Impact of Panel Size on Molecular Residual Disease (MRD) Assay Performance

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Background

- Sequencing cell-free DNA (cfDNA) is a promising method for monitoring cancer treatment response and detecting recurrence. However, sensitivity at the low tumor fractions typical of early-stage, post-treatment, and early-recurrent tumors is limited by the small number of variants targeted in commercially available assays.

- We developed a tumor-informed MRD assay using tumor-normal whole-genome sequencing (WGS) followed by interrogation of select somatic variants in cfDNA. The method was tested on a cohort of 30 patient samples, targeting 1,000 somatic single-nucleotide variants (SNV) per sample.

WGS Panel Design

- 20-50x tumor-normal WGS data was processed with a bioinformatic pipeline including somatic SNV calling, tumor purity estimation, and copy number anomaly calling.

- 1,000 target SNVs from each sample were selected to create a sample-specific panel for hybridization capture and deep sequencing.

- When fewer than 1,000 high-confidence targets were identified we backfilled with lower-confidence variants.

- We evaluated somatic calling performance by deep targeted sequencing of formalin-fixed paraffin-embedded (FFPE) tumor tissue, patient normal DNA, and pre-treatment patient cfDNA.

Circulating Tumor DNA Detection & Quantification

- Somatic targets were enriched and sequenced to high depth (>500x after UMI deduplication) in FFPE tumor tissue, patient normal DNA, and pre-treatment patient cfDNA.

- We developed a maximum likelihood statistical model to quantify tumor fraction (the genome-equivalents proportion of cfDNA derived from tumor tissue) and call MRD positive or negative status in patient cfDNA.

- We then created serial dilutions of patient cfDNA with non-patient cfDNA at a set of predetermined tumor fractions for 17 samples with sufficient cfDNA mass and tumor fraction.

- Bootstrap resampling of target sites was used to estimate sensitivity across the tumor fraction spectrum given varying target site counts (Figure 2).

- Tumor-fraction quantification accuracy was estimated by comparing model estimates to the expected dilution tumor fraction (Figure 3).

Conclusions

- WGS-driven panel design allows targeting up to 1,000 high-confidence somatic variants in an MRD assay across diverse cancer types and stages.

- Increasing the MRD panel size in turn leads to higher sensitivity at low tumor fractions and robust tumor-fraction quantification in patient cfDNA.

- High-sensitivity MRD has the potential to enable earlier recurrence detection and gives researchers and clinicians new tools for monitoring patient treatment responses.