Validation of NEO | PanTracer LBx, a liquid biopsy precision oncology pan-solid tumor comprehensive genomic profiling assay (CGP)

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Abstract # PP.05

Introduction

- In the era of precision oncology, the expanding list of biomarker-driven therapies necessitated a switch from small, targeted panels to large-scale comprehensive genomic profiling (CGP) panels to maximize clinically actionable findings while conserving limited tissue.
- While tissue is the gold-standard, risks associated with invasive biopsies, sampling errors, tumor inaccessibility, inadequate sample quality/quantity can limit its use.
- Liquid biopsy (LBx) CGP is emerging as an alternative approach overcoming such limitations for faster treatment decisions in patients with advanced cancer.
- Here we present the analytical (AV) and clinical validation (CV) of NEO | PanTracer™ LBx, a pan-solid tumor next generation sequencing (NGS) CGP assay for therapy selection/clinical trial enrichment.

Methods

- PanTracer LBx has been designed to detect key classes of somatic alterations across solid tumors, such as:
  - Small variants (SNVs/InDels; 514 genes)
  - Copy Number Variations (CNVs; 59 genes)
  - Fusions (23 genes)
  - Key immune signatures [Microsatellite Instability (MSI) and blood tumor mutational burden (TMB)]
- The assay has been analytically and clinically validated in a CAP/CLIA certified laboratory across the above variant classes to determine key assay performance characteristics:
  (1) Limit of Detection (LoD), **(2)** Limit of Blank (LoB), and **(3)** Precision
- Assessment of concordance (percent agreement) and overall accuracy involved orthogonal comparisons with:
  - An amplicon-based assay (InVisionFirst™-Lung) (N=146 pts) performed as part of the AV process, and
  - Four commercial liquid CGP assays (N=142 pts) performed as part of the clinical validation process (CV).

Results

Limit of Detection (LoD)

- LoD was assessed by measuring the detection of somatic variants present in SeraSeq® ctDNA Complete™ mutation mix at different %VAF levels (0.1%-2%) and DNA concentrations (10 and 30 ng).
- 10 replicates were tested for each VAF/input combination.
- A probit regression model was utilized in the LoD assessment for small variants; For fusions and CNVs, LoD calculations were based on the lowest VAF and fold-change, respectively.
- LoD90 and LoD95 values for detection of small variants, fusions and CNVs are shown in **Table 1**. LoD90 results for the different somatic alterations at different %VAF levels and DNA input are shown in **Figure 1**.

Table 1. LoD90 and LoD95 values for small variants, CNVs and fusions at different DNA input.

		Input	LoD_{90}	LoD_{95}
Probit	Small Variants	10 ng	0.49%	0.60%
		30 ng	0.23%	0.28%
	SNV	10 ng	0.29%	0.34%
		30 ng	0.17%	0.20%
	InDel	10 ng	0.69%	0.84%
		30 ng	0.31%	0.38%
Lowest to reach required detection	CNV (fold change)	10 ng	1.205	1.205
		30 ng	1.205	1.205
	Fusion	10 ng	0.50%	1.00%
		30 ng	0.50%	0.50%

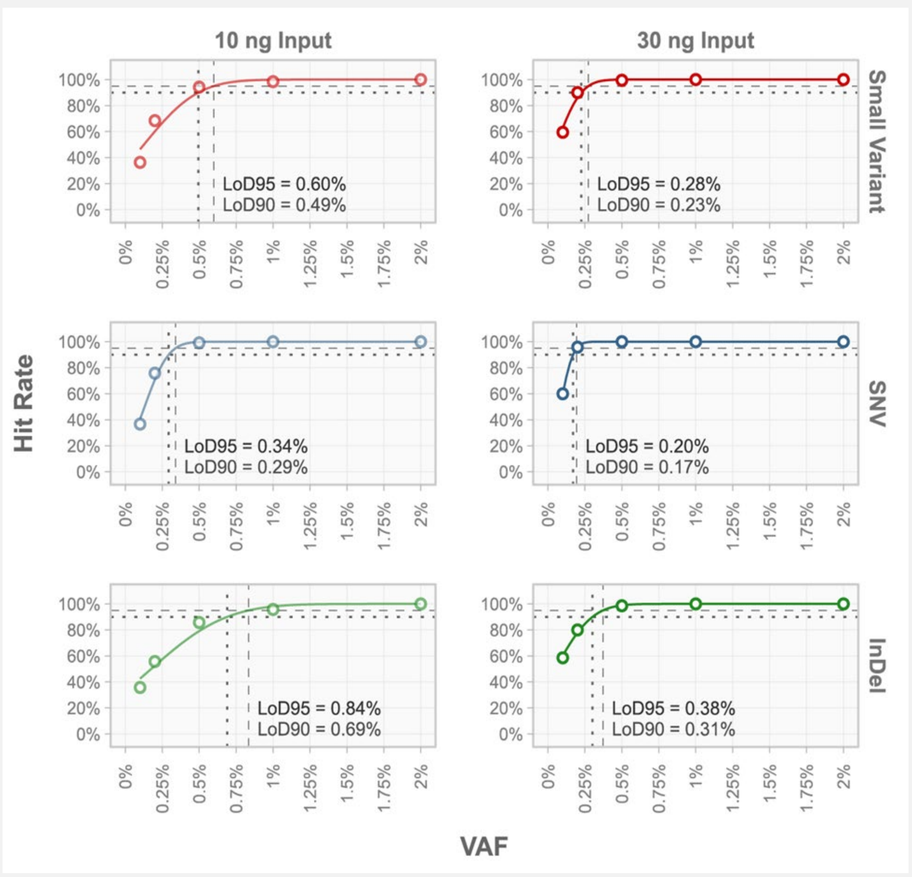

Figure 1. LoD90 and LoD95 for SNVs and InDels evaluated at two different DNA input concentrations and various %VAF levels.

Limit of Blank (LoB)

- 21/22 healthy donor samples included in the LoB assessment passed all QC steps and were considered in the final analysis.
- Buffy coat samples from 9 matching donors were also sequenced to verify germline and clonal hematopoiesis of indeterminate potential (CHIP) variants.
- LoB assessment demonstrated high specificity (absence of detection considered as a true negative) across all assessed variant classes (**Table 2**).

Table 2. LoB metrics for the different classes of somatic alterations

	Present in Healthy Donor plasma	Present in BC	FP	TN	Expected Total Variants	Specificity (%)	Passing Criteria
SNV/InDel	25	21	4	10853	10857	99.96	>99.5%
CNV	0	N/A	0	1239	1239	100	>95%
Fusion	0	N/A	0	483	483	100	>95%

BC: Buffy Coat, **FP**: False Positives, **TN**: True Negatives

Precision Testing

- Precision included contrived samples from the LoD study at 0.5% and 1.0% VAF tested at 10 ng and 30ng DNA input.
- For evaluation of **intra-run precision**, each sample was processed on two runs in triplicates (n=6 for each sample, total tested samples: 24).
- For evaluation of **inter-run precision**, samples were processed on six runs (n=6 for each sample, total tested samples: 24) performed on three different dates by five different operators with three different library prep reagents and three sequencing SBS reagent lots on five different sequencers.
- Precision testing demonstrated high repeatability (intra-run precision; 98.25%) and reproducibility (inter-run precision; 97.32%) results (**Table 3**).

Table 3. Intra- and inter-run precision testing results for the different variant classes evaluated as part of the assay's analytical validation

	Intra-run precision (Repeatability)	Inter-run precision (Reproducibility)	Precision (repeatability and reproducibility)
Small Variants	99.11%(446/450)	97.97% (2654/2709)	98.13% (3100/3159)
SNV	99.31% (286/288)	100% (1728/1728)	99.9% (2014/2016)
InDels	98.77% (160/162)	94.39% (926/981)	95% (1086/1143)
CNVs	100% (48/48)	100% (292/292)	100% (340/340)
Fusions	91.67% (66/72)	91.49% (398/435)	91.52% (464/507)
Total	98.25% (560/570)	97.32% (3344/3436)	97.45% (3904/4006)

Number are provided as percentage (concordant variants/total variants
Numbers in "total" row and "precision" column are the sum of SNVs, InDels, CNVs and fusions

Orthogonal Testing – Concordance (Percent Agreement) and Accuracy

(1) Analytical Validation

- 146 late-stage cancer samples tested with an amplicon-based assay (InVisionFirst™-Lung) were used for orthogonal comparison to the PanTracer LBx assay. These included:
  - Residual cfDNA from 44 clinical samples with both eTam-Seq (SNVs/InDels) and fusion results of the InVisionFirst™-Lung assay.
  - 102 commercial biobank samples – This cohort was tested only for SNVs/InDels using both assays – Fusion was not tested by InVisionFirst™-Lung.
  - Full results are summarized in **Table 4.**

Table 4. Positive and negative percent agreement and overall accuracy between PanTracer LBx and IVFL-Lung for small variants and fusions.

	Samples	Evaluated Variants	Total Variants	TP (PanTracer +/IVFL +)	FP (PanTracer +/IVFL -)	FN (PanTracer -/IVFL +)	TN (PanTracer -/IVFL -)	PPA	NPA	Accuracy
Small variants	**146**	141	20586	184	6	7	20389	96.33%	99.97%	99.94%
SNV	**146**	115	16790	150	4	6	16630	96.15%	99.98%	99.94%
InDel	**146**	27	3942	27	2	1	3912	96.42%	99.94%	99.92%
Fusion	**44**	4	176	1	0	0	175	100.00%	100.00%	100.00%

Total Variants: Number of evaluated variants x Total samples tested
PPA: Positive Percent Agreement; **NPA:** Negative Percent Agreement
Note: CNV from InVisionFirst™-Lung was not part of the analysis in the final validation

(2) Clinical Validation

- As part of the assay's clinical validation, single collection timepoint samples from 142 patients with various types of advanced cancer were tested with PanTracer LBx and four commercially available plasma-based CGPs assays.
- Concordance analysis between PanTracer LBx and the four orthogonal assays demonstrated >99% overall detection accuracy for the different variant classes – 99.56% for small variants (**Table 5**), 99.82% for CNVs and 100% for fusions.

Table 5. Positive and negative percent agreement and overall accuracy between PanTracer LBx and four commercially available liquid CGP assays.

Orthogonal assay	Count of samples	Variants per orthogonal	Total variants	TP *PanTracer+/ Orthogonal +*	FP *PanTracer+/ Orthogonal -*	FN *PanTracer-/ Orthogonal +*	TN *PanTracer-/ Orthogonal -*	PPA	NPA	Accuracy
Assay 1	19	182	3458	156	19	15	3268	91.23%	99.42%	99.02%
Assay 2	42	75	3150	52	21	5	3072	91.23%	99.32%	99.17%
Assay 3	77	547	42119	426	89	65	41539	86.76%	99.79%	99.63%
Assay 4	5	12	60	11	0	1	46	91.67%	100.00%	98.28%
Total	143	816	48787	645	129	86	47925	88.24%	99.73%	99.56%

Orthogonal Testing – MSI and bTMB

(1) MSI

- PanTracer LBx MSI status on 154 clinical samples was compared against several orthogonal methods:
  - 23 samples were verified as MSI-high (MSI-H) by PanTracer LBx and were concordant with the available orthogonal results
  - 131 PanTracer MSI-H not detected samples were concordant with the available orthogonal results from matching plasma testing.
- Overall, concordance for MSI detection was 100% among the 154 samples with no false positive and false negative detection, passing the required ≥95% acceptance criteria (**Table 6**).

Table 6. MSI concordance between PanTracer LBx and various orthogonal assays

Assay	TP	FP	FN	TN	PPV	NPV
MSI ddPCR	1	0	0	0	100%	-
Clinical MSI-PCR	1	0	0	0	100%	-
CGP LBx Assay 1	0	0	0	17	-	100%
CGP LBx Assay 2	2	0	0	40	100%	100%
CGP LBx Assay 3	0	0	0	70	-	100%
IHC	3	0	0	0	100%	-
CGP LBx Assay 4	0	0	0	4	-	100%
Tissue NGS	16	0	0	0	100%	-
Total	23	0	0	131	100%	100%

(2) bTMB

Plasma-Tissue TMB correlation

- Of the 36 matching plasma and tissue samples tested by PanTracer LBx and NeoComprehensive Solid Tumor assay, 14 pairs qualified for TMB correlation analysis per verification plan inclusion criteria (estimated tumor purity ≥ 1%).
- Pearson's correlation between the tissue and plasma TMB (**Figure 2**) was very good except for two outlier samples (*shown in blue*).

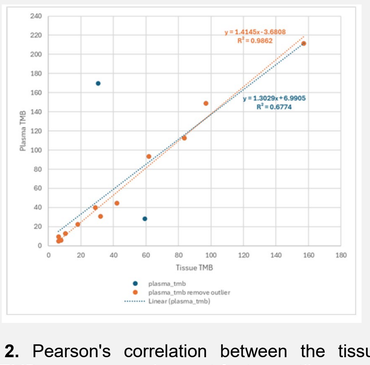

Figure 2. Pearson's correlation between the tissue and plasma TMB was very good except for two outlier samples.

Plasma-Plasma bTMB correlation

- bTMB was assessed across 73 clinical samples by one of three commercially available LBx CGP assays and results were used for orthogonal comparisons to those obtained with PanTracer LBx.
- The cohort was separated based on tumor content as estimated by PanTracer LBx (<1% or >1%) – A much better correlation with the orthogonal results was observed in the higher tumor content (blue) group (Pearson's correlation R2: 0.78 vs. 0.28), consistent with the reduction of robustness of bTMB outcome with reduced tumor content (**Figure 3**).

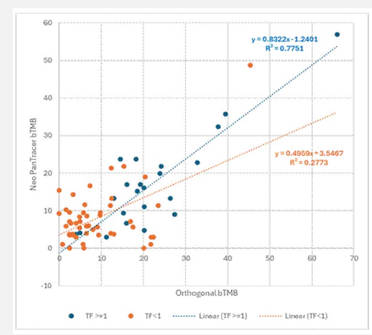

Figure 3. Pearson's bTMB correlation between PanTracer LBx and three orthogonal assays separated by tumor content

Conclusions

- All pre-specified AV and CV acceptance criteria were successfully met.
- Concordance analyses further demonstrated strong agreement with both a highly sensitive targeted panel and larger commercial LBx CGP assays.
- Investigation of variant call discrepancies between PanTracer LBx and the orthogonal liquid CGP assays is currently underway.
- Taken together, results confirm PanTracer LBx as a highly accurate and reproducible assay for guiding treatment selection.

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