Liquid biopsies for molecular profiling of mutations in non-small cell lung cancer (NSCLC) patients lacking tissue samples

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PURPOSE

Approximately 30% of patients with lung adenocarcinoma have an actionable driver mutation[1].

Access to tumour tissue to perform either the initial molecular profile or at the point of acquired resistance, however, is often limited. Further understanding the molecular mechanisms of acquired resistance to targeted therapies provides key information for determining subsequent treatment options.

Circulating tumour DNA (ctDNA) can be used as a non-invasive method for the detection and quantification of molecular abnormalities.

METHODS

We performed a prospective study in 174 NSCLC advanced patients in whom the diagnostic molecular profile or profile at relapse was unknown due to lack of tumour tissue biopsy or insufficient cellularity in the biopsy, to assess molecular alterations using ctDNA analysis.

Objectives of the study were:

- To assess the molecular alterations in the ctDNA of NSCLC patients in whom the initial molecular profile or profile at relapse was unknown
- To assess resistance mutations on treatment
- To assess the proportion of patients who receive personalised treatment based on these results

Patients were analysed as two separate cohorts; 1) unknown molecular profile or 2) diagnostic tissue EGFR+ known mutation and being treated with EGFR TKI therapy. All patients had to have received at least 1st line of therapy prior to study entry. Screening sample was taken irrespective of whether patient was in relapse or receiving treatment at time of sample.

10mls of blood (K2-EDTA) was collected and processed at the Gustave Roussy Cancer Campus to obtain plasma samples. Follow-up samples were collected on a subset of patients.

DNA was extracted from <4ml of plasma and analysed using Inivata's enhanced TAm-Seq™ assay covering regions from 34 cancer-related genes (Figure 1). eTAm-Seq analytical validation studies[2] demonstrated high sensitivity and specificity; identification of >99% mutations at allele frequency (AF)>0.5% and a lower limit of detection of 0.06%.

RESULTS

From June 2015 to August 2016, 174 patients were enrolled: 61% female; 78% never-smoker; 86% diagnosed with an adenocarcinoma subtype; 91% with stage IV disease; and 70 patients (40%) had known *EGFR* mutant tumours. (Table 1)

Sex	Male / Female	67 / 107 (39%/61%)
Age (years)	Median	64 (23-90)
Stage (study entry)	III / IIIA / IIIB / IV / NR	2 / 5 / 6 / 159 / 2
Histology	NR	5
Adenocarcinoma		149 (86%)
Squamous		3 (2%)
Other		17 (10%)
Smoking Status	NR	6
	Never-smoker	78 (49%)
	Former-smoker	68 (39%)
	Smokers	22 (13%)
Pack Years (PA)	Median	30 (2-100)
EGFR mutation (Tissue at diagnosis)		70 (40%)
Del19 (47) / L858R(17) / L861Q(1) / G719A(1) / NR(4)		
Prior lines of therapy	Median	2

Table1. Patient characteristics (NR=Not Reported)

Liquid biopsy ctDNA screening was successfully performed for 95% of all patients, and mutations were detected in 128 of 162 patients (79%) across 18 genes (Figure 2). TP53 was the most frequent mutation reported in this study population, with EGFR and KRAS within expected range for these patient cohorts.

Of the mutations detected, 63% were <1% allele fraction, with 43% between 0.06-0.5% (Figure 3).

ctDNA analysis confirmed diagnostic tissue EGFR wildtype and positive mutation status in 144 of 174 patients: 9 patients were identified with EGFR mutation not previously detected (confirmed in subsequent samples);12 of 21 patients in whom ctDNA analysis did not detect EGFR known mutation were on treatment with EGFR TKI at time of sample with no evidence of progression. Of the EGFR+ve patient cohort (n=48) detected by ctDNA analysis at screening, the most common mutations (n=92) detected were del19 (39) and L858R (9). Of note, resistance mutations T790M (25) and C797S were identified. In 2 cases, simultaneous T790M and C797S were reported.

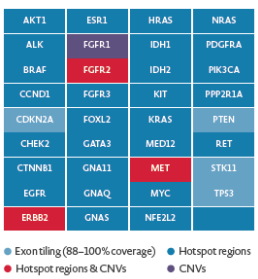

Figure 1. Inivata eTAm-Seq panel

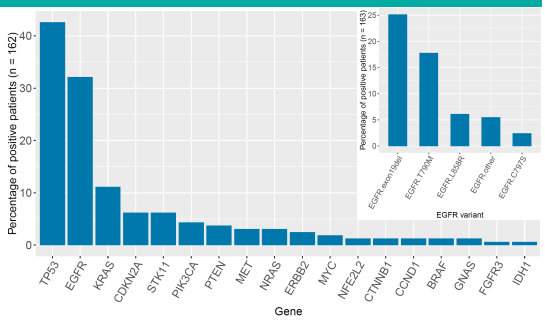

Figure 2. Mutations detected in ctDNA as screening profile and inset (3) for EGFR ctDNA +ve patients at screening or subsequent follow-up samples

Among *EGFR* mutant NSCLC patients (n=70) progressing on EGFR TKI at the time of liquid biopsy (n=42), *T790M* mutation was reported in 57% (24 of 42). 18 patients were subsequently treated with osimertinib (AZD9291) on compassionate basis.

In the patient cohort with unknown initial molecular profile, clinically relevant molecular alterations found were:

- 8 patients with *KRAS* mutations (2 with *STK11*)
- 5 patients with common *EGFR* mutations
- 1 patient with ERBB2 exon 20 insertion
- 4 patients with *MET* exon 14 mutation
- 2 patients with *BRAF* (*G469A, G466R)* mutations

Personalised treatment based on ctDNA molecular profiling was performed in 17% of patients (osimertinib (18, T790M), EGFR TKI (7), crizotinib (2, MET), afatinib (1, ERBB2).

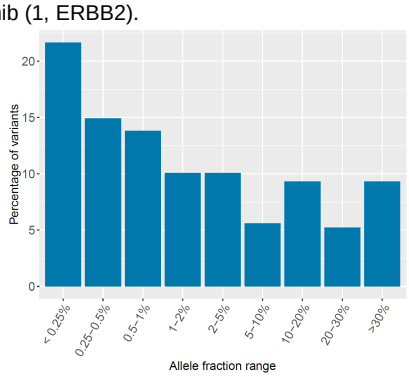

Figure 3. Allelic fraction of mutations detected

CONCLUSIONS

Circulating tumour DNA analysis provides an alternative method to invasive tissue biopsy as a 'liquid biopsy' for obtaining molecular profile of mutations in advanced NSCLC patients. This study demonstrated good correlation of eTAm-Seq broad panel ctDNA analysis in detecting known driver mutations, as well as identifying clinically relevant mutations in patients with unknown molecular profile, at low levels (20% at AF<0.25%). Moreover, ctDNA analysis identified the resistance mutations T790M and C797S in patients with relapsing disease to EGFR TKI therapy, with response to subsequent osimertinib therapy. Additional studies are underway to further validate and investigate the utility of ctDNA analysis to provide the clinician with actionable information to guide patient treatment.

Bibliography: (1) Kris – JAMA 2014 * (2) Gale Poster 3639, AACR 2016