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

C.J. Battey, PhD*; Ashley Acevedo, PhD*; Matt LaBella, PhD*; Sangita Ganesh, PhD; Elise Buser; Nafei Xu; Ravi Patel, PhD; Kiefer Haug; Kyle Trettin, PhD; Jeff Jasper, PhD; Jan Risinger; Genevieve Gould, PhD; Dale Muzzey, PhD

***Authors contributed equally**

Myriad Genetics, Inc. Salt Lake City, UT, USA

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 (**Figure 1**).

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.

Results

Figure 1. WGS Panel Design Variant Counts and Target Confirmation Rate

A median of 97.8% of targets were confirmed via high-depth tumor sequencing.

80% of samples contained over 1,000 high-confidence targets. The median number of high-confidence SNVs was 2,344.

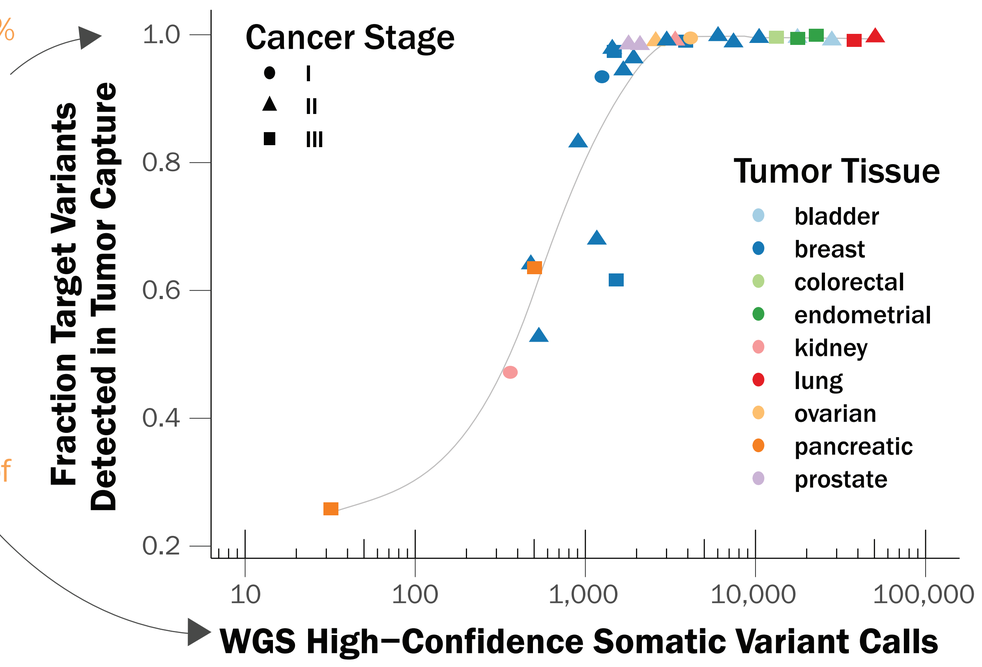


Figure 2. Assay Sensitivity as a Function of Tumor Fraction and Target Variant Count in 17 cfDNA Dilution Series

Models are tuned for 100% specificity across 136 negative controls.

A 1,000-target panel yielded >95% sensitivity at >99% specificity down to 0.002% tumor fraction.

Increasing target variant counts from 50 to 300 raised sensitivity at 0.01% tumor fraction from 50% to >99%.

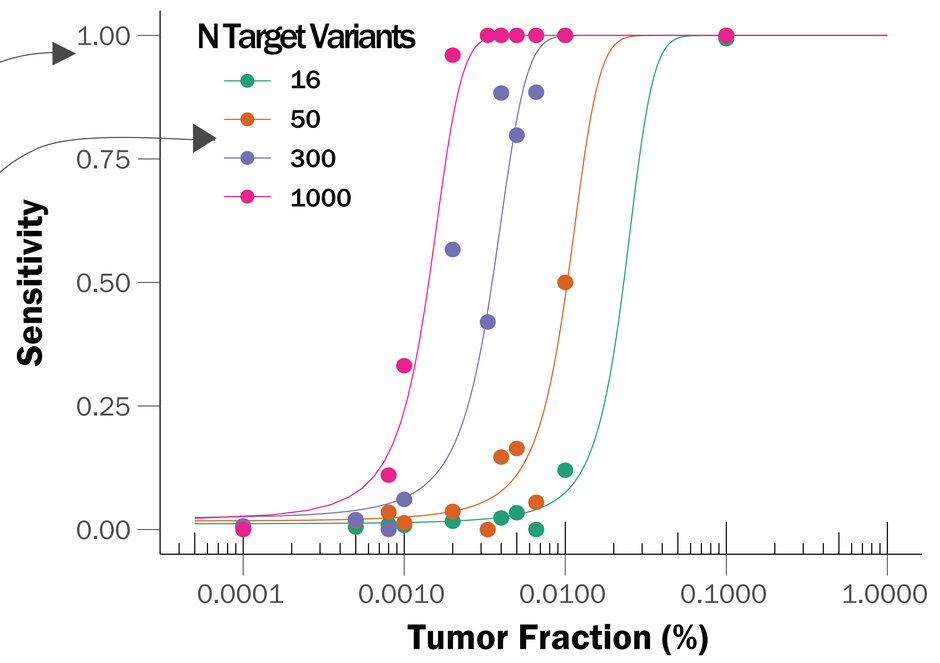
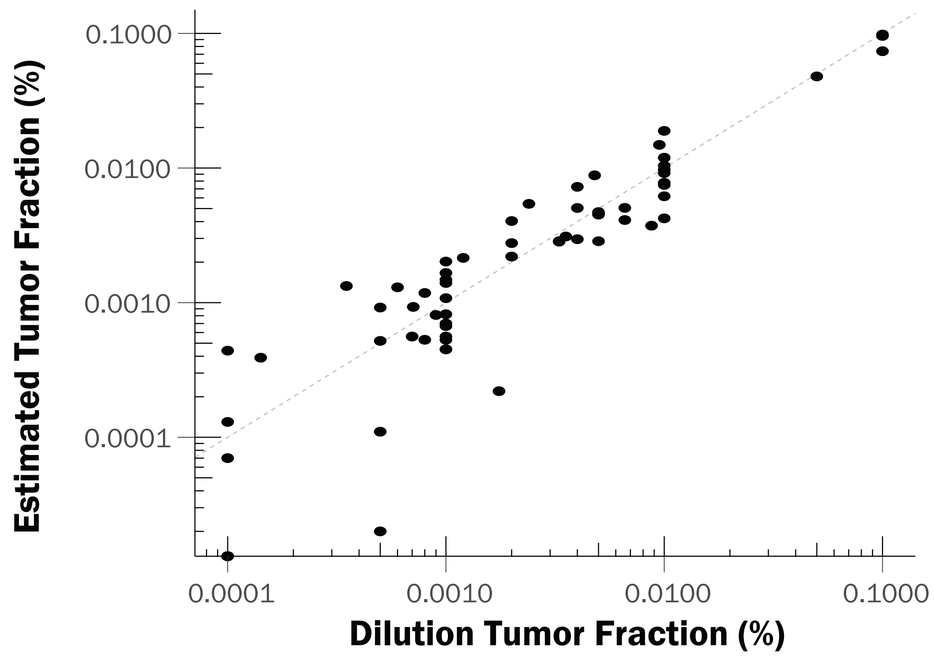


Figure 3. Tumor-Fraction Quantification in 17 cfDNA Dilution Series

Tumor-fraction quantification was linear down to 0.001%, with a mean absolute error of 0.0016%.



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