variants** in 15 genes implicated in lung and other cancers, using a proprietary, high-efficiency hybrid capture system and NGS sequencing of ctDNA from plasma. 392 patients (342 from the 42756493EDI1001 clinical trial screening, and 50 from a University of Washington study CC9372) were analyzed in the Resolution Bioscience CLIA laboratory. Patients had previously been diagnosed with one of ten histologies, including lung, breast, and ovarian cancer. The patient cohort was a clinically relevant sampling of treatment-naïve patients, those on therapy, and those with refractory disease after >= 1 therapy course.

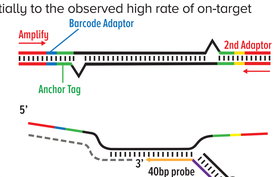
Indication	Patients
NSCLC Adenocarcinoma	133
Breast cancer	56
Ovarian cancer	50
Head & Neck	38
Glioblastoma Multiforme	30
Cholangiocarcinoma	28
Gastric cancer	18
NSCLC Squamous	17
Esophageal cancer	14
Urothelial cancer	7
unknown[3]	1
Total Patients	**392**

METHODS



Targeted NGS of cfDNA was performed at Resolution Bioscience as described in Paweletz et al (2015)[1], using an NGS panel targeting actionable mutations and rearrangements found in NSCLC. Peripheral blood was collected in 2x10mL K_2EDTA collection tubes and plasma was extracted on-site and shipped to Resolution Bio's CLIA lab.

Specimens were accessioned into Resolution's custom LIMS system. cfDNA was extracted, amplified genomic libraries were denatured and hybridized with 40nt targeting probes. Following hybridization and purification of probe-clone complexes, primer extension of the probe was used to copy the captured genomic sequence information as well as the adaptor. This step, which enriches for genomic clones that are in direct physical contact with probes, contributes substantially to the observed high rate of on-target sequence reads (> 95%), and also allows for efficient detection of gene fusion partners without any prior knowledge of the gene partner or breakpoint.



Following paired end sequencing of capture libraries, bioinformatics was performed with Resolution's customized bioinformatics platform.

Significantly, 386 of 392 specimens (98.5%) were evaluable with clinical reports returned in a median of 8.6 days.

ASSAY PERFORMANCE

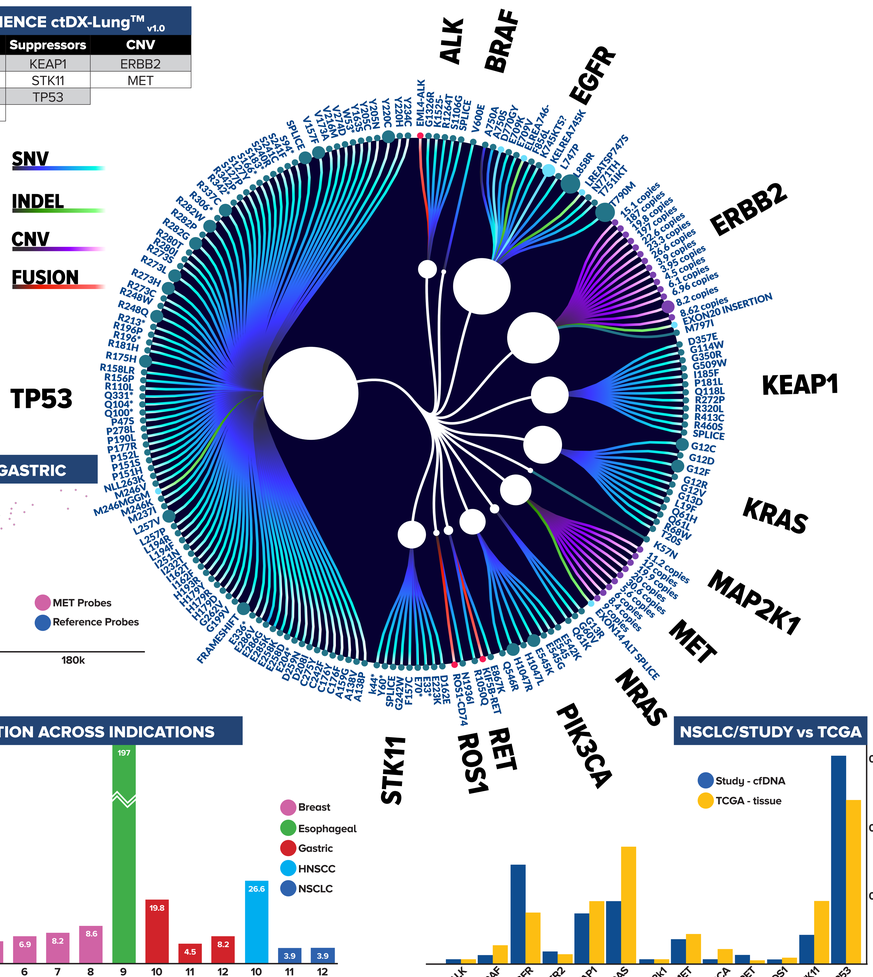
Median Turnaround TIme	8.6 days
Specimen Rejection Rate	0.26%[3]
Specimen Failure Rate	1.28%[2]
Patients with Mutations	61%
Median Mutations / Patient	1.7
Median Allele Frequence	2.7%

5 specimens[2] did not meet the required 500 genomic equivalents input threshold for the assay, even after combining cfDNA yield from both patient specimens, and 1 specimen[3] did not meet inclusion critera (patient was on treatment) and was rejected.

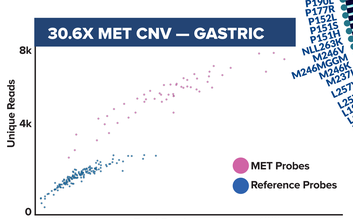
[1]Paweletz et al., Clinical Cancer Research 2016 22(4):915

RESOLUTION BIOSCIENCE ctDX-Lung™ v1.0

SNV/Indels	Fusions	Suppressors	CNV
ALK	ALK	KEAP1	ERBB2
BRAF	NTRK1	STK11	MET
EGFR	RET	TP53	
ERBB2	ROS1		
KRAS			
NRAS			
MAP2K1			
MET			
PIK3CA			
RET			
ROS1			



30.6X MET CNV — GASTRIC



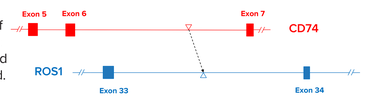
ERBB2 AMPLIFICATION ACROSS INDICATIONS

NSCLC/STUDY vs TCGA

CASE CC9372-15: ROS1 FUSION — DENIED NGS COVERAGE, FISH FALSE NEG

47 year old male, never smoker. Stage IIIB NSCLC Adenocarcinoma. Bronchoscopic biopsies were positive for adenocarcinoma on fine-needle aspirates of the right hilum and subcarinal lymph nodes. Molecular studies demonstrated EGFR wild type and ALK and ROS1 translocation negative. Due to the strong suspicion for a driver mutation and the minimal material from bronchoscopy, mediastinoscopy was done to obtain pathologic material for NGS, which was not performed as this was denied by insurance. **Testing of ctDNA detected ROS1-CD74 gene fusion.**

Initial IFISH testing of the mediastinoscopy specimen showed copy loss of 5'ROS1 plus one extra copy of ROS1 in some cells, and no rearrangement of ROS1 by IFISH. When the positive result from ctDNA was communicated to pathology, additional levels were tested and ROS1 fusion was detected. Additionally, single gene NGS confirmed a ROS1-CD74 fusion.



Due to delays in diagnosis, the patient was treated with carboplatin/pemetrexed chemotherapy followed by pemetrexed maintenance. He remains on therapy after 18 months. On progression or intolerance, he will be treated with crizotinib.

GENE	TYPE	MUTATION	AF%	LOCATION	
ROS1	FUSION	ROS1-CD74	2.8%	chr5:149782970-chr6:117646631	
TP53	SNV	I232T	0.6%		

CASE CC9372-40: EGFR L858R — BIOPSY INELIGIBLE

75 year-old male presented with dyspnea was found to have a right upper lobe mass and multiple bony metastases. Soon after presentation the patient had hemorrhage stroke, precluding timely biopsy as the patient was not felt to be candidate for biopsy due to high risk post-stroke. **ctDNA assay detected canonical EGFR L858R and p53 mutation.** The patient started on erlotinib while in outpatient rehabilitation. CT after initiation of therapy showed response and the patient responded symptomatically with decreased bony pain. Patient died 4 months later, likely from co-morbid conditions.

GENE	TYPE	MUTATION	AF%	LOCATION	
EGFR	SNV	L858R	8.10 %	chr7: 55259515	T / G
TP53	SNV	S241F	2.10 %	chr17: 7577559	G / A

CASE CC9372-41: EGFR INDEL & T790M, MET CNV — BIOPSY INEVALUABLE

75 year-old male, former light smoker, with a history of right upper lobe stage 1B NSCLC treated with lobectomy. He subsequently relapsed and was treated with thoracic radiation followed by two years of erlotinib for EGFR exon 19 deletion. On progression, pemetrexed was given but discontinued for intolerance. Patient had progressive bony metastases and poor performance status with dyspnea and hypoxia. Progressive disease in bone, not easily accessible and decay would not yield DNA for molecular testing. **ctDNA assay detected canonical driver EGFR indel, EGFR T790M resistance mutation, and a slight MET amplification.** Osimertinib was started and the patient had a radiographic and symptomatic response. At 9 months, treatment is ongoing.

GENE	TYPE	MUTATION	AF%	LOCATION	
EGFR	SNV	ELREA746-	5.1%	chr7: 55242465	GGAATTAAGAGAAGCA / G
EGFR	SNV	T790M	0.6%	chr7: 55249071	C / T
MET	CNV	5.5X			

CASE CC9372-45: EGFR INDEL & T790M — BIOPSY RELUCTANT

78-year-old woman who presented 3 years ago with R acetabular fracture which required prolonged inpatient hospitalization and left her wheelchair bound. Diagnosis was made from surgical bone specimens showing TTF1 positive metastatic adenocarcinoma. Not able to test for EGFR/ALK due to need to decalcify bone specimen. Patient unsuitable for biopsy of lung lesion. Due to a minimal and remote smoking history as well as her poor performance status, she was treated with erlotinib and remained on this treatment for 2 years. The disease progressed at bony sites and paclitaxel was started with response. After 6 months of treatment, she again progressed at bony site. The patient reluctant to undergo biopsy. **ctDNA testing revealed canonical EGFR indel & EGFR T790M mutation** and osimertinib was started with radiographic and symptomatic response. The patient now on treatment 7+ months.

GENE	TYPE	MUTATION	AF%	LOCATION	
EGFR	INDEL	KELREA745K	9.60 %	chr7: 55242464	AGGAATTAAGAGAAGC / A
EGFR	SNV	T790M	7.80 %	chr7: 55249071	C / T
TP53	SNV	M246V	4.60 %	chr17: 7577545	T / C

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