Abstract

Introduction: Next generation sequencing (NGS) of blood-derived nucleic acids is an emerging paradigm for determining the mutational status of cancer patients over time. Both circulating tumor cells (CTC) and cell-free circulating DNA have been proposed as possible sample types for extracting tumor DNA. Here we present data from a CTC enrichment modality that results in tumor cell purities of >90% and a high sensitivity NGS data analysis workflow that enables the use of standard amplicon panels typically used for primary tissue. This study is aimed at urological cancers (kidney, prostate), and adds to previously published data presented for bladder cancer patients using this technique.

Results: Multisite analytical validation data, based on spiking of cells into whole blood, and a matched molecular and bioinformatics approach demonstrate a detection limit down to 10 cells from a blood draw with a false positive rate of below 0.1% per sample. Clinical data from two different urological cancer pilot studies (prostate and kidney) demonstrates the detection of somatic variants for a majority of samples, and significant overlap between detected mutations and known somatic mutation sites. For example, in prostate patients, we detect common mutations (i.e. TP53, PTEN and APC genes) that are similar to the population distribution of mutation rates in tissue biopsies. Significant overlap between detected mutations and known somatic mutation sites. For

Conclusions: This assay makes possible the detection of somatic variants from urological cancer patients without the need for a tissue biopsy.

Somatic Mutation Detection from Liquid Biopsies via NGS: Urological Cancers

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Figure 2: Next generation sequencing workflow. Based on the IsoFlux NGS Kit that allows routine purification of CTC samples to >90% tumor DNA content, a workflow was developed for NGS characterization of patient samples utilizing an commercially available cancer hotspot panels and the PGM sequencing instrument (Life Technologies). CTC enrichment is followed by lysis, DNA amplification, library preparation / sequencing and a specialize variant filtering algorithm.

Figure 4: Analytical validation of the CTC to NGS assay. Using a mesenchymal-like cell line (MDA-MB-231) the limit of detection of the CTC-based NGS was determined by spiking cells into 14 mL healthy donor blood. IsoFlux enrichment, targeted amplification using the CHpO2 panel, and blinded NGS analysis to discover the variants present. The detection limit is below 2 cells / mL blood for all variants, with a very low false positive rate (no false positives in this data set). Note that the CTC purity for matched samples is in the range of 12-16%, with a good match between the expected and detected allele frequency of mutations.

Figure 5: Patient Samples and Enumeration Results. CTC counts are presented for the 15 patients enrolled in the pilot trial, as compared to a healthy normal cohort. The study consisted of prostate and kidney cancer patients, with enriched CTC samples sequenced at the CompanionDx lab using three different commercially available cancer gene panels. These advanced patients had high CTC counts as compared to healthy baselines.

Figure 6: Sample NGS Results - Patient Samples. The vast majority of the CTC-enriched samples processed through the analysis pipeline yielded genomic aberrations: the two examples shown utilized the Oncomine (left table, patient 1) and RCC (right table, patient 14) panels. While all germ line mutations were present in both enriched CTC and matched normal samples, the abnormalities presented here were present in the CTC sample but not in the normal sample.

Figure 7: Median variant numbers by panel. Median variant counts present in CTC samples as compared to matched normal samples were calculated for each of the three oncogene panels used. The larger pan-cancer panel Oncomine (Life Technologies) did an excellent job of detecting abnormalities, while the more limited hotspot panels detected mutations consistent with the panel coverage for these indications (prostate, renal cell carcinoma).

Summary

We present analytical and patient sample validation for a novel approach to molecular profiling of liquid biopsy samples from prostate and renal cell carcinoma patients via NGS.

The methods presented enabled the recovery of high CTC counts in all late stage patients participating in this study, as well as the isolation of matched normal samples by collecting the white blood cell fractions resulting from the same separation. Both CTCs and leukocyte fractions were analyzed using a sensitive sequencing and NGS data analysis pipeline capable of detecting variants down to 0.5% allele frequency with high fidelity. Abnormalities found in the tumor cell samples were not present in matched normals, and were similar to abnormalities typically detected in tissue based studies for similar patient cohorts and gene panels. In relation to other liquid biopsy methods, the high sample purity provided by this workflow enables use of standard genomic panels as opposed to highly specialized sequencing and analysis methods.

This study demonstrates the detection of somatic mutations in urological cancers from a blood draw. Ongoing studies include sequencing of CTCs cultured post enrichment, mutational profile changes over time, and correlation to matched biopsy samples.