NeXT Dx TEST

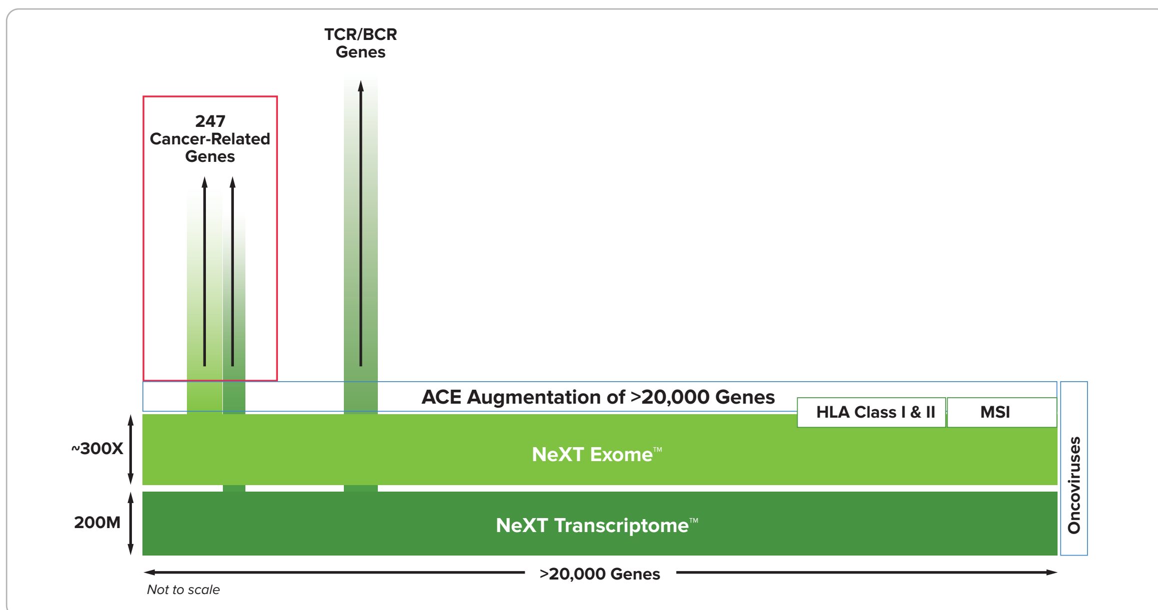
ANALYTICAL VALIDATION STUDY SUMMARY

Personalis®

Introduction

Personalis NeXT Platform®, an augmented, exome/transcriptome-based platform can simultaneously profile the tumor and immune microenvironment from a single FFPE sample across all of the approximately 20,000 genes, including 247 cancer-related genes with >1,000X boosted coverage (Figure 1). This boosted coverage enables the generation of the NeXT Dx Test; a laboratory developed test (LDT) that provides enhanced sensitivity and specificity to single nucleotide variants (SNVs), insertions/deletions (indels), and copy number alterations (CNAs) from DNA, and novel and known gene fusions from RNA in these 247 cancer-related genes listed in Table 1. The exome-wide footprint also enables accurate microsatellite instability (MSI) detection and tumor mutational burden (TMB) assessment, all included as part of the NeXT Dx Test. The NeXT Dx Test 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. The NeXT Dx test is offered by Personalis Clinical Laboratory.

Figure 1: Personalis NeXT Platform®: Comprehensive Solution for Precision Oncology



Gene Selection

Genes were selected for the NeXT Dx Test based on a comprehensive review of the medical and scientific literature, information from oncology knowledge bases, and targeted and immunotherapy-related clinical trials registered in ClinicalTrials.gov. The list includes genes recommended (Table 1) for testing in guidelines from professional societies including the National Comprehensive Cancer Network (NCCN), the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP), while also incorporating genes associated with sensitivity and resistance to targeted therapies.

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*	

Here we describe an analytical validation study to assess the performance characteristics of the NeXT Dx Test using both commercially available, pre-characterized reference materials as well as clinical FFPE samples.

Materials and Methods

To evaluate variant detection performance, clinical FFPE samples (n = 53) consisting of 11 different tumor types and tumors of unknown primary (Table 2) were used for this validation study along with commercially-available control cell lines (HCC1187, HCC1395, and NCI-H2126 from ATCC), constructs (various from Horizon Discovery and SeraCare), and CAP proficiency samples. MSI status was evaluated by using a set of 48 specimens (38 colorectal and 10 gastric tumors). All specimens evaluated for this validation met the ≥20% tumor content requirement.

Table 2: FFPE Clinical Samples

Tumor type	Number of samples	Tumor type	Number of samples
Solid Neoplasm	1	Neuroendocrine	2
Breast	2	Non-small cell lung cancer	16
Colorectal	7	Pancreatic	1
Esophageal	3	Prostate	8
Head & Neck	4	Renal cell	1
Melanoma	3	Tumor of unknown primary	5

Genomic DNA and RNA dual extraction was performed using AllPrep DNA/RNA FFPE Tissue Kit (Qiagen). The assay utilizes ≥100ng DNA and ≥100ng RNA co-extracted from a single FFPE sample. We then created an indexed genomic library using our proprietary library preparation protocol. These libraries were pooled and enriched using our patented ACE enrichment technology. The resulting enriched pools were then sequenced on Illumina NovaSeq next-generation sequencing instruments with paired-end reads measuring 150 base pairs in length. To ensure the quality of the library preparation and sequencing, clinical-grade sequencing quality control metrics were implemented (Table 3).

Table 3: Sequencing Quality Metrics

DNA Quality Metrics	RNA Quality Metrics
Total ≥50 Gb (≥167M read clusters)	≥100M read clusters
≥Q30 average base quality	≥75% of total read pairs post rRNA removal
≥90% reads mapped to the genome	≥70% of post rRNA reads mapped to reference genome
≤0.5 duplicate read pairs	≥70% read pairs mapping to exons
≥0.5 capture specificity	
≥80% of genes finished at 99% at 20x	

The quality of the DNA data was confirmed by determining the percent of reads that map to the reference genome, the number of unique reads, and the percent of reads within the footprint of the NeXT Dx enrichment. Quality of the RNA data was confirmed by determining the total number of reads after removing ribosomal RNA (rRNA) transcripts and the total number of reads that map to the reference genome. DNA sequencing yield of ≥50 Gb per tumor was obtained to achieve ~300X mean exome coverage, and >1,000X mean coverage over the 247 clinical gene footprint. Alignment to human genome assembly (version hg19) and somatic variant calling, filtering and annotation were performed using the Personalis bioinformatic pipeline and GenomOncology Clinical Workbench. Visual review of each reportable small variant was performed by a team of variant scientists through manual assessment of read pileup data for pass/fail decisions. CNA variants were assessed for completeness of genes involved in the event (only whole gene amplifications and deletions are reported) and fusion variants were evaluated to verify that reported sequences occurred in clinically-reportable transcripts.

Analytical Validation Study Design

The study was designed to evaluate variant detection performance in 247 cancer-related genes as well as to assess the Test's ability to accurately determine the status of two immunotherapy-relevant biomarkers, MSI and TMB. The validation study was performed by the Personalis Clinical Laboratory, College of American Pathologists (CAP)-accredited and Clinical Laboratory Improvement Amendment (CLIA)-certified laboratory. The NeXT Dx Test detects four major classes of alterations: SNVs, indels, CNAs, and gene fusions. 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. Use of orthogonal technology was considered where possible to resolve any discrepancies between the two platforms. The MSI classification was validated using clinical samples with MSI status predetermined by orthogonal technologies (IHC or PCR). The exome-wide TMB calculation was compared between pipeline computed TMB with manually counted non-synonymous small variants across the whole exome footprint from each specimen run in this validation study. The validation design summary is shown in Table 4. Specimens evaluated for SNVs, indels, and fusions met the ≥20% tumor content requirement, while those evaluated for CNAs met the ≥30% tumor content requirement.

Table 4: 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 • 39 clinical samples (FFPE tissue from 11 different tumor types)	Tumor DNA	958 SNV events 132 indel events
Copy Number Alterations	• Two cell lines • Two commercial constructs • 17 clinical samples	Tumor DNA	41 CNA events
Gene Fusions	• Two fusion constructs • 16 clinical samples (FFPE)	Tumor RNA	52 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

Analytical sensitivity was calculated as a Positive Percent Agreement (PPA) based on the number of true positives (TP) and false negatives (FN) using the equation below. TPs are determined by the number of calls in the observed variant call-set that have matching results in the expected call-set. FNs are determined by calls that are in the expected call-set and that have missing or non-matching results in the observed variant call-set.

$$PPA = TP*100/(TP+FN)$$

The positive predictive value (PPV) of detection of all variants was calculated based on the number of true positives (TP) and false positives (FP). TP is the number of somatic variants detected among all expected variants. FP is the number of detected variants that were not among the expected variants.

$$PPV = TP*100/(TP+FP)$$

Small Variant Detection Performance

The assessment was carried out on a total of 958 SNVs and 132 indels (up to 50 bp in size) within 247 cancer-related genes. The study included 70 SNVs and 4 indels from three tumor cell lines and six Horizon constructs (HD301, HD728, HD730, HD731, HD753, HD802). Expected variants were determined for the cell lines from an analysis of COSMIC and CCLE, while expected variants for the constructs were established by the relevant vendor. In addition, a total of 888 SNVs and 128 indels from 19 CAP proficiency samples and 39 clinical FFPE samples were assessed with the expected variants determined from an analysis by the previously validated ACE CancerPlus Test.

Detected SNVs and indels from all samples were compared to expected variants. The analytical sensitivity was calculated to be 99.8% (956/958; 99.3%-99.9%) at 95% confidence interval) for SNVs with an allelic frequency (AF) of ≥5% and 99.2% (131/132; 95.6%-99.9%) confidence interval) for indels with an AF of ≥10%.

Positive predictive value (PPV) was assessed for the CAP and clinical FFPE specimens. Based on observed variant calls, the PPV was calculated to be >99.9% (886/886; 99.6%-100%) for SNVs with an AF of ≥5% and >99.9% (127/127; 97.1%-100%) for indels with an AF of ≥10%.

Copy Number Alterations Detection Performance

The performance of CNAs was assessed for samples with a tumor content ≥30%. A set of two cell lines (HCC1395 and NCI-H2126) with four known whole gene deletions, two constructs (SeraCare) with six known amplifications, and 17 clinical FFPE samples (from seven known tumor types plus one sample that was tumor of unknown origin) with 26 amplifications and 15 deletions, run on a previously-validated assay were used for this validation study. A tumor purity adjusted copy number threshold of ≥8 for amplifications and 0 for deletions was used for detection. Detected CNAs among all samples were compared to expected CNA calls for the total of 41 variants from 21 samples and the analytical sensitivity was determined to be 97.6% (40/41; 87.1%-99.9%).

Based on the observed variant calls, the PPV was estimated to be >99.9% (40/40; 91.2%-100%).

Gene Fusion Detection Performance

The analytical performance for the detection of gene fusions was determined from 20 events from two fusion construct samples (SeraCare) and 32 events from 16 clinical FFPE samples (from six known tumor types and two samples with tumor of unknown origin) that had been previously tested by the ACE CancerPlus Test. Detected fusion events among all samples were compared to expected fusion calls for the total of 52 fusion events.

Based on the comparison of the observed fusion events with those expected to be present in the reference samples, the analytical sensitivity (PPA) was estimated to be 96.2% (50/52; 86.8%-100%) and the PPV was estimated to be >99.9% (50/50; 92.9%-100%).

Microsatellite Instability (MSI) Determination Performance

Utilizing the NeXT Dx Test, MSI status was determined from the five standard MSI loci: BAT25, BAT26, NR-21, NR-24, and NR-27. Classification was based on the following criteria: two or more unstable markers in a given specimen was classified as MSI-high (MSI-H), and any sample with no unstable markers detected was classified as stable (MSS). Cases with a single unstable marker and four stable markers were classified as MSI-low (MSI-L). Cases with uninformative markers were considered inconclusive unless the same sample has at least two or more unstable markers, in which case it is reported as MSI-H.

The performance of the NeXT Dx Test in determining MSI status was evaluated by using a set of 48 specimens (38 colorectal and 10 gastric tumors) that had been previously orthogonally tested. This set included 24 MSI-H and 24 MSS specimens. The samples previously classified by IHC lack MSI-L classification, and therefore only have an MSI-H or MSS designation as the “expected” status compared to the NeXT Dx Test, which classifies samples by MSI-H, MSI-L, and MSS categories. Therefore, MSI-L classification was merged with MSS for the purpose of this validation study, due to the nature of the comparator methodology (IHC).

Based on the comparison of the observed MSI classifications with those expected to be present in the reference samples, the concordance was determined to be 97.9% (47/48).

Tumor Mutational Burden (TMB) Determination Performance

Utilizing the power of the Personalis NeXT Platform®, TMB was calculated automatically by exome-wide analysis of non-synonymous somatic mutations identified in the coding region. The TMB is simply the sum of detected small variants divided by the total coding footprint or 34.86Mb. Each specimen run in this validation study had its TMB generated by pipeline from the exome data. To validate the pipeline computed TMB, we manually counted non-synonymous small variants across the whole exome and confirmed if the manually calculated value and pipeline generated value were identical for a given sample. There were no inconsistencies detected.

Conclusion

The results of this validation study demonstrate that the NeXT Dx Test, a laboratory developed test (LDT) developed 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. The overall analytical validation results summary of NeXT Dx Test is provided in Table 5. The Test also enables MSI status and exome-wide TMB determination to inform immunotherapy selection.

Table 5: NeXT Dx Validation Results Summary

	Variant	Specification
Analytical Sensitivity	Single Nucleotide Variants (at mutant allele frequency ≥5%)	99.8% (CI 99.3-99.9)
	Small Insertions and Deletions (at mutant allele frequency ≥10%)	99.2% (CI 95.6-99.9)
	Copy Number Alterations (at tumor content ≥30%)	97.6% (CI 87.1-99.9)
	Gene Fusions	96.2% (CI 86.8-100)
Analytical Specificity (PPV)	Single Nucleotide Variants (at mutant allele frequency ≥5%)	>99.9% (CI 99.6-100)
	Small Insertions and Deletions (at mutant allele frequency ≥10%)	>99.9% (CI 97.1-100)
	Copy Number Alterations (at tumor content ≥30%)	>99.9% (CI 91.2-100)
	Gene Fusions	>99.9% (CI 92.9-100)
MSI	Five Bethesda loci (at tumor content ≥20%)	97.9% concordance
TMB	Exome-wide	Reported as mutations per megabase

Due to biological factors and/or technical limitations, the following regions have less than adequate coverage, which may result in decreased sensitivity for variants in these regions: AKT1 (14:105239214-105239428); AREG (4:75318270-75318353); CEBPA (19:33792644-33792994); FGF2 (4:123747930-123748506); MAGEA3 (X:151935872-151936166); and SDHD (11:111965528-111965693).

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