Validation of a Clinical 1400-Gene Assay for Genomic Profiling of Cancer from DNA and RNA

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FINANCIAL DISCLOSURE: Jonathan Beck, Michael J Clark, Martina Lefterova, Elena Helman, Ravi K Alla, Deanna M Church, Sean M Boyle, Shujun Luo, Massimo Morra, Jason Harris, Nan Leng, Christian Haudenschild, Richard Chen, and John West are employees of Personalis, Inc.
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Overview

Genomic assays are increasingly used in oncology to guide clinical management and assess tumor responsiveness to novel therapeutics. As the number of cancer genes with clinical relevance expands, broader mutational profiling will be necessary. Here we present the analytical validation of the ACE Cancer Panel, the largest gene panel currently available for clinical use.

ACE Extended Cancer Panel Design

We created a targeted cancer panel that covers 1438 cancer and pharmacogenomic genes for enrichment and sequencing with DNA and RNA at high depth (>500x). This panel interrogates all known cancer genes with therapeutic implications, frequently mutated genes, and genes in commonly perturbed cancer pathways.

Cancer Reference Standards

Comprehensive cancer reference standards were created to account for various cancer mutation types and test limits of detection. We procured 38 cell lines containing the following:

Cancer Cell Lines		
	28	Small Variants
	19	Copy Number Alterations
	15	Gene Fusions

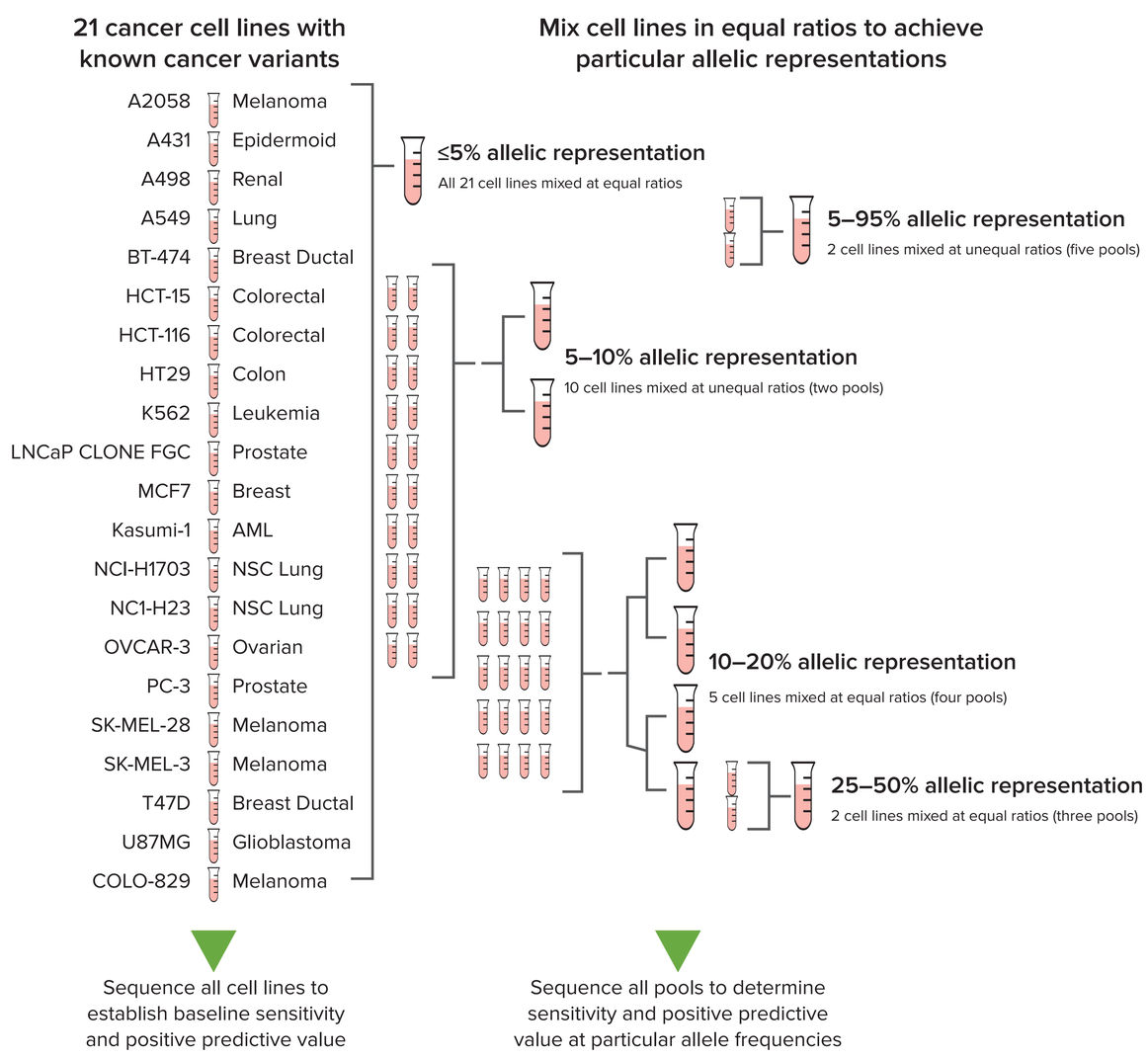
DNA Validation

LOD for Copy Number Alterations

Tumor biopsies are frequently contaminated with surrounding normal tissue. To simulate different levels of normal contamination, cell lines containing CNAs were diluted according to the following:

Limit of Detection (LOD) for Small Variants

Cell lines were diluted into mixtures to generate minor allele frequencies (MAF) ranging from ≤5% to 95%; ~16,000 SNVs; 675 indels.



RNA Validation

Cell lines (15) were identified containing known fusions events characterized by at least two independent studies. These events included ALK fusions, BCR-ABL1, and ROS1 fusions. The cell lines used are listed in the table below:

Cell Line	Tumor Type	Cell Line	Tumor Type
A-498	Renal	SR	Lymphoma
A673	Ewings Sarcoma	THP-1	Lymphoma
HCC78	Lung	U-937	Lymphoma
K562	Leukemia	HCC38	Breast
Kasumi-1	Leukemia	K562	Leukemia
MCF7	Breast	LNCaP	Lung
NCI-H2228	Lung	A-431	Epidermoid Carcinoma
SJCRH30	Rhabdomyosarcoma		

Results

Fusions Verified in RNA Validation Study

In total, eighteen well described, previously known fusions were verified by RNA sequencing using the ACE Extended Cancer Panel. The validated fusion events are listed in the table below:

Cell Line	Fusion	Cell Line	Fusion
A-498	INVS-TGFBR1	SR	NPM1-ALK
A673	EWSR1-FLI1	THP-1	KMT2A-MLLT3
HCC78	SLC34A2-ROS1	U-937	PICALM-MLLT10
K562	BCR-ABL1	HCC38	NOTCH2-SEC22B
Kasumi-1	RUNX1-RUNX1T1	K562	NUP214-XKR3
MCF7	SMARCA4-CARM1	LNCaP	RERE-PIK3CD
NCI-H2228	EML4-ALK	NCI-H2228	ALK-PTPN3
NCI-H2228	PTCH1-LUC7L2	THP-1	SNAPC3-KMT2A
SJCRH30	PAX3-FOXO1	A-431	EGFR-PPARGC1A

Calculating Limits of Detection

Analytical Sensitivity Analytical Sensitivity = TP*100/(TP+FN)

LOD Sensitivity Positive Predictive Agreement (PPA) = TP/(TP+FN)

LOD Sensitivity Positive Predictive Value (PPV) = TP/(TP+FP)

ACE CancerPlus Performance Specifications		
Requisition Required	Yes	
Specimen Types	FFPE, Fresh Frozen, ≥20% tumor content	
Regions Analyzed	Coding regions of 181 genes	
Type of Sequencing	DNA and RNA using Illumina NGS	
Typical Median Depth	>500x	
Turnaround Time	~3 weeks	
Specifications		
Sensitivity	Base Substitutions	(AF ≥5%) >99%
	Indels	(AF ≥10%) >99%
	Copy Number Alterations	96% for tumor content ≥30%
	Gene Fusions	>95%
Specificity (PPV)	Base Substitutions	AF ≥5%) 99%
	Indels	(AF ≥10%) >99%

Conclusion

Robust validation of the ACE Extended Cancer Panel demonstrated high sensitivity and specificity for all variant types. This assay can be used as a versatile diagnostic tool to test core clinically actionable genes, but also implicate new cancer genes as clinically relevant.

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