Analytical performance of an ultra-sensitive, tumor-informed liquid biopsy platform for molecular residual disease detection and clinical guidance

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BACKGROUND

Early detection of cancer recurrence and treatment monitoring using non-invasive procedures such as circulating tumor DNA (ctDNA) profiling is an emerging new strategy for cancer patient care. ctDNA is shed by primary or metastatic tumor cells and contains tumor-specific somatic mutation signatures. However, most ctDNA-based molecular residual disease (MRD) detection methods leverage a limited genomic footprint, restricting detection sensitivity to 10^{-4} ~ 10^{-5} tumor fraction and thus their utility in many clinical settings [1, 2]. In the post-diagnosis situation, early detection of cancer, especially those with low tumor mutational burden (TMB) [3], may become challenging if they do not harbor sufficient variants in these limited footprints to produce detectable signals. Further, insights into tumor evolution, including actionable mutations may be missed.

Here we report a performance update of the NeXT Personal® platform. NeXT Personal utilizes hybridization-capture-based technology to quantify ctDNA in liquid biopsy samples. Its unified probe panel design and workflow simultaneously detects and monitors MRD, surveys known cancer-related clinical variants, and quantifies and tracks investigational variants of interest, by leveraging both tumor-informed and tumor-agnostic genomic information (Fig. 1). The tumor-informed MRD content is dedicated to personalized MRD detection and longitudinal monitoring, which utilizes cumulative tumor-derived signals to achieve ultra-high sensitivity down to 1 ~ 3 parts per million (PPM) limit of detection (LOD). The tumor-agnostic clinically relevant content surveys known and validated actionable genomic variants in 90 clinically relevant genes, and serves to inform therapy selection and detect existing or potentially emerging events such as new subclones or metastases. The investigational content provides capacity to track important cancer-related variants for research or investigational purposes.

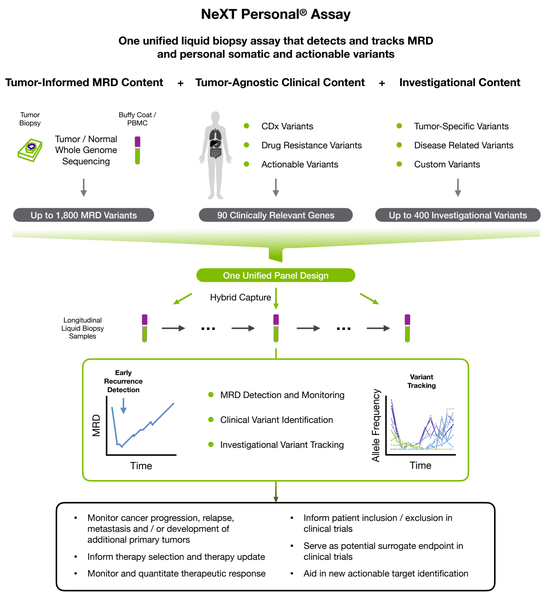

Figure 1: NeXT Personal Assay Overview.

References:

1. Odegaard, J. I. *et al.. Clin Cancer Res.* 24, 3539–3549 (2018).
2. Reinert, T. *et al.. JAMA Oncol.* 5, 1124–1131 (2019).
3. Merino, D. *et al.. J Immunother Cancer.* 8, e000147 (2020).

METHODS

The NeXT Personal assay requires a single tube of peripheral blood (1 ~ 8 mL plasma or 2 ~ 50 ng cfDNA) for each monitoring time point, as well as 1 upfront tumor biopsy sample (fresh frozen or formalin-fixed paraffin-embedded sections, 1 mm^3 in size) paired with a matched normal sample (e.g. buffy coat or peripheral blood mononuclear cells) to identify tumor-specific genomic content. All experiments were performed in the Clinical Laboratory Improvement Amendments-certified and College of American Pathologists-accredited laboratories at Personalis, Inc. following standard operating procedures. The tumor-informed MRD content (up to 1,800 variants) and Investigational content (up to 400 variants) for each panel were designed by identifying high quality and low noise tumor-specific genomic variants based on matched tumor-normal whole genome sequencing using proprietary algorithms. The tumor-agnostic clinically relevant content was predesigned for all panels (subject to periodic review and update), which surveys known and validated actionable genomic variants such as companion diagnostic (CDx) variants, drug resistance hotspots and other actionable variants in 90 clinically relevant genes. Data analysis was performed using the proprietary production analysis pipeline for NeXT Personal.

RESULTS

Analytical Performance of MRD Detection

In a series of 75 cfDNA samples with artificially constructed tumor fractions (TF) ranging from 10^{-6} to 5 x 10^{-1}, the assay detected signals as low as 1.95 PPM (Fig. 2, p-value < 1 x 10^{-4}). The median LOD was 1.45 PPM (range: 1.17 - 1.69 PPM). Our results showed a very high linear correlation (Pearson R^2 = 0.999) between observed and expected MRD signals that span over 5 orders of magnitude. The detected MRD signals were also orthogonally confirmed by ddPCR (Fig. 3, Pearson R^2 = 0.917, PPA = 100%, NPA = 100%). Note the high linear correlation between the NeXT Personal MRD signals and the tumor signals detected by ddPCR, and that NeXT Personal detects signals 100 ~ 1000X lower than ddPCR's LOD (solid circles, actual ddPCR readings; hollow circles, imputed ddPCR readings from higher concentration measurements due to ddPCR's LOD). In addition to the ultra high sensitivity, high MRD detection specificity (100% observed, 95% CI: 98.22-100%) was also demonstrated by assaying 205 patient-specific panels (representing 12 distinct tumor types) on unrelated healthy donors who are expect to display no signal at any target loci (Table).

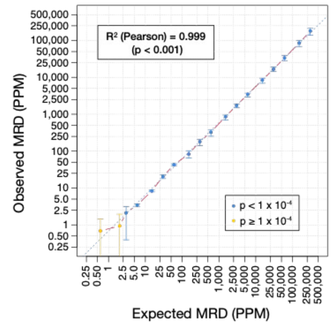

Figure 2: MRD Detection Sensitivity.

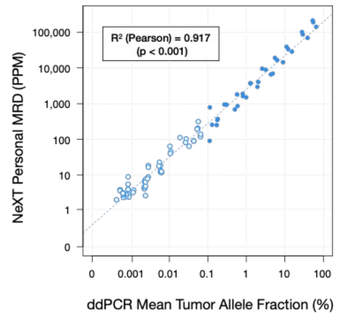

Figure 3: ddPCR Confirmation of MRD Signals.

Number of Patient-Specific Panels	True Negative MRD Detections	False Positive MRD Detections	Specificity
205	205	0	100% (95% CI: 98.22-100%)

Analytical Performance of Genomic Variant Tracking

The NeXT Personal assay reports individual genomic variant detection with VAF >= 0.1% (recommended p-value threshold < 1 x 10^{-6}). High detection PPV, NPV, sensitivity, specificity and overall accuracy were observed in all VAF ranges, with high measurement precision shown by observed / expected VAF ratios (Fig. 4). In addition, VAF measurement precision and signal linearity was orthogonally confirmed by ddPCR for 17 clinical variants (Fig. 5), showing 100% agreement (Cohen's κ = 1.00).

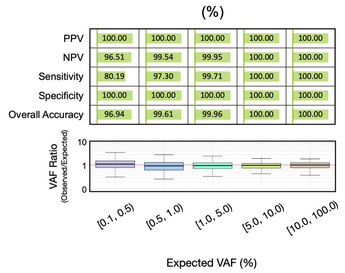

Figure 4: Variant Detection Performance.

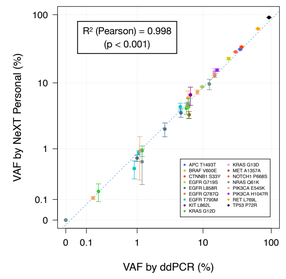

Figure 5: ddPCR Confirmation of VAF Calls.

Clinical Applications

A retrospective analysis was undertaken in an advanced liver cancer (low TMB) cohort of 11 patients undergoing immunotherapy. ctDNA levels in longitudinal plasma samples were measured with NeXT Personal. Our results (Fig. 6) demonstrated that changes in ctDNA levels during therapy correlated highly with disease status [patients with progressive disease (PD) vs. patients with disease control i.e. partial response (PR), complete response (CR) or stable disease (SD): 3wk to baseline % change, Wilcoxon signed rank test (one-sided) p = 0.026; 6wk to baseline % change, p = 0.011; 9wk to baseline % change, p = 0.0022], and were detected prior to clinical response confirmation by RECIST 1.1.

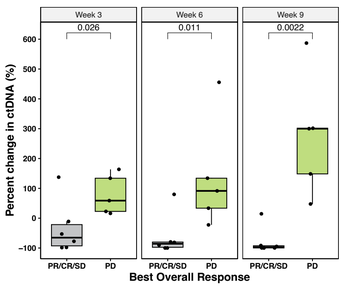

Figure 6: ctDNA Change Observed During Immunotherapy.

CONCLUSION

In summary, we present the analytical performance of NeXT Personal, a highly sensitive and specific personalized liquid biopsy assay for MRD and individual clinical and investigational mutation detection and monitoring. With verified LOD of 1 ~ 3 PPM for MRD and 0.1% VAF for individual mutations, our assay is sufficiently sensitive for residual or recurrent cancer detection in even challenging situations such as early stage, low shedding, or low TMB cancers, at earliest possible time points with low input cell-free DNA amount required. Moreover, the demonstrated high assay specificity and signal linearity ensures that the detected signals are true and accurate measurements of the residual disease. Mutation level information from 90 clinical genes also helps identify actionable targets and informs therapy selection and therapy update. The assay benefits from both tumor-informed and tumor-agnostic genomic information, making NeXT Personal unique in its capability to detect and monitor MRD while informing and guiding clinical decisions.