biomarkers

**SERIAL NEXT GENERATION SEQUENCING OF CFDNA TO MONITOR PHASE I TARGETED DRUG ADMINISTRATION**

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**Aim:** Plasma from patients with cancer contains cell free tumour DNA (cfDNA) that may be utilized for genotyping tumours in real time. We evaluated the use of cfDNA next generation sequencing (NGS) as a multipurpose response biomarker in advanced cancer patients receiving Phase I targeted drugs.

**Methods:** Between 12/2012 and 11/2013, 135 plasma samples from 24 patients with identified mutations in cfDNA and completing at least 2 courses of investigational targeted therapy were sequenced serially during treatment. Targeted NGS was performed on the Ion Torrent PGM platform with a 50-gene panel with a target coverage of at least 500 X per amplicon.

**Results:** The mean sequencing coverage across the experiments was 1,685 X. Overall, 24 patients with various tumour types receiving inhibitors of the PI3K-AKT-mTOR pathway (n = 18), MEK (n = 4), or in combination (n = 2) and others targets (n = 5) were included. Five patients received two consecutive Phase I trials. The mean number of mutations identified in cfDNA per patient was 2 (range 1-5) and the mean mutation allele frequency (AF) within the samples was 26% (Range 2-58%). TP53, PIK3CA and KRAS were the top 3 mutated genes identified, with respectively 19 (42%), 9 (20%) and 7 (16%) different mutations identified. The monitoring of mutation AF in consecutive plasma samples during treatment showed dynamic modifications related to treatment. In the 10 patients with multiple mutations in cfDNA, similar dynamic changes in serial plasma samples were generally observed but in some cases, evidence suggesting clonal heterogeneity was observed, whereby certain mutations dominated in the plasma during the course of the treatment. Patients having a fall in AF of cfDNA mutation after 2 cycles of treatment by >30% (n = 9) had a significantly better time to progression of 111 days compared to 53 days (n = 11) (p = 0.0169) for patients having an increasing AF of >20% compared to baseline.

**Conclusions:** Serial sequencing of cfDNA during targeted therapy allows monitoring of AF cfDNA mutations that can be associated with response and time to progression. This biomarker warrants further evaluation in the setting of developmental therapeutics.

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