6094

Detection of MRD assessment with the Personalis NeXT Personal assay using MATRIX plasma-in-plasma contrived samples

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BACKGROUND

Detection of circulating tumor DNA (ctDNA) has been shown to correlate with clinical outcome of oncology patients. Molecular residual disease (MRD) profiling by ctDNA is being rapidly deployed. Current tumor-informed MRD tests assess a relatively small number of personalized variants, placing limits on their detection sensitivity and ultimately causing a significant proportion of the patient population to receive false-negative results due to insufficient assay limit of detection (LOD). Recent studies have shown that increasing the overall number of trackable variants can significantly improve LOD and MRD detection sensitivity, and multiple vendors are developing and commercializing "ultra-sensitive" MRD assays that rely on tumor whole genome sequencing (WGS) to inform panel selection.

Here we report a performance evaluation (LOD, sensitivity, specificity) of the Personalis NeXT Personal tumor-informed MRD assay. To understand the improvement in sensitivity of this and other "ultra-sensitive" MRD assays, we have used a set of contrived plasma-in-plasma samples in a dilution series of plasma tumor fractions.

METHODS

Experimental design: Commercial matched tumor and plasma samples were used for this study. All samples were properly consented for exploratory analysis. **STUDY 1:** Four unique patients were used to create the contrived samples and represented three different cancer types (melanoma, lung, colorectal). **STUDY 2:** Four additional unique patients were used to create the contrived samples, with each patient sample representing a different cancer type (lung, breast, colorectal, and bladder). The cancer plasma was diluted into a background of healthy volunteer plasma to create the 72 *plasma-in-plasma* samples in each study (total of 144). The dilution scheme (Figure 1) shows how the STUDY 1 dilutions were modified to allow for more replicate samples at the lowest dilution levels in the STUDY 2 study.

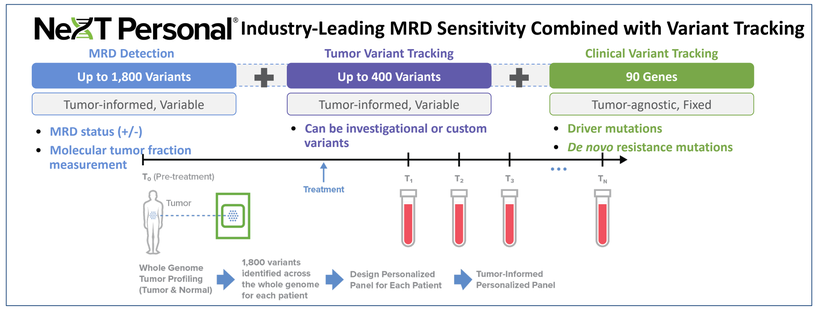
Personalis assay: The NeXT Personal assay is a tumor-informed next-generation sequencing assay[1]. WGS was performed on the matched solid tumor and normal samples from each patient. Up to ~1800 somatic variants were selected for each patient. A personalized panel was designed and used to enrich for the selected targets in the individual plasma samples. An aggregated positive variant score was evaluated to determine the presence of circulating tumor DNA in each plasma sample. A pre-release version of the Personalis analysis pipeline was used for STUDY 1. STUDY 2 analysis used the production release pipeline with a lower p-value threshold and an updated error model for improved specificity.

Matrix study description: The MATRIX sets built for STUDY 1 and STUDY 2 are derived from individual patient tumor plasma samples diluted into healthy donor plasma backgrounds. These MATRIX samples are commutable to true clinical samples and retain all the biomarker characteristics of the tumor samples, which can be diluted to any desired level. The figure below shows the dilution scheme for the four samples in the two MATRIX sets[1]. STUDY 2 replicates were included at the predicted LoD of the evaluated MRD assays, as the STUDY 1 study informed. In total, 144 samples were tested as part of these studies. A small number of negative samples were also provided to minimally assess specificity.

	PPM	2000	1000	500	200	100	50	20	10	0
	% Tumor Plasma Dilution	0.200%	0.100%	0.050%	0.020%	0.010%	0.005%	0.002%	0.001%	0.000%
# Replicates	STUDY 1	3		3	3		3	3		3
	STUDY 2		1	2		4	5		4	2

NeXT Personal assay design & workflow

The NeXT Personal assay uses a single panel consisting of MRD content, tumor-informed variant tracking, and fixed clinical variant tracking content. WGS is performed on patient tumor and normal samples. Up to 1800 targets are selected for MRD, up to 400 targets from exonic regions, and 2130 SNVs from curated fixed content. Panels are manufactured, QC'd, and available for sequencing plasma-extracted cfDNA.



RESULTS

Overall performance

All examined tumor/normal pairs successfully completed the assay design. STUDY 1 panels had an average of 1863 variants (range: 1818 to 1888) selected for MRD tracking, plus 152 clinically relevant variants for an average of 2206 variants assessed per patient. STUDY 2 panels had an average of 1836 variants (range: 1819 to 1867) selected for MRD tracking, plus 2130 clinically relevant variants for an average of 4094 variants assessed per patient.

Extracted cfDNA yields ranged from 1.6 to 13.5 ng/mL plasma per sample. STUDY 1 patient samples had an average of 4.2, 5.6, 7.7, and 11.0 ng total cfDNA. STUDY 2 patient samples had an average of 4.8, 5.1, 6.2, and 14.8 ng total cfDNA. Almost all samples were below the target NeXT Personal assay cfDNA input amount of 15 ng.

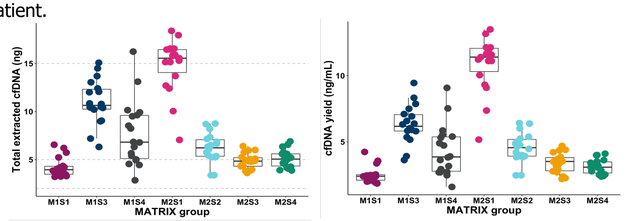
In STUDY 1 a total of 45 positive samples were analyzed after removing set 2 that had been over-diluted. Tumor signal was detected in all analyzed samples, including nine samples at 0.002% tumor plasma dilution. Thus, the assay sensitivity was 100%.

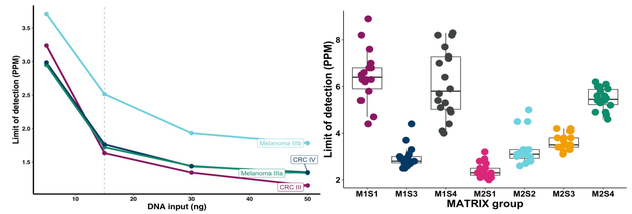
STUDY 2 included a total of 64 positive samples. Tumor signal was detected in 62 of 64 positive samples, including 14 of 16 samples at 0.001% tumor plasma dilution. Two false negatives were reported at the 0.001% tumor plasma dilution. Thus, the assay sensitivity was 87.5% at the lowest dilution (0.001%) and 96.8% overall.

STUDY 1 had one false positive detection (1 of 9 samples analyzed) using pre-release product parameters, but re-analysis with the released commercial product used in STUDY 2 resulted in zero false positive calls. STUDY 2 also had zero false positives (0 of 8 samples).

Plasma extraction & Limit of Detection

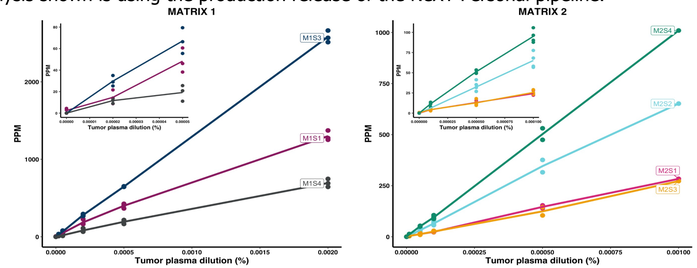
Total extracted cfDNA values are shown below. Horizontal dashed lines represent target input to achieve 1-3 PPM LOD (15ng), lower bound (5ng), and absolute minimum (2ng). cfDNA yield is consistent with observations reported in literature[1]. NeXT Personal's LOD as a function of input amount using 4 patient panels. Plasma samples were diluted from 50 to 5ng. Vertical dashed line represents target input to achieve 1-3 PPM LOD. Reported LODs for each set of plasma samples (18), by patient.

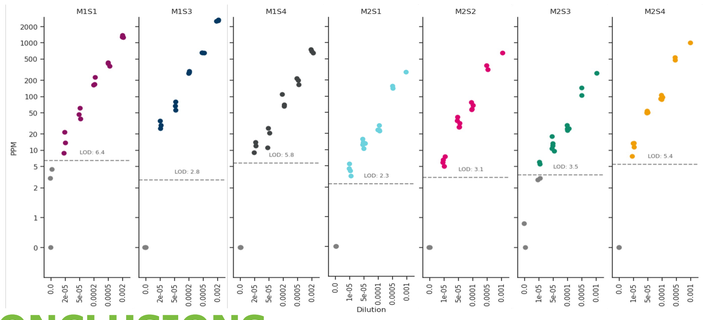




NeXT Personal demonstrates ultra-high sensitivity

NeXT Personal shows excellent linearity across the entire dilution range from 0% to .02% for both STUDY 1 and STUDY 2, with a mean LOD as low as 2.3 PPM for highest input samples. STUDY 1 sensitivity is 100%, with lowest positive dilution at .002%. STUDY 2 sensitivity is 96.8%, with false negatives in 2 out of 4 .001% dilutions for M2S3 (low mean input of 4.8 ng). Analysis shown is using the production release of the NeXT Personal pipeline.





CONCLUSIONS

MATRIX *plasma-in-plasma* samples are a robust option for assessing the sensitivity of MRD assays to ultra-low levels of ctDNA. This approach addresses all parts of an MRD assay for both the plasma and tumor/normal tissues and is the closest thing to real clinical samples while allowing direct interrogation of sensitivity.

MATRIX contrived samples assessment using the Personalis NeXT Personal assay shows ultra-high sensitivity with reproducible data down to the 1-3 PPM range.

Expansion of the personalized panel fixed clinical content allows tracking of more driver and resistance mutations with no impact to the MRD sensitivity.

References

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2. Stetson D., Labrousse P., Russel H., Shera D., Abbosh C., Dougherty B., Barret J., Hodgson, D, Hadfield J. Next-generation MRD assays: do we have the tools to evaluate them properly? arXiv:2311.00015. https://doi.org/10.48550/arXiv.2311.00015