Analytic validation of an ultra-sensitive tumor-informed circulating tumor DNA assay based on whole genome sequencing

#5034

Josette Northcott[1], Gabor Bartha[1], Jason Harris[1], Conan Li[1], Fabio C.P. Navarro[1], Rachel Marty Pyke[1], Manqing Hong[1], Qi Zhang[1], Shuyuan Ma[1], Tina X. Chen[1], Janet Lai[1], Nitin Udar[1], Juan-Sebastian Saldivar[1], Erin Ayash[1], Massimo Mora[1], Joshua Anderson[1], Jiang Li[1], Tiange Cui[1], Tu Le[1], Ruthie Chow[1], Randy Jerel Velasco[1], Chris Mallo[1], Rose Santiago[1], Robert C. Bruce[1], Laurie J. Goodman[1], Yi Chen[1], Dan Norton[1], Richard O. Chen[1], and John M. Lyle[1]

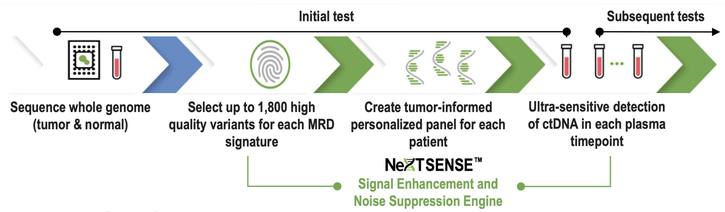
Contact: john.lyle@personalis.com

1 Personalis, Inc. | 6600 Dumbarton Circle, Fremont, CA 94555, USA

BACKGROUND

Circulating tumor DNA (ctDNA) is a powerful noninvasive tool for detecting residual disease, monitoring for recurrence, or tracking therapy response. Detection of ctDNA raises the possibility of escalating treatment for patients earlier, with the goal of improving outcomes. The absence of ctDNA potentially allows for the de-escalation of treatment, thereby avoiding unnecessary toxicity and cost to the patient.

We describe the analytical validation of **NeXT Personal®**, an ultra-sensitive, tumor-informed ctDNA assay for use in patients diagnosed with solid tumor cancers. NeXT Personal uses whole genome sequencing (WGS) of tumor and matched normal samples combined with NeXT SENSE™ advanced analytics (shown below) to accurately identify up to ~1,800 somatic variants specific to the patient's tumor. A personalized panel is created, targeting these variants, and then used to sequence cell-free DNA extracted from patient plasma samples, allowing ultra-sensitive detection of ctDNA.



METHODS

WGS and panel design

DNA libraries were prepared from tumor and matched normal samples from two cell lines, and from 118 patients across nine cancer types. WGS was performed on NovaSeq instruments (Illumina) to a depth of at least 30x. Somatic variant calling, ctDNA target selection, and probe design were completed using the algorithms of the NeXT Personal platform. Up to ~1,800 targets are selected genome wide from the high-quality and low-noise somatic variants for inclusion in the personalized panel.

Cell-free DNA samples

The Seraseq ctDNA MRD Panel Mix (LGC SeraCare) was used for the accuracy study. HCC1954/ HCC1954BL patient-matched cell lines were used to create serially diluted ctDNA for the analytical range measurements; detection threshold, LOD, precision, linearity, and LOQ studies. Except as noted, all pre-enrichment libraries prepared from these sources were created with 15 ng of cfDNA. Normal plasma was isolated from blood collected in Streck Cell-Free DNA BCTs from 131 healthy donors (Stanford Blood Center) for the LOB and specificity studies. Patient plasma from 118 donors was sourced from multiple vendors (BOCA Biolistics, BioOptions, Cureline, Discovery Life Sciences, DxBioSamples, and Us4Cure) and used to assess sample processing success rate only. Clinical sample pre-enrichment libraries were prepared with 2 to 30 ng of cfDNA.

Enrichment, sequencing and MRD analysis

NeXT Personal panels were used to enrich up to 1500 ng of library DNA using a hybridization-capture approach. The post-enrichment libraries were sequenced deeply on NovaSeq instruments to optimize the number of unique molecules observed. Following noise suppression via consensus read formation, the individual ctDNA signals were aggregated across the targets in each panel to obtain the total ctDNA signal in PPM. A ctDNA positive call is made when the p-value < 0.001 (false positive rate < 1 in 1,000).

Specificity evaluation using simulated panels

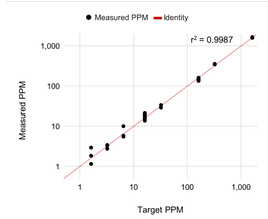
First, 23,600 in silico panels were generated by replacing the target variants in the clinical panels with random bases from within the 50 bp flanking the target (Abbosh et al. 2023). Second, 23,600 in silico panels were generated by re-shuffling the targets used across the clinical panels into new combinations. Both approaches maintained the size and trinucleotide error context of each panel. Simulated panels were used to analyze the specificity study data for false positive calls.

RESULTS

Accuracy & specificity

Quantitative accuracy. Samples of known concentration were prepared through serial dilution of the Seraseq reference into its matched normal control and then measured with NeXT Personal.

Qualitative accuracy. Positive samples: Seraseq 0.05% and 0.005% ctDNA controls (20 each). Negative samples: Seraseq 0% ctDNA control (3), normal cell line cfDNA (46), and healthy donor cfDNA (239). A total of 120 different panels were used. All positive and negative control samples were correctly classified by NeXT Personal.



		Sample Values	
		Positive	Negative
NeXT Personal	Positive	40	0
	Negative	0	288

Specificity. 118 healthy donor cfDNA samples were enriched with a different, unrelated patient panel to detect the presence of targeted tumor variants. All samples were correctly classified "Not Detected" by NeXT Personal. Two in silico approaches, each using 23,600 simulated panels, resulted in 4 and 31 false positives.

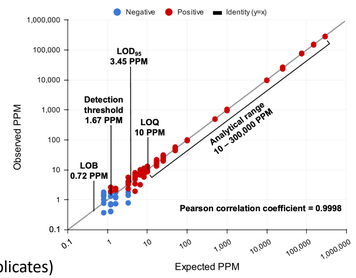
Approach	Panels	Specificity (95% CI)
Empirical measure	118	100% (96.8%, 100%)
In silico flanking	23,600	99.98% (99.96%, 100%)
In silico reshuffling	23,600	99.95% (99.92%, 99.98%)

Analytical range measurements

A NeXT Personal panel was created from WGS of tumor and normal cell line gDNA. The panel variants were verified to ensure that the SNV substitutions and allele frequencies closely mimicked a typical clinical panel. This panel was used on a contrived sample set created from cell line cfDNA, as well as on a set of healthy donor normal cfDNA samples. To capture assay variability, cfDNA samples in each study were run by 2 operators over ≥2 days using two different enrichment kit reagent lots.

Limit of blank (LOB). 121 donor normal cfDNA samples and 46 normal cell line cfDNA samples. The higher value of the two reagent lots was taken as the LOB.

	Enrichment Reagents	
	Lot A	Lot B
Upper 95th percentile of signal on blank specimens	0.493 PPM	**0.719 PPM**



Detection threshold. NeXT Personal computes the ctDNA signal detection threshold for each plasma sample. For the 190 measurements performed in the analytical range measurement studies, the detection threshold ranged from 1.47 to 1.87 PPM (mean 1.67 PPM).

Limit of detection (LOD_{95}). 70 cfDNA samples (7 dilutions x 10 replicates) with known ctDNA concentrations ranging from 0.8 to 8.2 PPM were analyzed using the precision profile approach. The highest value of the two reagent lots was taken as the LOD_{95} determination.

	Enrichment Reagents	
	Lot A	Lot B
LOD (PPM)	1.80	**3.45**

Limit of Quantification. ctDNA samples at concentrations of 6.6, 8.2, 10, 17, 25 and 50 PPM were assayed 10 times. The limit of quantification is set as the lowest ctDNA concentration for which total error is <25% for both reagent lots.

Precision. 24 cfDNA samples were run at ctDNA concentrations selected as representative of low (25 PPM), medium (1,000 PPM), and high (25,000 PPM) ctDNA signals in clinical samples. The overall precision was calculated as the coefficient of variation (CV). The total allowable error for measurements in this study (25%) was set by the upper 95% confidence interval of the highest CV value.

			Enrichment Reagents Lot A, LotB	
Target PPM	Bias between measured and target PPM	SD of measured values (PPM)	Total Error in PPM (root mean square)	Total Error, %
50	0.87, -2	3.77, 3.78	3.86, 4.27	7.73%, 8.55%
25	0.64, 1.35	3.05, 2.83	3.11, 3.13	12.5%, 12.5%
17	1.78, -0.31	2.13, 2.35	2.78, 2.37	16.4%, 13.9%
10	0.74, 0.26	1.1, 1.73	1.33, 1.75	13.3%, 17.5%
8.2	1.13, -0.23	2.07, 0.99	2.36, 1.02	28.8%, 12.5%
6.6	-0.13, 1.56	1.93, 0.88	1.93, 1.79	29.3%, 27.1%

Sample	Total runs	Mean conc., measured (PPM)	SD (PPM)	CV (%)
High conc. (25,000 PPM)	24	25,017	1,038.2	4.15%
Medium conc. (1,000 PPM)	24	991.0	35.18	3.55%
Low conc. (25 PPM)	24	26.02	3.323	12.77%

Linearity. Determined using a minimum of 3 replicate samples at each of 19 different ctDNA concentrations ranging from 0.8 to 300,000 PPM. A regression fit yielded a Pearson correlation coefficient of 0.9998 (p<0.001).

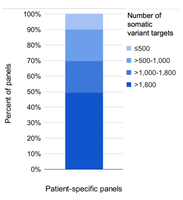
Clinical panels and contrived sample characterization

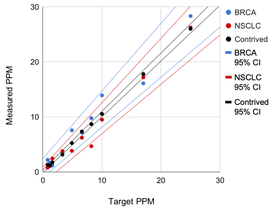
Clinical panels. 118 clinical sample sets were assayed, representing nine different cancer types. Nearly half of the patient-specific panels had >1,800 somatic variant targets. The median detection threshold of the panels was 2.0 PPM (range 1.0 - 12 PPM).

Contrived Sample Functional Characterization Study. Measured versus target ctDNA concentration of contrived samples and 2 ctDNA positive clinical samples (BRCA and NSCLC). Patient cfDNA was serially diluted with donor normal cfDNA to ctDNA concentrations from 1.2 to 25 PPM. The 95% confidence intervals (CI) are shown as lines. There was no significant difference between the 95% CI of the slopes and intercepts for both clinical and the contrived sample, therefore the use of cell line samples in the validation study is appropriate.

	Stage				Type
Type	I	II	III	IV	Total
Lung	4	10	6	1	28
Colorectal	5*	7	12	0	24
Bladder	3	7	8	4	22
Breast	9	12	6	1	21
Melanoma	1	3	5	0	9
Prostate	0	3	1	2	6
Ovarian	0	1	2	0	3
Renal	0	0	2	1	3
Stomach	0	0	2	0	2
Stage Total	22	43	44	9	**118**

**Two specimen sets were found to be from the same patient.*





SUMMARY

Specifications and analytical validation performance

Metric	Description	Measured performance
Panel size	Number of tumor-specific targets	Up to ~1,800 somatic variants
Accuracy		
Quantitative accuracy	Agreement between measured and target value	1.15 to 1,617 PPM (r^2 = 0.9987)
Sensitivity	Rate of true positive detections of known positive samples	100% (98.5%–100%)
Positive predictive value	Rate of true positive detections of total positive detections	100% (CI 96.0%–100%)
Negative predictive value	Rate of true negative detections of total negative detections	100% (CI 98.5%–100%)
Specificity	Rate of negative calls on normal samples	100% (CI 99.92%–100%)
Analytical range measurements		
Detection Threshold*	Signal threshold for making a positive call	1.67 PPM
Limit of Blank*	Upper 95th percentile of signal on blank specimens	0.719 PPM
Limit of Detection*	Lowest concentration detectable in 95% of replicates	3.45 PPM
Precision	Coefficient of variation	4.15% (25,000 PPM) 3.55% (1,000 PPM) 12.8% (25 PPM)
Linearity	Range 0.8 to 300,000 PPM	Pearson correlation, r = 0.9998
Limit of Quantification	Lowest concentration measured with total error <25%	10 PPM
Clinical sample processing		
Processing success rate	Rate of success across 9 tumor types, stages I–IV	99.1%
Effect of interfering substances		
Genomic DNA (≥1.5 kb)	Consequence of leukocyte lysis in collected blood sample	Robust up to 25%
Cell-free DNA input amount		
Sample input quantity	Input range of clinical samples returning results within the defined total allowable error	2 to 30 ng

**Representative panel, performance of individual panels may vary.*

CONCLUSION

The **NeXT Personal** tumor-informed ctDNA detection approach, combining WGS with NeXT SENSE, results in an ultra-sensitive and highly specific quantitative diagnostic assay. The high level of sensitivity and specificity of NeXT Personal has the potential to improve both lead times to recurrence detection and detection of molecular residual disease. The quantitative aspect of the assay is a differentiating feature which can enable more robust disease and treatment monitoring. These results suggest strong potential for clinical use of the assay in ctDNA monitoring of solid tumor cancers.

Reference

Northcott, et al. "Analytical validation of NeXT Personal®, an ultra-sensitive personalized circulating tumor DNA assay." Oncotarget (2024) 15: 200-218. Web. 26 Mar. 2024