Evaluation of stored liquid biopsies for molecular profiling in non-small cell lung cancer (NSCLC) patients

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BACKGROUND

Molecular profiling is often limited by access to sufficient tumour tissue for comprehensive analysis, and due to tumour heterogeneity, the complete range of tumor DNA abnormalities may not be represented nor accurately reflect the clinical evolution of disease

Circulating tumour DNA (ctDNA) can be used as a liquid biopsy for molecular abnormalities detection, quantification and monitoring for personalised treatment strategies

We evaluated ctDNA analysis of archived plasma collected prospectively as part of a clinical trial evaluating a second generation EGFR TKI versus placebo, in heavily pretreated patients with advanced NSCLC

METHODS

Patients had advanced incurable NSCLC, and had previously received standard of care systemic therapy with chemotherapy and at least one prior line of treatment with an EGFR TKI

Archival tumour samples were collected prospectively and analysed for EGFR mutation and KRAS mutation

Plasma was collected prospectively between Dec 2009-Jun 2013 at baseline and at every second four weekly cycle, in a phase III clinical trial evaluating a 2nd generation EGFR TKI versus placebo

Blood was collected in an EDTA tube and allowed to stand for 30 minutes before centrifugation at 2,500g for 15 minutes then aliquoted into cryovials and stored at -80 °C

ctDNA was analysed by InVision (enhanced tagged-amplicon sequencing) using a 34-gene panel including cancer hotspots, entire coding regions and copy number variants. InVision combines efficient library preparation and statistically-based analysis algorithms to identify and quantify cancer mutations

An initial sample set of n=360 (50% of randomized patients) was selected for this preliminary study to assess correlation of ctDNA for EGFR and KRAS analysed on archived plasma samples compared to EGFR and KRAS status from diagnostic archival tumour tissue

Primary endpoint

- Correlation of ctDNA for EGFR and KRAS analysed on archived plasma samples compared to EGFR and KRAS status from diagnostic archival tumour tissue

Secondary Endpoints

- ctDNA EGFR and KRAS status in patients with unknown EGFR or KRAS status
- Incidence of additional gene mutations identified from ctDNA

RESULTS

Table 1: Patient Characteristics

Variable	Characteristic	Number (%)
Sex	Female Male	184 (51) 176 (49)
Age years	Median (range)	63 (32-90)
Histology	Adenocarcinoma	245 (68)
	Squamous	57 (16)
	Other	58 (16)
Performance Status	0	1 (<1)
	1	92 (26)
	2	243 (68)
	3	24 (7)
Smoking History	Never	143 (40)
	Former	188 (52)
	Current	29 (8)
Race	Caucasian	189 (53)
	East Asian	136 (38)
	Other Asian	21 (6)
	Other	4 (1)
Prior chemotherapy	≤2	336 (93)
	>2	24 (7)
Prior EGFR TKI	Adjuvant	1 (<1)
	Palliative	359(100)

Median time lapse between tissue biopsy and liquid biopsy collection was 714 days

Patients had received a median of 3 lines of therapy prior to baseline plasma sample

Despite the lapse between tissue sampling and extensive prior therapy, 216 results of EGFR from tissue analyses (wildtype or mutant) were confirmed by ctDNA analysis (Figure 1a)

In addition, 47 T790M mutations were identified not detected previously in tissue, presumed to be acquired resistance due to prior therapy

206 KRAS tissue mutation results (wildtype or mutant) were confirmed by ctDNA analysis (Figure 1b)

RESULTS

Figure 1a
EGFR Concordance Summary

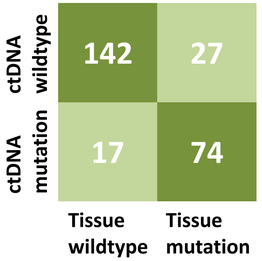
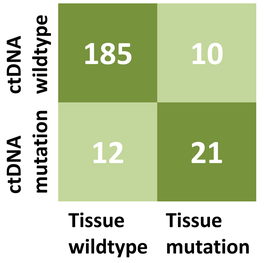


Figure 1b
KRAS Concordance Summary

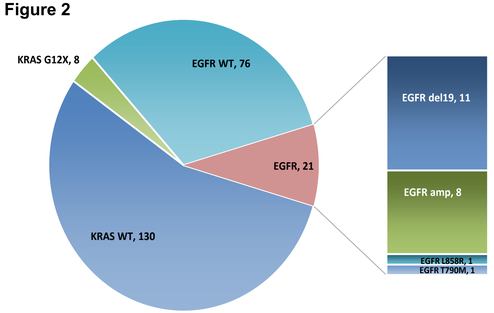


Patients with unknown tissue EGFR and KRAS profiles were analysed to detect driver mutations present at time of enrollment (Figure 2)

EGFR mutations were detected by ctDNA analysis in 13 patients

KRAS mutations were detected by ctDNA in 8 patients

Figure 2



RESULTS

Table 2
Other (number of patients) ctDNA reported mutations by gene

Gene	Number (%)	Gene	Number (%)
ALK	1 (<1)	KIT	2 (<1)
BRAF	5 (1)	MED12	2 (<1)
CCNDI	0 (0)	MET	6 (2)
CDKN2A	13 (4)	MYC	0 (0)
CTNNB1	8 (2)	NFE2L2	4 (1)
ERBB2	21 (6)	NRAS	3 (<1)
ESR1	3 (<1)	PDGFRA	1 (<1)
FGFR1	12 (3)	PIK3CA	11 (3)
FGFR2	2 (<1)	PPP2R1A	1 (<1)
FGFR3	1 (<1)	PTEN	10 (3)
FOXL2	1 (<1)	RET	1 (<1)
GATA3	7 (2)	STK11	24 (7)
GNAS	2 (<1)	TP53	223 (62)
HRAS	1 (<1)	U2AF1	4 (1)
IDH1	5 (1)		

Figure 3: Number of patients with concurrent mutations as detected by ctDNA

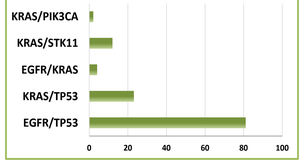
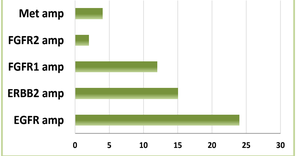


Figure 4. Number of patients with amplifications (amp) detected by ctDNA



CONCLUSIONS

- Banked archival plasma samples can yield acceptable concordance for ctDNA EGFR mutations and KRAS mutations compared with tumour tissue analysis
- Concordance between plasma and tumour analysis from banked samples is complex and must include consideration of assays used, sensitivity of the assays and collection time points with respect to prior treatments
- ctDNA can identify a range of mutations on banked plasma samples
- Analyses of additional baseline samples and serial samples is planned