Abstract #7014

Kinase Fusions in Non-Small Cell Lung Carcinoma by Hybrid-Capture Based ctDNA Assay

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Abstract

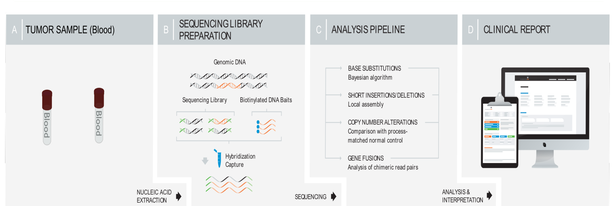
Background: For the detection of genomic driver alterations in NSCLC, comprehensive genomic profiling (CGP) or focused molecular testing of biopsied tissue is a well-accepted approach for matching targeted therapies in first line treatment. For NSCLC patients where invasive biopsy represents a serious risk, assessment of circulating tumor DNA (ctDNA) is an emerging alternative.

Methods: In patients with clinically advanced NSCLC, two 10 mL aliquots of peripheral, whole blood were collected and plasma was isolated. ctDNA was extracted to create adapted sequencing libraries prior to hybrid capture and sample-multiplexed sequencing on an Illumina HSQ2500 to >5000x unique coverage. Results were analyzed with a proprietary pipeline to call substitutions, indels, rearrangements and copy number amplifications.

Results: In 269 NSCLC patients evaluated, 20 (7.4%) harbored kinase fusions. 17/20 (85%) were adenocarcinomas, all stage IV. Median patient age was 61 years (range 41-81), and 59% were female. 13 (4.5%) cases harbored ALK fusions with partners as follows: nine EML4 (one each of novel partners PPFIBP1 and CACNB4), and two with unidentified partners. All but one case had breakpoints in ALK intron 19, the remaining harboring a novel intron 17 breakpoint. Three cases (1%) harbored KIF5B-RET (canonical breakpoint intron 12), three (1%) had CD74-ROS1 (breakpoints: ROS1 intron 33(2) and intron 32(1)), and one had FGFR3-TACC3. ALK, RET, and ROS1 fusions were observed by tissue testing of NSCLC in the FoundationCore database with similar frequencies. Five patients had a biopsy with insufficient tissue for CGP; three had both sufficient tissue and ctDNA available. The remainder had no tissue available. For one patient, EML4-ALK fusion was detected in both ctDNA and tissue, collected six days apart. For another, CGP identified EGFR L858R + EGFR L709K and the patient had a durable response to afatinib/cetuximab. After progression, ctDNA assay identified FGFR-TACC3 as well as EGFR L858R. For a pre-menopausal, therapy naïve never smoker, female of east Asian heritage, both assays detected a CD74-ROS1 fusion, whereas ROS1 rearrangement was not identified by the prior use of another commercially available ctDNA test. The patient had a major radiographic response by the second cycle of crizotinib treatment.

Conclusion: Hybrid capture based ctDNA assay can identify kinase fusions in NSCLC when CGP of biopsied tissue cannot be performed and can direct rational use of first line TKIs. This series identified a novel mechanism of acquired resistance to EGFR inhibitors, novel fusion partners and intronic breakpoints for ALK, and a case of false negative testing by another ctDNA assay.

Materials and Methods



Sample requirements
- 10-20 ml blood
- 5-10 ml plasma
- ≥50 ng extracted ctDNA
- Smear analysis to quantify cfDNA content

Laboratory process
- Adaptor-ligation library construction
- Molecular and sample barcodes
- Hybridization capture with biotinylated DNA oligonucleotides
- 2x175 paired-end sequencing on Illumina HiSeq 2500 platform.

Analysis methods
- Error correction to <0.05%
- Target depth post correction: ≥5,000x
- Variant calling
  - Subs: down to <0.5% VAF
  - indels and fusions: down to 1% VAF
  - Hi-level amplifications

Reporting approach
Interpretation without a matched normal
- Known driver alterations (COSMIC, documented fusions)
- Likely driver alterations (hotspots & truncations)

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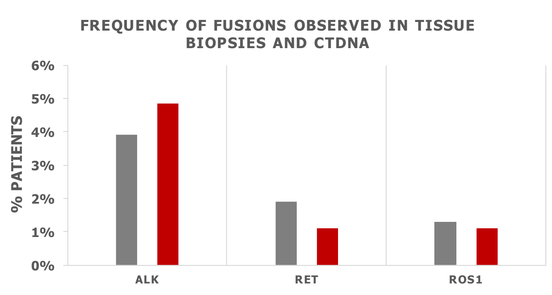
Results

Table 1. Clinicopathologic features of patients with NSCLC with evidence of tumor harboring kinase fusions in ALK, RET, ROS1, and FGFR3

	All Cases with Evidence of Tumor	Kinase Fusion Positive
Total number of cases	269	20
Median Age in years (range)	67 (24-87)	61 (41-81)
Gender (%)		
Male	128 (48)	8 (41)
Female	141 (52)	12 (59)
Histology (%)		
Adenocarcinoma	211 (78)	17 (85)
NSCLC (NOS)	39 (15)	2 (10)
Lung Cancer (NOS)	17 (6)	1 (5)
Adenosquamous	2(1)	0 (0)
Stage# (%)		
II	1 (1)	0 (0)
III	14 (5)	0 (0)
IV	194 (72)	18 (90)
Unknown	60 (22)	2 (10)
NSCLC NCCN gene alt (%)		
Yes	95 (35)	20 (100)
No	174 (65)	0 (0)

NSCLC: Non-small cell lung cancer; NOS: not otherwise specified; NCCN: National Comprehensive Cancer Network; alt: alteration.

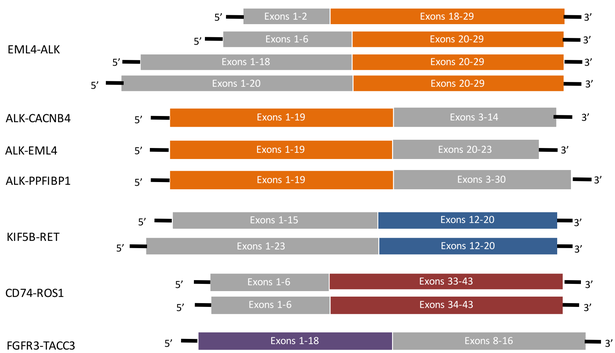
Figure 1. ALK, RET, and ROS1 fusions observed by tissue testing in the FoundationCore database at similar frequencies to those observed in ctDNA



Results

Figure 2. Identification of ALK, RET, ROS1 and FGFR3 fusions and a novel ALK fusion breakpoint

- 13 total *ALK* fusions were identified with *ALK*, all but one with a canonical intron 19 breakpoint. 9 with *EML4* as a partner, 1 with *PPFIBP1*, 1 with *CACNB4*, and two with unidentified partners.
- 1 *EML4-ALK* fusion harbored a novel breakpoint on the edge of intron 17 and exon 18.
- 3 *ALK* fusions were observed with *ALK* as the 5′ partner; 1 *ALK-EML4*, 1 *ALK-PPFIBP1*, and 1 *ALK-CACNB4*.
- 3 *KIF5B-RET* fusions were identified with *RET* as the 3′ partner and canonical breakpoints in intron 12.
- 3 *CD74-ROS1* fusions were identified with *ROS1* as the 3′ partner and canonical breakpoints in intron 32 or intron 33.
- 1 *FGFR3-TACC3* fusion was observed with a breakpoint in exon 18.



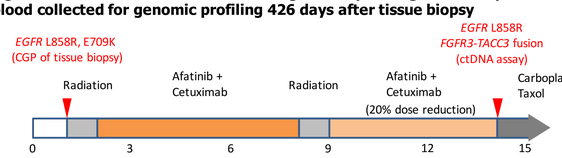
Index cases with matched tissue biopsy

- 6 patients (30%) with an actionable kinase fusion detected from ctDNA had a tissue biopsy with insufficient tissue for genomic profiling
- 3 patients (15%) with a actionable kinase fusion detected from ctDNA had a tissue biopsy with sufficient tissue for genomic profiling with FoundationOne.

PATIENT 1: Tissue and blood were collected 6 days apart and both harbored an *EML4-ALK* fusion.

PATIENT 2: Blood was collected post-progression on an EGFR targeted therapy, 464 days after tissue biopsy. *FGFR3-TACC3* fusion observed in ctDNA only as possible acquired resistance mechanism (Figure 3). [P3.02c–024]

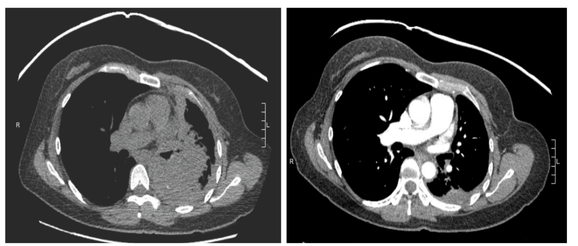
Figure 3. Timeline of clinical course and genomic profiling of NSCLC patient with blood collected for genomic profiling 426 days after tissue biopsy



Index cases with matched tissue biopsy

PATIENT 3: Blood was collected after one cycle of carboplatin, pemetrexed and bevacizumab; 37 days after tissue biopsy (Figure 4).

Figure 4. Antitumor response to crizotinib after *ROS1* fusion identified on tissue and ctDNA genomic profiling assays.



This patient is a 41-year-old never-smoker female of Southeast-Asian descent with advanced lung cancer. Imaging revealed diffuse pleural thickening and nodularity, a left pleural fluid collection, significant LLL collapse, and mild mediastinal and hilar LAD, with a liver mass invading the hemi-diaphragm, and axial bone lesions. Repeat PET-CT scan two weeks later showed complete collapse of the LLL and partial encasement of the LUL bronchi with extensive metastatic disease involving the left pleura and diffuse lymphadenopathy. At the time of diagnosis another CLIA ctDNA assay was negative for genomic alterations, but CGP (FoundationOne) of left subclavicular lymph node demonstrated a CD74-ROS1 fusion. The patient was started on carboplatin, pemetrexed and bevacizumab before the CGP results were available. After one cycle FoundationACT was performed and also identified the CD74-ROS1 fusion. The patient's therapy was switched to crizotinib and she had a significant radiographic and clinical response after 8 weeks.

Conclusions

- Fusions in kinase genes were detected using hybrid-capture based genomic profiling of ctDNA in 7.4% of NSCLC cases with evidence of tumor
- *ALK, RET, ROS1, and FGFR3* fusions were identified in 20 cases, including 1 *EML4-ALK* fusion with a novel breakpoint on the border of intron 17 and exon 18, and novel *ALK* fusion partners
- Fusions in *ALK, RET,* and *ROS1* were observed in ctDNA of NSCLC cases at frequencies similar to that observed by tissue testing in the FoundationCore database
- *FGFR3-TACC3* fusion was identified as a potential mechanism of acquired resistance in the ctDNA of 1 case of EGFR-driven lung cancer post-progression on an EGFR targeted therapy
- One patient with a *CD74-ROS1* fusion detected by FoundationACT (and by FoundationOne on a tissue biopsy collected 37 days prior to blood) had a major radiographic response by the second cycle of crizotinib treatment. This patient tested negative for *ROS1* fusion with another commonly available ctDNA assay
- CGP of ctDNA can identify kinase fusions in NSCLC when CGP of tissue cannot be performed, and can direct rational use of TKIs