An exome and transcriptome based NeXT Dx™ test enables therapy selection for cancer patients and offers insight into emerging composite biomarkers for immunotherapy

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Introduction

The emergence of immune checkpoint inhibitors has highlighted the potential of immuno-oncology therapeutics to produce unprecedented beneficial responses in cancer patients. However, diagnostic biomarkers that consistently predict patient response to immunotherapies have remained elusive in spite of increased use of these treatments.

There is an unmet need for the development of integrative, composite biomarkers that can model the complex biology driving response and/or resistance to immunotherapy more effectively than existing single-analyte approaches. However, current cancer diagnostic panels, with their focus on a small set of genes, have limited scope and variability in Tumor Mutational Burden (TMB) assessments for certain cancer types, and limited utility to support emerging composite biomarkers that can predict the immune response. To address these limitations, we developed and validated NeXT Dx™, a comprehensive whole-exome and transcriptome based diagnostic test designed to simultaneously characterize tumor and immune microenvironment from a single limited FFPE sample.

Methods

The NeXT Dx test is built upon the Personalis NeXT Platform™, which is an augmented exome/transcriptome based platform that improves uniformity of coverage across all ~20,000 genes, including boosted coverage of 247 cancer-related genes (Figure 1). This boosted coverage enables the generation of the NeXT Dx test, a laboratory developed test (LDT) that detects single nucleotide variants, small insertions and deletions, copy number alterations (CNAs) and gene fusions in 247 cancer-related genes (Table 1). The exome-wide footprint enables accurate tumor mutational burden (TMB) assessment and microsatellite instability (MSI) detection.

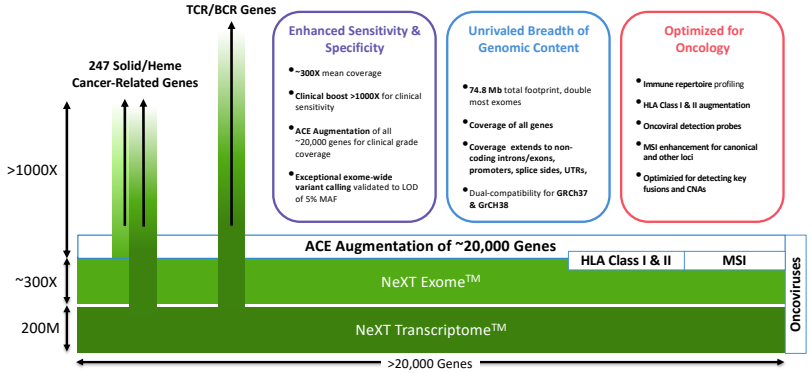

Figure 1. Personalis NeXT Platform™: A Comprehensive Solution for Precision Medicine

Table 1: NeXT Dx Gene List

ABL1*	BCOR	CDK4*	DDR2*	FANCD2*	FOLR1	KDM6A*	MKL1	NOTCH2	PMS2*	RET*	SRSF2	VHL*
AKAP9	BCORL1	CDK6*	DEK	FANCE*	FOXL2	KDR*	MLH1*	NPM1*	POLE*	RICTOR*	STAG2	WEE1
AKT1*	BCR*	CDK9	DKK1	FANCF*	FYN	KIT*	MLLT3	NRAS*	PRAME	ROS1*	STAT3*	WT1*
AKT2*	BRAF*	CDKN1A*	DLL3	FANCG*	GATA1	KLB	MPL	NTRK1*	PRKACA	RPN1	STAT5B*	XPO1
AKT3*	BRCA1*	CDKN1B*	DNMT3A	FANCI*	GATA2	KMT2A	MRE11A*	NTRK2	PSCA	RUNX1*	STK11*	XRCC1*
ALK*	BRCA2*	CDKN2A*	EGFR*	FANCL*	GNA11	KRAS*	MS4A1	NTRK3	PTCH1*	RUNX1T1	SULT1A1	YES1*
APC*	BRIP1*	CDKN2B*	EML4	FANCM*	GNAQ	LAG3	MSH2*	NUP214	PTEN*	SDHB	SYK	ZRSR2
AR*	BTK	CEBPA*	EP300	FBXW7*	GNAS	MAGEA3	MSH6*	PALB2*	PTK2	SDHC	TERT*	
ARAF	CALR	CHEK1*	EPCAM	FCER2	GPNMB	MAGEA4	MSLN	PARP1	PTPN11	SDHD	TET2*	
AREG	CBFB	CHEK2*	ERBB2*	FGF19*	HNF1A	MAP2K1	MTOR	PDCD1	PVRL4	SETBP1	TGFBR1	
ARID1A*	CBL*	CREBBP	ERBB3*	FGF2*	HRAS*	MAP2K2	MUTYH*	PDCD1LG2*	RAD21*	SF3B1	TGFBR2	
ASXL1*	CCND1*	CRKL*	ERBB4	FGFR1*	HSP90AA1	MAP2K4*	MYC*	PDGFRA*	RAD50*	SHH	TMPRSS2	
ATM*	CCND2*	CRLF2	ESR1*	FGFR2*	IDH1	MAP3K1*	MYCN*	PDGFRB	RAD51*	SLX4*	TNFRSF4	
ATR*	CCND3*	CRTC1	ESR2	FGFR3*	IDH2	MAPK1*	MYD88	PGR	RAD51B*	SMAD4*	TNFRSF8	
ATRX*	CCNE1*	CSF1R	ETV6	FGFR4*	IGF1R	MCL1*	MYH11	PIK3CA*	RAD51C*	SMARCA4*	TP53*	
AURKA*	CD274*	CSF3R	EWSR1	FH	IKZF1*	MDM2*	NF1*	PIK3CB*	RAD51D*	SMARCB1*	TSC1*	
AXL*	CD276	CTAG2	EZH2*	FLCN	IL2RA	MDM4*	NF2*	PIK3CD	RAF1*	SMC1A	TSC2*	
BAP1*	CD40	CTLA4	FANCA*	FLT1*	JAK1	MECOM	NFE2L2	PIK3CG	RARA	SMC3	U2AF1	
BCL2*	CDH1	CTNNB1	FANCB*	FLT3*	JAK2*	MEN1*	NKX2-1*	PIK3R1*	RB1*	SMO*	VEGFA	
BCL6*	CDH3	CUX1*	FANCC*	FLT4*	JAK3	MET*	NOTCH1	PML	RBM15	SRC*	VEGFB	

Genes for which copy number alteration is assessed are indicated by an asterisk ("*").

The clinical report includes mutations from these cancer-related genes as well as important diagnostic markers for targeted therapy and/or immunotherapy selection, clinical trial matching, and prognostic prediction.

We validated the NeXT Dx test using tumor-derived cell-lines (HCC1187, HCC1395, NCI-H2126), constructs from Horizon Discovery and SeraCare®, clinical FFPE samples, and proficiency testing samples (Table 2). TMB was calculated using the gold standard approach based on exome-wide data from non-synonymous variants (SNVs and indels). The test requires >4 FFPE slides to co-extract DNA and RNA that were sequenced using Illumina NovaSeq instruments at our CAP-accredited, CLIA-certified laboratory. Additional assay enhancements for HLA, immune repertoire, and oncoviruses were designed to further optimize the platform for immunotherapy biomarker discovery applications.

Table 2: NeXT Dx Validation Plan Summary

Variants Validated	Validation Samples	Source of Validation	# of Variants for Validation
SNV and Indels	• Three tumor cell lines • Six commercial constructs • 19 CAP proficiency samples • 43 clinical samples (FFPE tissue from 12 different tumor types)	Tumor DNA	755 SNV events 81 indel events
Copy Number Alterations	• Two cell lines • Two commercial constructs • 19 clinical samples	Tumor DNA	36 CNA events
Gene Fusions	• Two fusion constructs • 16 clinical samples (FFPE)	Tumor RNA	39 fusion events
MSI	48 specimens orthogonally tested for MSI status	Tumor DNA	Five canonical loci: BAT25, BAT26, NR-21, NR-24, NR-27
TMB	All samples utilized for small variant, CNA, and MSI detection assessments	Tumor DNA	Reported as mutations per megabase

Results

Specimens evaluated for small variants and fusions met the ≥20% tumor content requirement, while those evaluated for CNAs met the ≥30% tumor content requirement. Typical median coverage depth was >1,000X for 247 cancer-related genes, and ~300X for the remaining (whole exome) footprint. Experiments were performed for SNVs, indels, CNAs, and fusions independently, with analytical sensitivity and specificity evaluated for each variant class. Performance characteristics were established using commercially-available, pre-characterized control materials as well as clinical FFPE samples and the results were compared with the previously-validated ACE CancerPlus® Test. TMB was calculated automatically and manually by exome-wide analysis of non-synonymous somatic mutations identified in the coding region. The TMB is the sum of detected small variants divided by the total coding footprint or 34.86Mb.

Validation of NeXT Dx demonstrated overall >99% specificity for all variant types and 99.5% sensitivity for SNVs at >= 5% AF; 98.7% sensitivity for indels at >= 10% AF; 97.2% sensitivity for CNAs in samples with >= 30% tumor content for 247 genes footprint; and a 97.9% concordance rate for MSI compared to IHC/PCR (Table 3). RNA fusion testing showed a 94.9% sensitivity (Table 3).

Table 3: NeXT Dx Performance | Validation Results Summary

	Variant	Specification
Analytical Sensitivity	Single Nucleotide Variants (at mutant allele frequency ≥5%)	99.5% (CI 98.6 -99.8)
	Small Insertions and Deletions (at mutant allele frequency ≥10%)	98.7% (CI 93.3-99.9)
	Copy Number Alterations (at tumor content ≥30%)	97.2% (CI 85.4 -99.9)
	Gene Fusions	94.9% (CI 82.6 -99.4)
Analytical Specificity (PPV)	Specificity	>99%
MSI	Five Bethesda loci (at tumor content ≥20%)	97.9% concordance
TMB	Exome-wide	Reported as mutations per megabase

Conclusion

The results of this validation study demonstrate that the NeXT Dx Test, a laboratory developed test (LDT) run in a CAP-accredited and CLIA-certified laboratory, is a highly-sensitive, specific, and accurate test for the detection of small variants, CNAs, and fusion events in 247 cancer-related genes for targeted therapy selection, clinical trial matching, and prognostic prediction. Additionally, whole-exome and transcriptome data offers an opportunity to further research novel biomarkers which may enable improved stratification of patient response to immunotherapies for all cancer types.

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