NeXT Personal®
Specificity study

UNCOMPROMISED MRD PERFORMANCE

NeXT Personal® delivers 1-3 PPM analytical sensitivity and 99.98% analytical specificity

Introduction

The promise of circulating tumor DNA (ctDNA) as a biomarker for disease prognosis, therapy response, and disease recurrence has been hampered by low sensitivity and poor specificity of the available assays. Multiple factors (tumor type, stage, and ctDNA shedding kinetics) influence the level of ctDNA present in the blood. Therefore ultra-high sensitivity is required to detect circulating tumor molecules at the biologically relevant levels. To be truly useful, an assay must achieve both ultra-high sensitivity and ultra-high specificity. High sensitivity allows for earlier and more robust detection; high specificity ensures that the detected signal corresponds to the tumor rather than biological or assay noise. Lack of specificity can lead to erroneous cancer recurrence diagnoses and therefore unnecessary clinical interventions. Even when considering MRD at a single time point, for example when evaluating residual disease after surgical resection, a false positive can lead to improper risk assessment and over-treatment. This becomes even more impactful when monitoring patients over time (Fig. 1). Therefore, **when developing a diagnostic tool for clinical use, it is essential to prioritize specificity alongside sensitivity, ensuring accurate and reliable results.**

Effect of assay specificity on false positive rate

Figure 1. Impact of false positive rate over 10 timepoints

NeXT Personal ensures exceptionally low false-positive ctDNA detections.

NeXT Personal is an ultra-sensitive, tumor-informed liquid biopsy assay designed to detect molecular residual disease (MRD) at the earliest timepoints, with detection levels as low as 1 part per million (PPM). Here, we describe results demonstrating high analytical specificity and repeatability in an MRD assay with ultra-high analytical sensitivity.

Empirical study

Evaluation of analytical specificity: NeXT Personal detects MRD based on signal aggregation across up to 1,800 tumor-specific somatic variants identified with Tumor-Normal whole genome sequencing (WGS). To evaluate the analytical specificity of MRD detection, we used 205 cancer patient-specific panels and measured tumor signal in plasma-derived cell-free DNA (cfDNA) from healthy donors (Fig. 2). Healthy donors should not yield tumor signal using patient-specific cancer panels.

Repeatability measurement: We used 6 of the cancer patient panels to sequence the cfDNA of 28 to 30 different healthy donors.

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Figure 2. Schematic of study workflow

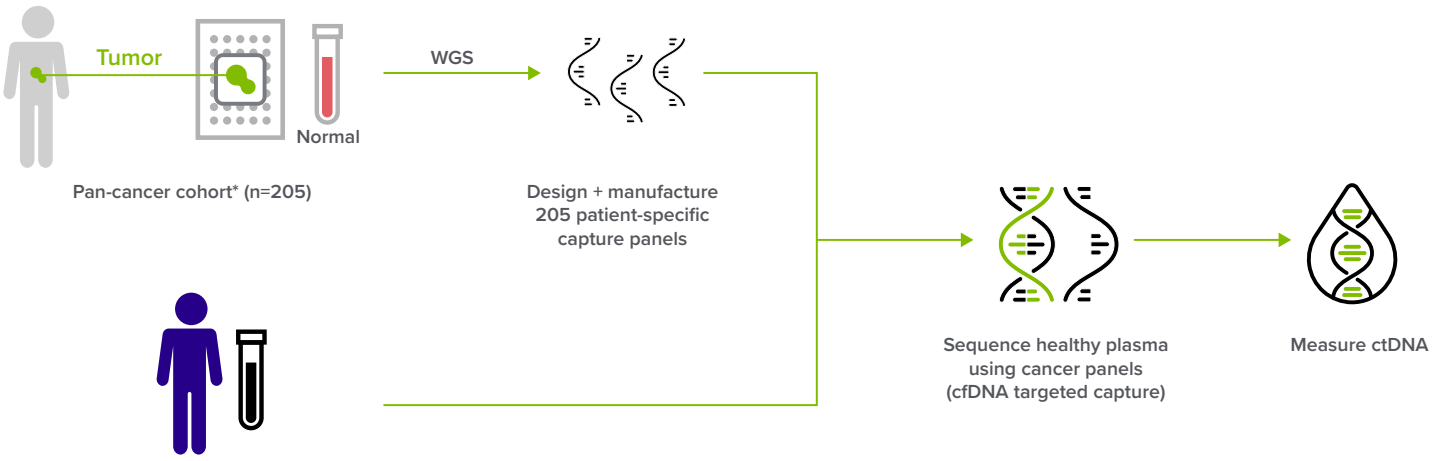


*Fig. 3; **Fig. 4

Figure 3. Cancer patient metadata

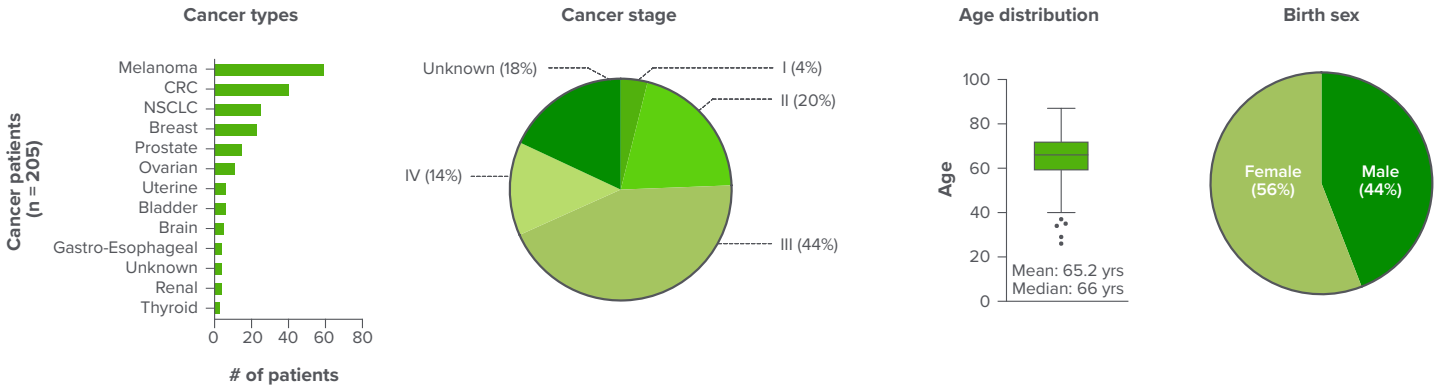
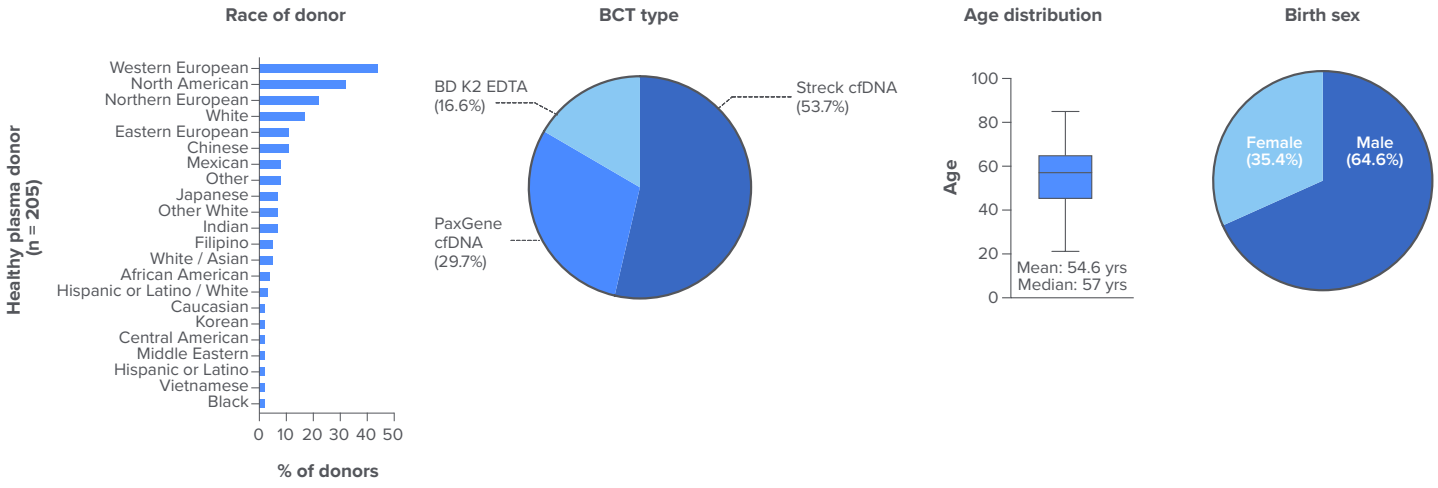


Figure 4. Healthy donor metadata



Results

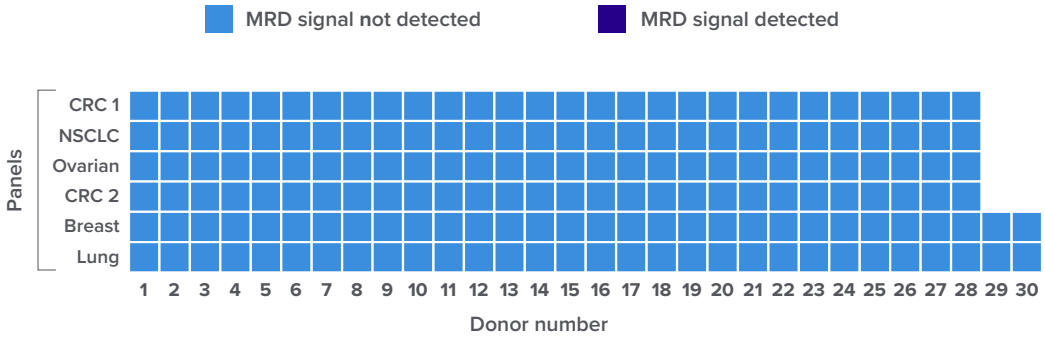
100% specificity observed across 205 cancer panels / healthy donors.

The pan-cancer cohort was composed of predominantly stage II–IV patients from 12 distinct tumor types. Across all patient panels and healthy donors, **no MRD was detected (100% specificity)** (Fig. 5). In addition to the 100% specificity observed, the median detection threshold was 2.6 PPM, with the lowest achieved detection threshold measuring 1.4 PPM, demonstrating high analytical sensitivity. No correlation was observed between noise and tumor type or healthy plasma donor age (data not shown). Furthermore, we observed 100% repeatability across six cancer patient panels that were each used to sequence at least 28 unique plasma donors, with MRD not being detected in any of the replicates (Fig. 6).

Figure 5. Specificity

Of the 205 unique NeXT Personal cancer panels and healthy donor plasma-derived cfDNA pairings, no MRD was detected, resulting in 100% specificity (CI: 98.22-100%).

Figure 6. Repeatability



6 patient-specific panels were used to sequence at least 28 unique healthy donor plasma samples. No signal was observed across all panel/healthy donor combinations, demonstrating 100% repeatability in the experiment.

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Verification of specificity with an alternative design analysis

99.98% specificity observed using an alternative design analysis across 40,600 synthetic panels

Creation of 40,600 synthetic panels: To significantly increase the sample size, we created over 40 thousand synthetic panels as described by Abbosh et al[1], and adapted for platform differences. Briefly, we used random sampling with replacement in order to substitute targeted variants with trinucleotide context-matched high-quality bases (covered by sequencing reads with comparable depth, passing all NeXT Personal quality filters and not present in dbSNP Build 146) present within +/- 50 bp of an actual target.

MRD specificity evaluation: We used sequencing data covered by each patient-specific or synthetic panel. As no tumor signals should be expected at these positions in the healthy donor samples, any positive signal that passed NeXT Personal's MRD detection threshold (p-value < 1×10^{-3}) would be considered as a false positive.

Results

The alternative design analysis yielded an observed specificity of 99.98% (CI: 99.96-99.99%).

Figure 7. Extrapolation using an alternative design analysis demonstrates ultra–specificity of NeXT Personal MRD assay

Specificity analysis	# Panels	# False positives	Observed specificity	Lower 95% CI	Upper 95% CI
Empirical	205	0	100%	98.22%	100%
Alternative design analysis	40,600	7	99.98%	99.96%	99.99%

Discussion & conclusions

MRD false positives may lead to unnecessary treatment in the clinical setting. Therefore, it is imperative to develop an MRD assay that maintains high analytical specificity to deliver reliable data. These studies demonstrate that NeXT Personal is a robust MRD assay that consistently delivers analytical specificity close to 100% (100% empirically, 99.98% in the alternate design method, and 100% repeatability), while maintaining ultra-high sensitivity (median 2.6 PPM LOD). Such high performance may ultimately become critical when making important decisions on which MRD assay to use in clinical studies, and ultimately patient care.

1. Abbosh, C., Frankell, A.M., Harrison, T. et al. Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. Nature 616, 553–562 (2023). https://doi.org/10.1038/s41586-023-05776-4

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