Targeted next-generation sequencing of cell-free tumor DNA to longitudinally monitor cancer burden and progression

Jonathan Irish1, Cassie A Schumacher1, Navya Nair2, Olga Camacho-Vanegas2, Sukhinder Sandhu1, Laurie Kurihara1, Peter Dottino3, Melissa Schwartz1, Timothy Hinkins5, John Martignetti2, Vladimir Makarov1

1Swift Biosciences, 5 Parkland Plaza, Suite 100, Ann Arbor, MI 48103
2Iahn School of Medicine at Mount Sinai, Departments of Genetics and Genomic Sciences and Obstetrics/Gynecology & Reproductive Sciences, 1425 Madison Avenue New York, NY 10029

Abstract

Ultrasound and ovarian cancers are the fourth and fifth, respectively, most common cancers in women in the United States and combined are estimated to account for nearly 23,000 deaths in the US each year. The use of circulating free-tumor DNA (cfDNA) to monitor disease burden during and post-treatment has implications for effectively treating the disease. Combination therapy is targeted according to the mutational landscape of the tumor DNA over time. This requires careful assessment of tumor DNA from a drawing to detect tumor cell free DNA and cell-free DNA that may be associated with the target specific drug. We performed a pilot study to retrospectively study the cfDNA collected from 11 women with gynecological cancers. Each had undergone primary surgery and chemotherapy to treat their tumors. We assessed the mutational profile of the tumor DNA from 2 to 6 months apart. Samples were sequenced using a next-generation sequencing alignment panel covering 56 oncology-related genes. We observed a correlation between tumor-specific mutation frequency in the cfDNA and survivability. In all 11 women, no reappearance of the primary tumor mutations was observed and these women (9) remain in remission or living with disease (1). In 2 of 11 women, while the frequency of the primary tumor mutation in the first time point was less than 1%, a recurrence of the mutation(s) detected in the primary tumor was observed at the second time point. With multiple active targets to treat the tumor, this may be an opportunity to identify potential reagents or modalities.

Tumor Mutational Profiling

Figure 2. Preliminary Study to Determine Tumor Mutational Profiles. 57 tumor samples and corresponding normal samples derived from blood cells were obtained and sequenced using Accel-Amplon-5G. 2 samples were excluded after not passing quality filters. 3 tumors were sequenced on an Illumina MiSeq to > 500x coverage for somatic variant calling, normal samples were sequenced to > 100x coverage for germline variant calling. Alkaline tetrazolium were determined bioinformatically using LoFreq.

Accel-Amplon-5G Oncology Panel v2

- Single-tube assay
- 2-hour workflow
- 10ng input DNA
- Continuous, covering overlap of 56 oncology-relevant genes
- v2 panel adds coverage of selected germline SNP targets to track sample “fingertip”
- > 95% coverage uniformity
- > 95% of aligned reads on-target

Accel-Amplon-5G Oncology Panel v2 Specifications. The Accel-Amplon workflow consists of two steps. Step 1 is a multiplex PCR step, containing of oncology-specific primer pairs as well as primer pairs for germline mutations to generate the germline fingerprint. After a bead-blast cleanup, the second step adds a unique index and the sequencing adapters to each library to allow multiplexing on the sequencing instrument. The table depicts the 56 genes that are represented in this panel. Continuous, covering overlap of selected areas is included for these genes. Full indexing exon coverage is included for this gene.

cfDNA Quantification and QC

Figure 5. Allelic PCR Assay. Accurate cfDNA quantification of cfDNA is imperative for successful binary preparation. Fluorescent methods such as Qubit do not quantitate amplifiable DNA and cannot distinguish cfDNA from high molecular weight (HMW) genomic DNA (gDNA). An allelic PCR assay targeting both 115 bp and 247 bp (none of the Allelic PCR assay above can quantify amplifiable DNA. The Assay1 primer quantify both cfDNA and HMW gDNA, while the Assay2 primer quantify only HMW gDNA. The ratio of 247:115 determines a DNA integrity score; the expected score for HMW gDNA is 1, and the expected score for cfDNA is between 0.20 and 0.65, but can vary with cancer types. This assay is based on Max et al, 8th J Cancer 2014 Oct 14; 11(18): 1482-92.

Figure 4. Longitudinal Study of cfDNA. cfDNA was retrospectively isolated from blood samples at various times after tumor resection for 11 individuals from Figure 2. Accel-Amplon-5G Barcodes were made from 10 ng of cfDNA (exception: Juv, 7.0 ng and juv (1.11) and sequenced on an MiSeq to an average 15,000x coverage. All allele frequencies (AF) were determined using LoFreq and compared against the AF’s detected in the tumor sample. All AFs below 1% were determined by visual inspection using the Integrated Genome Viewer (IGV) software from the Broad Institute. Examples are in orange and green boxes and illustrated in Figure 5.

Ultra-Low Frequency Variant IdentifID

Figure 5. Ultra-low Frequency Variant Identification. For tumor mutations which LoFreq did not identify as the cfDNA, 500x coverage (shown to be able to implicate the tumor DNA around PIK3CA H1047R, PIK3CA around TP53 Leu238Asn; Reads identified with HMM (DNA is 1), and the expected score for cfDNA is between 0.20 and 0.65, but can vary with cancer types. This assay is based on Max et al, 8th J Cancer 2014 Oct 14; 11(18): 1482-92.

Conclusion

- 10 ng of tumor DNA and normal DNA from blood can be used to identify somatic variants present in ovarian and uterine tumors.
- Sequencing cfDNA derived from women with gynecological cancer can identify the same variants seen in the tumors and at various times after resection.
- There is an observed correlation between resurgence of tumor mutations in the cfDNA of individuals with gynecological cancers and outcome. More and broader studies should be done with more frequent and consistent time points to further probe the utility of this technique. Other factors such as age, treatment, type of cancer, etc should also be considered when interpreting results.
- Sample ID is critical to allow samples derived from the same individual to be properly paired during analysis.