Analytical Validation of oncoReveal CDx as a FDA approved, Pan-Cancer Solid Tumor IVD Assay.

Nicholas J Lodato, Seok-Hong Lim, Sean Marris, Zonghan Liu, Momalu David, Yi Cao, Ian Flockhart, Jordan Aldersley, James Song, Sean Polvino, Yue Ke, Zhaohui Wang

Pillar Biosciences, Inc. 9 Strathmore Rd Natick, MA 01760
(800) 514-9307 | www.pillarbiosci.com

ABSTRACT

Introduction The oncoReveal CDx pan-cancer solid tumor IVD is the first FDA approved distributed kit-based assay for use as a companion diagnostic test and for tumor profiling purposes. oncoReveal CDx uses amplicon-based target enrichment for detection of SNVs and indels in 22 genes using FFPE DNA to be used on the Illumina MiSeqDx platform. Here we report results from validation studies used to support the FDA approval of the assay.

Methods Clinical FFPE tumor tissues representing 10 different tumor types positive or negative for variants covered by oncoReveal CDx were used to assess non-clinical performance of the assay. The DNA input range for the assay was established using DNA from FFPE samples containing CDx or tumor profiling variants tested between 5-160+ ng. Min tumor content and max tolerated necrotic tissue fraction were determined over multiple tumor types. Limit of detection (LoD) was established using a probit approach for all CDx markers and select tumor profiling variants. Analytical accuracy was performed to demonstrate concordance between oncoReveal CDx and two externally validated comparator methods (evNGS-A & B). A total of 271 samples from 10 tumor types with variants across 15 genes were tested and 213 samples from 10 tumor types with variants across 19 genes were tested for oncoReveal CDx vs evNGS-A or evNGS-B, respectively. A multi-site reproducibility study was performed to measure assay precision for CDx and tumor profiling variants across multiple tumor types.

Results oncoReveal CDx was robust (100% sample valid rate, PPA and NPA for variant calling) when tested using 10-80 ng of input for CRC and NSCLC tissues and at 30 ng for eight additional tumor types. Four FFPE tumor samples positive for CDx variants were diluted with tissue-matched non-tumor FFPE tissues resulting in tumor content as low as 3.3%. oncoReveal had a 100% detection rate for all samples with >10% tumor content at 30 ng of DNA input. The LoD of the assay for CDx variants is between 1.7-2.6% VAF and range from 1.4-4.7% for tumor profiling variants. Results from accuracy show >99% PPA for the evNGS-A and >98% PPA evNGS-B comparisons on the variant level. In the 3-site precision study, overall positive call rate was 96% for all variants across all sites and >95% for all sites.

Conclusions oncoReveal CDx pan-cancer solid tumor IVD is a rapid and robust kitted assay for the detection of >3660 CDx and tumor profiling variants in 22 genes. Given the FDA's recent Final Rule on LDTs, oncoReveal CDx will be an attractive FDA approved kitted option for laboratories and pharma partners to quickly assess key oncogenic driver genes for multiple solid tumor types.

TECHNICAL SPECS

Enrichment chemistry	Multiplex PCR using tiled amplicons
Number of pools	1
Number of genes/amplicons	22/103
Number of targets	>3600 DNA variants
Variant types	SNVs and indels
Recommended DNA Input	30-80 ng
Sample Type	DNA from FFPE
Validated NGS platform	Illumina MiSeqDx

ASSAY DESIGN FOR TUMOR PROFILING EVALUATION

Indication	Gene	Variant	Targeted therapy
Colorectal Cancer (CRC)	*KRAS*	KRAS wild-type (absence of mutations in codons 12 and 13)	ERBITUX® (cetuximab), or VECTIBIX® (panitumumab)
Non-Small Cell Lung Cancer (NSCLC)	*EGFR*	Exon 19 In Frame Deletions and Exon 21 L858R Substitution Mutations	EGFR Tyrosine Kinase Inhibitors approved by FDA

Table 1 (above) Variants with supported CDx claims in oncoReveal CDx

AKT1	*ALK*	*BRAF*	*CTNNB1*
DDR2	*ERBB2*	*ERBB4*	*FBXW7*
FGFR1	*FGFR2*	*FGRR3*	*MAP2K1*
MET	*NOTCH1*	*NRAS*	*PIK3CA*
PTEN	*SMAD4*	*STK11*	*TP53*

Table 2 (left) The 20 additional genes covered by oncoReveal CDx. More than 3600 variants within these genes were evaluated for tumor profiling purposes, including additional variants within *KRAS* and *EGFR* not covered in the CDx claims.

Gene	Variant(s)	Gene	Variant(s)
ALK	C1156; F1174; G1202R	*EGFR*	ex 20 ins; T790M; G719
	G1269A; I1170; I1171N		D761Y; L861Q; S781I
	L1196M; L1198F; M1166R	*FGFR3*	G370C; R248C; S249C; Y373C
	R1275Q; Y1278S	*ERBB2*	ex 20 ins; L755; I767; I841V
BRAF	V600E; V600K		L869R; T862A; V842I
KRAS	A146; Q61E	*NRAS*	G12; G13; Q61; A146T
MET	D1246N; R988C; Y1248H	*PIK3CA*	N345; E542; E545; H1047; R88

Table 3 Select clinically actionable variants that are covered in oncoReveal CDx.

Colorectal Cancer (CRC)	Breast Cancer	Liver Cancer	Non-Small Cell Lung Cancer (NSCLC)	Renal Cancer
Bladder Cancer	Endometrial Cancer	Melanoma	Pancreatic Cancer	Thyroid Cancer

Table 4 Tumor tissues from 10 cancer indications were used in analytical validation experiments to support a solid tumor, pan-cancer claim.

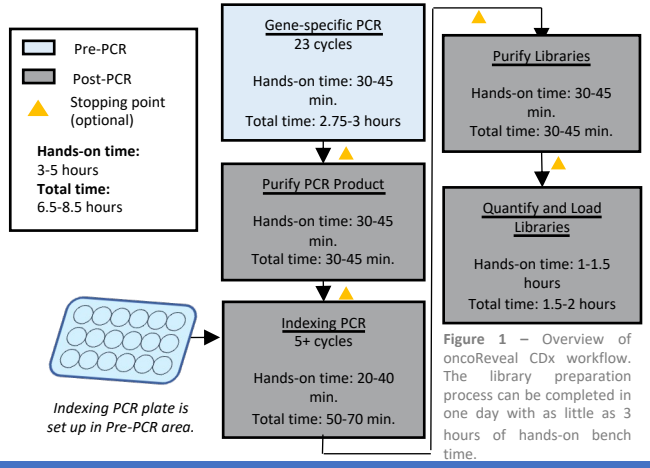

Figure 1 – Overview of oncoReveal CDx workflow. The library preparation process can be completed in one day with as little as 3 hours of hands-on bench time.

RESULTS AND CONCLUSIONS

Tumor Type	N	Test + Comp +	Test + Comp -	Test - Comp +	Test - Comp -	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
Bladder	8	16	0	0	23090	100.0% (80.6%, 100.0%)	100.0% (99.98%, 100.0%)
Breast	10	6	4	0	35340	100.0% (61.0%, 100.0%)	99.99% (99.97%, 100.0%)
Endometrial	12	42	0	0	42378	100.0% (91.6%, 100.0%)	100.0% (99.99%, 100.0%)
Liver	12	12	0	0	26886	100.0% (75.8%, 100.0%)	100.0% (99.99%, 100.0%)
Melanoma	12	12	0	0	42408	100.0% (75.8%, 100.0%)	100.0% (99.99%, 100.0%)
Pancreatic	6	12	6	0	21192	100.0% (75.8%, 100.0%)	99.97% (99.94%, 100.0%)
Renal	12	0	0	0	26898	Not evaluable	100.0% (99.99%, 100.0%)
Thyroid	12	6	0	0	42414	100.0% (61.0%, 100.0%)	100.0% (99.99%, 100.0%)
Total	84	106	10	0	260606	100.0% (96.5%, 100.0%)	99.996% (99.993%, 100.0%)

Table 5 Eighty-four libraries were prepared from eight tumor types using 30 ng of DNA input. A total of 106 positive variants were detected across all libraries and verified with an externally validated evNGS assay. Assay concordance was determined by PPA and NPA analysis.

Gene	Amino Acid Change	LoD (%VAF)
EGFR	p.Gly719Cys	1.6
	p.Thr790Met	3
	p.Pro772_His773dup	2.2
	p.Ser768Ile	4.8
KRAS	p.Gln61Leu	2.2
	p.Ala146Thr	2.8
PIK3CA	p.Glu542Lys	4.4
SMAD4	p.Ser178Ter	3.7
TP53	p.Glu294Ter	4.5
	p.Arg273His	4.7
	p.Arg273Cys	4.1
	p.Glu298Ter	4.7
BRAF	p.Val600Glu	1.4
PIK3CA	p.His1047Arg	4.1

	(+) Detected	Total (+)	(-) Detected	Total (-)	(+) call Rate; 95% CI (-) call rate; 95% CI
SNV	60	60	8340	8340	100.0% (94.0%, 100.0%) 100.0% (100.0%, 100.0%)
Ins	41	42	4344	4344	97.6% (87.7%, 99.6%) 100.0% (99.9%, 100.0%)
Del	41	42	7830	7830	97.6% (87.7%, 99.6%) 100.0% (100.0%, 100.0%)
ALL	142	144	20514	20514	98.6% (95.1%, 99.6%) 100.0% (100.0%, 100.0%)

Table 6 (Left) Eleven NSCLC and CRC specimens containing 14 tumor profiling variants (13 SNVs, and 1 insertion) were evaluated in this study. The LoD for tumor profiling variants were estimated using the hit rate approach. A minimum of 5 titration levels were tested with 20 replicates were tested for each sample. The LoD for each of the 14 variants in the study was defined as the VAF detected at ≥95% hit rate. **Table 7 (Above)** LoD for tumor profiling variants were confirmed using a minimum of six replicates across 20 samples representing the 10 tumor types described in Table 4.

(evNGS-A)

Var Type	# of Var	PPA (n/N) (95%CI)	NPA (n/N) (95%CI)
All	243636	99.6% (245/246) (97.7%, 99.9%)	99.9% (243380/243390) (99.9%, 99.9%)
SNV	141864	99.6% (228/229) (97.6%, 99.9%)	99.9% (141629/141635) (99.9%, 99.9%)
MNV	31354	100.0% (4/4) (51.0%, 100.0%)	100.0% (31350/31350) (99.9%, 100.0%)
Del	42148	100.0% (11/11) (74.1%, 100.0%)	99.9% (42134/42137) (99.9%, 99.9%)
Ins	28270	100.0% (2/2) (34.2%, 100.0%)	99.9% (28267/28268) (99.9%, 99.9%)

Table 8 A total of 271 samples represented by 10 tumor types were tested against evNGS-A for analytical accuracy. Of the 22 genes covered by oncoReveal CDx, 15 genes were also covered by evNGS and were considered for analysis. Agreement presented is binned by variant type as well as overall for the study.

(evNGS-B)

Var Type	# of Var	PPA (n/N) (95%CI)	NPA (n/N) (95%CI)
All	661045	98.6% (345/350) (96.7%, 99.4%)	99.9% (660677/66 0695) (99.9%, 99.9%)
SNV	250954	98.7% (308/312) (96.8%, 99.5%)	99.9% (250627/250642 (99.9%, 99.9%)
MNV	37587	100.0% (6/6) (61.0%, 100.0%)	100.0% (37581/375 81) (99.9%, 100.0%)
Del	238051	100.0% (21/21) (84.5%, 100.0%)	99.9% (238028/23 8030) (99.9%, 99.9%)
Ins	134453	90.9% (10/11) (62.3%, 98.4%)	99.9% (134441/13 4442) (99.9%, 99.9%)

Table 9 A total of 212 samples represented by 10 tumor types were tested against evNGS-B for analytical accuracy. Of the 22 genes covered by oncoReveal CDx, 19 genes were also covered by evNGS-B, including 6 additional genes not covered by evNGS-B, and were considered for analysis. Agreement presented is binned by variant type as well as overall for the study.