Analytical validation of PanTracer LBx performance, a comprehensive pan-solid tumor liquid biopsy assay

Christodoulos Pipinikas[1], Faqiang Wu[2], Daniel Whang[2], Rebecca Hong[2], Samuel Koo[2], Qinqin Zha[2], Jieying Chu[2], Rachel Schell[2], Jiannan Guo[2], Giovanni Marsico[1], Tim Forshew[1], Amber Chevalier[3], Andrew Lukowiak[3], Heng Xie[2], Gregory Jones[4]

[1] NeoGenomics, Cambridge, United Kingdom,[2] NeoGenomics, Aliso Viejo, CA,[3] NeoGenomics, Fort Myers, FL,[4] NeoGenomics, Durham, NC

NeoGenomics Laboratories, Aliso Viejo, CA

Abstract # 4090

Introduction

- Pan-solid tumor comprehensive genomic profiling (CGP) assays have gradually replaced single gene and smaller, tumor-specific panels to identify actionable somatic mutations in patients with advanced cancer.
- While tissue-based CGP remains important for clinical decisions, invasive biopsies can be challenging in terms of material quantity/quality and/or risk to patients.
- Plasma-based CGP assays provide the potential for rescuing cases where tissue fails, providing faster results to enable treatment decisions, monitoring treatment through repeated sampling, or capturing tumor heterogeneity.
- As such, CGP via liquid biopsy (LBx) represents an asset to patients, offering testing options that can analyse hundreds of genes.
- Here we describe initial results of the analytical validation (AV) process of a large deep sequencing panel aiming to establish the initial performance characteristics of NeoGenomics' PanTracer Liquid Biopsy Clinical CGP assay (**PanTracer LBx**).
- We also provide initial comparison data to an established highly sensitive targeted panel.

Methods

- Based on TSO500 ctDNA v2 chemistry, PanTracer™ LBx, is a next-generation sequencing (NGS), pan-cancer CGP assay for clinical and research applications.
- The assay has been designed to detect key classes of somatic alterations across solid tumors, such as:
  - Small variants (SNVs/InDels; 514 genes)
  - CNVs (59 genes)
  - Fusions (23 genes)
  - Key immune signatures (MSI and bTMB)
- PanTracer LBx was been analytically validated in a CAP/CLIA certified laboratory across the above mutation classes to determine key assay performance characteristics:
  - Limit of Detection (LoD)
  - Limit of Blank (LoB)
  - Precision

Abbreviations

CGP: Comprehensive Genomic Profiling, **LBx**: Liquid Biopsy; **AV**: Analytical Validation; **SNV**: Single Nucleotide Variants; **InDel**: Insertions and Deletions: **CNV**: Copy Number Variants; **CAP/CLIA**: College of American Pathologists/Clinical Laboratory Improvement Amendments; **MSI**: Microsatellite Instability; **bTMB**: Blood Tumor Mutation Burden, **VAF**: Variant Allele Frequency

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 input (10 and 30 ng).
- 10 replicates were tested for each VAF/input combination.
- For small variants, LoD assessment utilized a probit regression model.
- 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**. **Figure 1** shows the LoD90 results for the different somatic alterations at different %VAF levels and DNA input.

Table 1. LoD90 and LoD95 values for small variants 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%

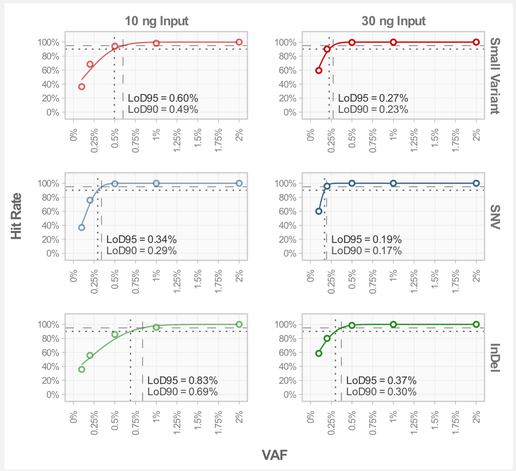

Figure 1. LoD90 and LoD95 for SNVs and InDels evaluated at two different DNA input concentrations and various %VAF levels.

All LoD test acceptance criteria successfully met

Limit of Blank (LoB)

- A total of 22 healthy donor samples were included in the LoB study.
- 21/22 samples 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.
- Detection across the different classes of somatic alterations is summarized in **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

LoB assessment passed all pre-specified acceptance criteria

Precision Testing

- Precision testing included samples at two VAF and two input levels.
- For evaluation of **intra-run precision**, each sample was processed on two runs in triplicates (total 24 replicates).
- For evaluation of **inter-run reproducibility**, samples were processed on six runs (total 24 replicates) 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.
- Intra-run precision (**Table 3**) and inter-run reproducibility (**Table 4**) across all comparisons, was 98.25% and 97.32%, respectively.

Table 3. Intra-run precision

	CNV	Fusion	InDel	SNV	Small variants (SNV and InDels)	Total
Concordant variants	48	66	160	286	446	560
Total variants	48	72	162	288	450	570
Precision (%)	100	91.67	98.77	99.31	99.11	98.25%

Table 4. Inter-run reproducibility

	CNV	Fusion	InDel	SNV	Small variants (SNV and InDels)	Total
Concordant variants	292	398	926	1728	2654	3344
Total variants	292	435	981	1728	2709	3436
Reproducibility(%)	100	91.49	94.39	100	97.97	97.32%

All precision testing acceptance criteria successfully met

Accuracy (Analytical Sensitivity & Specificity)

- A total of 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.
  - CNV from InVisionFirst™-Lung was not part of the analysis in the final validation.
  - Assay performance metrics are summarized in **Table 5** and **Figure 2**.

Table 5. Performance characteristics of PanTracer LBx assay for the accuracy study.

	InVisionFirst Lung (n=146)								
Orthogonal assay	Evaluated Variants	Total Variants *	TP	FP	FN	TN	Sensitivity	Specificity	Accuracy
Small variants (SNV & InDel)	141	20586	182	8	7	20389	96.30%	99.96%	99.93%
SNV	114	16644	148	6	6	16484	96.10%	99.96%	99.93%
InDel	27	3942	34	2	1	3905	85.71%	99.12%	98.92%
Fusion genes tested	4	176	1	0	0	175	100.00%	100.00%	100.00%

* **Total Variants:** Number of evaluated variants x Total samples tested
For small variants total samples tested, *N*=146; For fusions, total number of samples tested, *N*=44

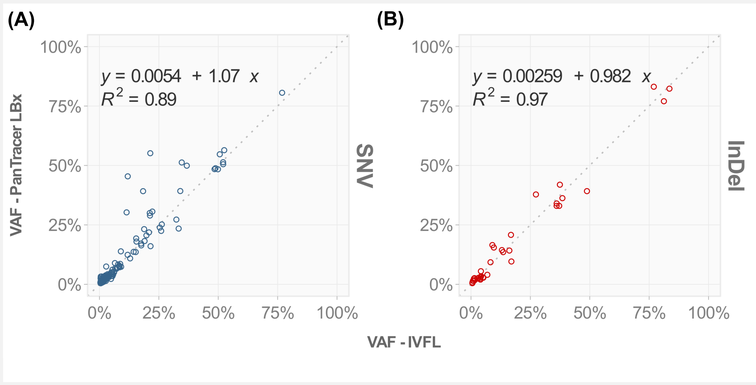

Figure 2. Correlation plot between PanTracer LBx %VAF levels and those obtained with InVisionFirst-Lung for (**A**) SNVs, and (**B**) Indels.

All accuracy testing acceptance criteria successfully met

Conclusions

- Performance evaluation of PanTracer LBx assay met all pre-specified AV acceptance criteria.
- Based on a large panel targeting different classes of somatic variants across multiple genes, it offers results consistent with a smaller, established targeted panel with the addition of more biomarkers that may be informative or actionable.

Acknowledgements: The authors would like to thank the NeoGenomics Medical, Laboratory and R&D teams for their support in the entire PanTracer AV process