Liquid Biopsy Analysis of NSCLC Patient Plasma using ddPCR and NGS

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Introduction

The use of liquid biopsy in clinical practice has become a reality. The currently approved liquid biopsy tests utilize real-time PCR technology and allow for positive/negative detection of EGFR mutations (L858R; exon19del) in cfDNA when NSCLC tissue is not available or is inadequate. The use of liquid biopsy continues to be explored for a wide range of additional questions and applications including patient screening for combination therapies and allele burden monitoring. Therefore, the future use of liquid biopsy in clinical practice may require technology platforms that enable the identification of a higher number of targets and/or the absolute quantification of mutant cfDNA molecules. Here we use both EGFR droplet digital PCR (ddPCR) and the Oncomine Lung cfDNA Assay (NGS panel) to evaluate allele burden and assess a broader mutational profile, respectively, in a set of plasma samples collected from NSCLC patients.

Methods

Plasma samples (n=2-7) were collected at Dana-Farber Cancer Institute. cfDNA was extracted using the QIAamp DSP circulating NA kit. EGFR ddPCR assays were designed by DEI (PMO-1931174). One-third of ddPCR extracted from 3 ml plasma was used for each EGFR ddPCR assay (L858R, T790M, L858R, and T790M). The L858R and T790M ddPCR assay detects mutations using mutant specific probes. The ex19del/19del allows for the detection of a broad range of exon 19 deletions via the loss of wild-type exon 19 signal. Of these plasma samples from patients with EGFR WT tumors were analyzed to assess the background noise (limit of blank). The other 37 plasma samples were analyzed in a blinded fashion. ddPCR analysis was performed on the QX200 system and data was analyzed using QuantaSoft software.

A subset of 12 plasma samples had sufficient cfDNA for additional analysis using the Oncoremine Lung cfDNA Assay on the Ion 55. This NGS panel is designed to detect 15 hotspot mutations in 11 lung cancer-related genes (ALK, BRAF, EGFR, FBXW7, KRAS, MET, NAB2, PIK3CA, ROSI, and TP53). Terrent Software Suite 5.2 was used for NGS data analysis. The Oncoremine Liquid cfDNA Multiplex Reference Standard (MAF 5%, 1%, 0.1% and WT) was also included in the ddPCR and NGS studies.

Results

Positive/Negative Assay Cut-off for EGFR ddPCR: The plasma samples from patients with WT tumors showed large variations in the amount of cfDNA extracted ranging from 60 copies to 2023 total copies of wild-type EGFR in triplicate wells. No positive droplet was observed in all samples for L858R and del19 assays. For the T790M assay, sample TRL_28 showed one double positive droplet and TRL_30 and TRL_32 showed one single positive droplet. Based on the ddPCR assessment from 16 EGFR negative plasma samples, the cut-off for calling positive mutation is set at: Poisson extended mutant copy number x (the actual number of positive droplets x 2) wild-type copy is > 1000; Poisson extended mutant copy number x 4.5 (the actual number of positive droplets x 2) wild-type copy is > 3000; Poisson extended mutant copy number x 4.5 (the actual number of positive droplets x 2) wild-type copy is > 10,000; Poisson extended mutant copy number x 4.5 (the actual number of positive droplets x 2) wild-type copy is > 30,000. These cut off values were applied to the data generated in other 32 samples. When the number of copies was below the established positive/negative cut off, it was reported as ‘0%’ for EGFR assay.

Comparison to ddPCR results from external lab: Plasma samples (n=33) from NSCLC patients that had been previously genotyped for EGFR L858R, Ex19del and T790M mutations using ddPCR assays were blinded and tested. MolecularMD analysis determined 12 samples (36%) to be L858R positive, 19 samples (58%) to be T790M positive and 20 samples (59%) to be T790M positive. The MAF ranged from 0.08-0.46% for L858R, 0.3-0.39% for T790M and 0.02-0.77% for Ex19del. The ddPCR results between the 2 labs were 100% concordant for L858R and 94% concordant for both T790M and ex19del. In all discordant cases, the MolecularMD ddPCR result was positive and the external lab result was negative. When sufficient cfDNA was available, either NGS or an alternative ddPCR assay was used to resolve discordant results (Table 7). The discordance for these samples could be caused by sampling variation during ddPCR extraction or assay testing to capture the few copies of mutant molecules present in the plasma samples.

For Further Information

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