Mutation detection in circulating free DNA (cfDNA) using a targeted next generation sequencing platform in lung cancer patients.

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Solid tumors frequently shed DNA into bloodstream, possibly because of spontaneous cell death or through exosomes. Thus, circulating free DNA (cfDNA) provides a unique opportunity for detecting genomic alterations in cancers, and can serve as non-invasive biomarkers for cancer early diagnosis, for monitoring treatment response or disease recurrence, and for genotype-based precision medicine. Currently, mutations in cfDNA can be detected relatively easily by droplet digital PCR (ddPCR) but only for cancer driver mutations previously known to be present in primary tumors. Therefore, this method is useful for monitoring treatment response or disease recurrence but not for discovery. Using next generation sequencing (NGS) to detect mutations in cfDNA could potentially be an optimal choice; however, the use of this massive sequencing technology remains challenging for detecting mutations in relatively a large set of target genes, because the yield of cfDNA is often not sufficient for such analyses and the DNA is highly fragmented. We hypothesized that methods improving the yields of cfDNA will facilitate mutation analysis with NGS. To evaluate that, we used a customized phenol-extraction and ethanol precipitation approach to isolate cfDNA from 1 mL of plasma from lung patient samples. We were able to obtain at least 50 ng of cfDNA from the samples tested. That was sufficient material to process the DNA in a highly customized targeted sequencing pipeline developed in the Institute for Personalized Cancer Therapy (IPCT) laboratory at MD Anderson called T200.1. This platform screens 263 genes (all exons) for mutations and provides global copy number data. To reduce the noise frequently observed in next gen sequencing data obtained from challenging samples such as cfDNA, we also sequenced patient's germline DNA (from blood white cells). The data comparison between germline DNA, cfDNA and respective primary tumors from lung cancer patients previously sequenced using the T200.1 platform will be presented.