A high sensitivity, tumor-informed liquid biopsy platform, designed to detect minimal residual disease at part per million resolution

#5163

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Introduction

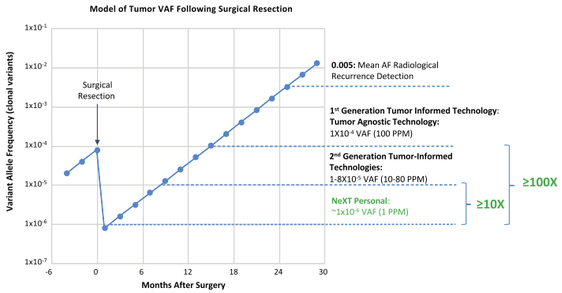
While circulating tumor-derived DNA (ctDNA) is an emerging biomarker for many cancers, the limited sensitivity of current detection methods reduces its utility for diagnosing MRD across a variety of clinical applications. Standard-of-care (SOC) radiological-based technologies, including CT, PET and MRI scans, also remain limited in their ability to detect residual disease during or after surgical or systemic therapy due to the minimum tumor volume required. NeXT Personal™, an advanced, personalized, and tumor-informed liquid biopsy assay, is designed to detect molecular residual disease (MRD) and cancer recurrence at the earliest timepoints — prior to, during or after treatment — in patients previously diagnosed with cancer.

Methods

NeXT Personal leverages tumor/normal whole genome sequencing (WGS) to design personalized, targeted MRD liquid biopsy panels for each patient. The MRD portion of the panel is composed of up to 1800 somatic tumor variants, enabling higher sensitivity MRD detection in plasma through tracking of high quality and lower noise variants. This allows the platform to achieve high sensitivity across cancer types and stages, including early-stage cancers and low mutational burden tumors, requiring only a single tube of blood (4mL plasma/15ng cfDNA), and 1mm^3 of FFPE tumor tissue.

Results

Delivering Industry-Leading Sensitivity to Detect Residual Disease at the Earliest Timepoints



Model Assumptions: Median breast tumor size detected by mammography: 1.3cm, Median shedding per NSCLC TRACERx study; Residual tumor from surgery: 1%; Volume doubling time (actual VAF time): 2 months

Figure 1: MRD Sensitivity: Targeting detection at the earliest timepoints

Tumor-informed liquid biopsy approaches have proven promising for detecting MRD and recurrence of cancer following surgical resection or other therapy. However, current liquid biopsy MRD assays typically detect ctDNA in a range above 30 to 300 PPM, leaving a significant fraction of MRD cases undetected, particularly soon after surgery and in early stage cancers where ctDNA can be at very low levels. To address this, we have developed NeXT Personal™, a tumor-informed liquid biopsy assay that reproducibly achieves sensitivity down 1-3 PPM, therefore enabling earlier detection of MRD and recurrence.

References:
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4. Phylogenic tracking and minimal residual disease detection using ctDN A in early-stage NSCLC: A lung TRACERx study. Abbosh et al., AACR 2020, Abstract CT023
5. Analytical development of the ReDaB™ assay, a highly sensitive and specific assay for the monitoring of minimal residual disease. Marsico et al., AACR 2020, Poster 309
6. Phylogenic ctDNA analysis depicts early-stage lung cancer evolution. Abbosh et al., Nature, 2017
7. Detection of circulating tumor DNA in early- and late-stage human malignancies. Bettegowda et al., Science Translational Medicine, 2014

Delivering Industry-Leading Sensitivity to Detect Residual Disease at the Earliest Timepoints

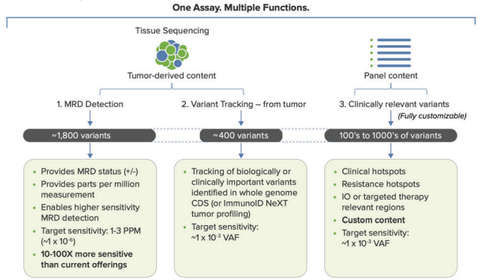


Figure 2: NeXT Personal Assay Features: Advanced, higher sensitivity, personalized, tumor-informed liquid biopsy for MRD and variant tracking

The MRD component of NeXT Personal leverages up to 1,800 WGS tumor derived somatic variants to provide a highly sensitive and aggregated measurement of tumor burden in plasma. Additionally, the ability to simultaneously track individual variants longitudinally can also be utilized to further our understanding of tumor biology and its dynamic response to therapy. NeXT Personal provides the ability to track and annotate individual variants over the whole evolution of a cancer patient's trajectory in a single panel design. Variants tracked by NeXT Personal in the blood are derived from those detected in the tissue, a Personalis-curated list of guideline-driven and resistance mutations, as well as those specified for panel inclusion by the user.

NeXT Personal Leverages up to 1,800 WGS Derived Somatic Variants to Achieve MRD Sensitivity Down to 1 PPM

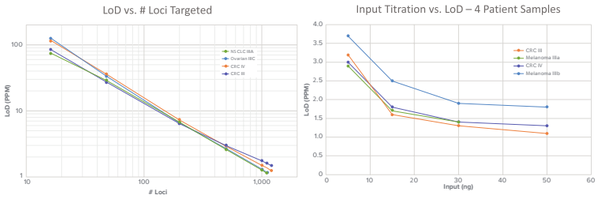


Figure 3: NeXT Personal's expansive set of patient-specific WGS derived variants greatly improves MRD LOD on a platform designed to profile variants using only 5-50 ng of input material

Central to the MRD performance of NeXT Personal is the number of tumor-derived mutations interrogated in patient plasma samples. Analytical and clinical MRD sensitivity can be increased by expanding coverage of loci containing tumor-specific mutations. Here, through an in-silico analysis, we highlight performance benefits that can be achieved by leveraging large numbers of high-quality somatic variants for MRD detection. Additionally, the platform performance is consistent using 15-50 ng of input material, which allows our platform to be applied with only a single tube of blood across a range of tumor types and stages.

NeXT Personal Yields Best-In-Class MRD Performance in Plasma, to a Lower Limit of 1-2 PPM

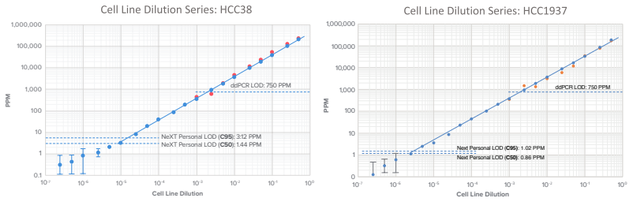


Figure 4: Cell line dilution series, HCC38, HCC1143, and HCC1937, demonstrates the linearity of tumor signal across dilution series down to the 1-3 PPM

We applied NeXT Personal to track MRD in a set of three well-characterized tumor-normal cell line systems. For each cell line, we serially diluted tumor cell line DNA with paired normal DNA, applying NeXT Personal to determine MRD signal (in PPM). Our platform reproducibly achieves an MRD limit of detection (LOD) of between 1 and 3 PPM. Furthermore, applying ddPCR, we corroborate our findings down to the detection limit of that technology.

NeXT Personal Effectively Tracks MRD Signal in Serially Diluted Patient Samples

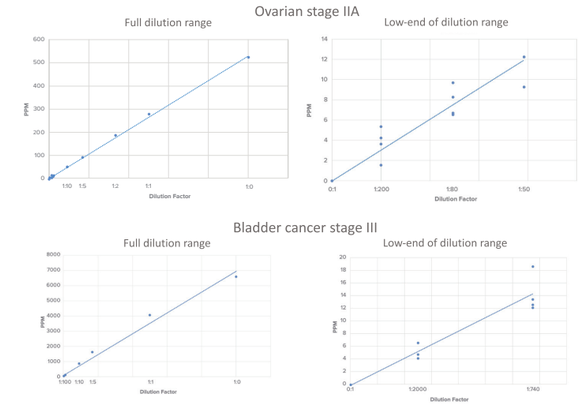
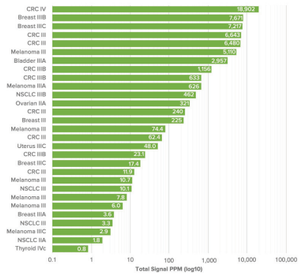


Figure 5: NeXT Personal characterizes MRD LOD in a set of serially diluted patient samples

By serially diluting paired tumor-normal-plasma patient samples, we were able to reproducibly and systematically demonstrate NeXT Personal's ability to track variants down to the 1-3 PPM range on real patient samples. Here we demonstrate the relationship between dilution factor and detected PPM using both ovarian and an bladder cancer patient samples.

Applying NeXT Personal to Profile MRD For a Broad Set of Cancer Types and Stages



NeXT Personal identified a range of positive PPM detection signals from a diverse set of cancer types and stages. Others have reported that PPM levels generally increase with cancer stage but there is a wide range, leading to heavy overlap across stages. Our results are consistent with this, here stage III melanoma, as an example, ranges from 2.9 to 5110 PPM. It is also notable that our lowest PPM detection of .8 PPM is for a stage IV tumor. The biology of tumor shedding is uncertain but it may be that it is an episodic process. If so, this places an added premium on sensitivity so as to detect the cancer even when shedding is at a low point.

Figure 6: Patient T/N/P trios across 8 tumor types and 3 stages were sequenced demonstrating range of detection; 0.8- ~19,000 PPM

NeXT Personal Demonstrates 100% MRD Specificity When Tested on Healthy Donor Samples

Number of Healthy Donors	True Negative MRD Detections	False Positive MRD Detections	Specificity
40 Unique Individuals	40	0	100%

Figure 7: NeXT Personal detected no positive MRD detections when applied on healthy normal donors

We applied cancer patient panels to profile MRD in 40 healthy donor samples, NeXT Personal reported no false positive signals. Rather than using a predetermined minimum number of mutations to define MRD status, NeXT Personal uses advanced, proprietary statistical analyses to distinguish signal from noise for each individual patient panel and sample. Guided by a P-value threshold derived from a specificity target of >99.9%, NeXT Personal is optimized for both sensitivity and specificity.

Conclusion

NeXT Personal achieved highly sensitive and specific MRD detection, reproducibly demonstrating a LOD down to 1 PPM in different cancer types and cell line dilutions, representing approximately 10 to 100 times higher sensitivity than other liquid biopsy MRD approaches. The high sensitivity of NeXT Personal potentially enables MRD detection across a broad variety of cancers and stages, including typically challenging early stage, low mutational burden, and low-shedding cancers.

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