NEO GENOMICS

PRECISION ONCOLOGY

WHITE PAPER

Analytical validation of the PanTracer™ LBx assay

Liquid biopsy-based comprehensive genomic profiling for advanced-stage pan-solid tumors

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In the era of precision oncology, treatment success hinges on the ability to accurately identify genomic alterations that inform the selection of targeted therapies. Traditional tissue biopsies have long served as the gold standard for comprehensive genomic profiling (CGP), but their use is often limited by insufficient sample quantity and quality, anatomical inaccessibility, and tumor heterogeneity.

Liquid biopsies offer a minimally invasive alternative that overcomes many of these limitations. By analyzing circulating tumor DNA (ctDNA) shed into the bloodstream by tumor cells, liquid biopsies can provide a real-time snapshot of the tumor's molecular landscape from a simple blood draw. They can also detect genomic alterations across multiple tumor sites.[1] This supports the utility of liquid biopsy tests not only at initial diagnosis but also at disease recurrence, aiding treatment selection, often with faster results than tissue-based testing.[2]

Liquid biopsy is increasingly recognized as a vital tool in the molecular profiling of solid tumors, emphasizing the growing significance of ctDNA analysis, primarily through a broad panel-based approach using next-generation sequencing (NGS).[3] Its clinical validity has been confirmed both as a standalone method and as a complement to traditional tissue-based testing. Clinical strategies for combining plasma and tissue analysis have been developed to include tissue-first, complementary, and plasma-first approaches, creating more flexible and personalized testing pathways.[3] Incorporation of these strategies into clinical practice guidelines for managing various solid tumor types highlights plasma testing as a key element in the diagnostic, therapeutic, and prognostic management of cancer.[3-6]

This white paper outlines the analytical validation of PanTracer™ LBx, an NGS assay designed for pan-solid tumor liquid biopsy applications, focusing on the sensitivity, specificity, accuracy, and orthogonal detection of single nucleotide variants (SNVs), insertions/deletions (InDels), copy number variations (CNVs), and fusions.

The PanTracer™ LBx assay

PanTracer LBx is an NGS assay that enables comprehensive genomic profiling (CGP) of plasma from patients diagnosed with advanced-stage solid tumors. The assay leverages minimally invasive peripheral blood samples as a complement or alternative to tumor tissue. This test is designed and validated to achieve highly sensitive detection with minimal input, extracted from up to two tubes of peripheral blood. (Figure 1)

The PanTracer LBx assay is designed to detect genomic alterations that are identified as guideline-recommended, actionable biomarkers relevant across solid tumor types for therapy selection, prognosis, and clinical trial options. The assay targets 514 genes to assess major types of DNA sequence variant classes such as SNVs, InDels, CNVs, and fusions. The assay additionally assesses blood tumor mutation burden (bTMB) and microsatellite instability (MSI) status, the validation of which is outside the scope of this white paper.

Key results

- **PanTracer LBx is a highly sensitive liquid biopsy assay**, detecting variants in ctDNA down to 0.45% variant allele frequency (VAF).
- During analytical validation studies, PanTracer LBx showed high concordance to an orthogonal liquid biopsy test, InVisionFirst®-Lung, **with an overall sensitivity of 96.3% and specificity of 99.96%.**
- In the real-world clinical setting, **PanTracer LBx demonstrated >99% accuracy compared to four leading commercial liquid biopsy assays.**

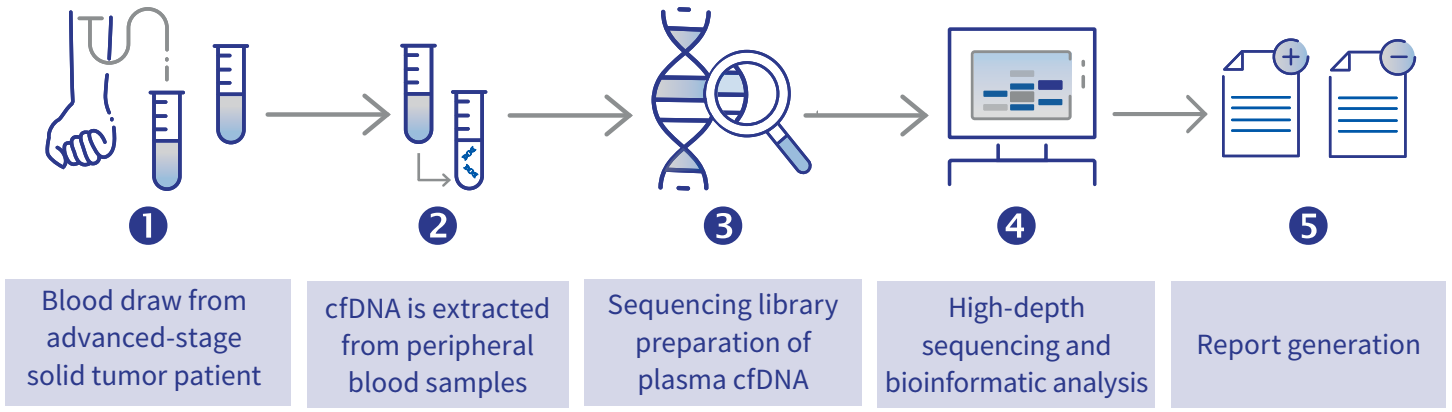

Figure 1: PanTracer LBx workflow
1. *Whole blood is drawn from advanced-stage cancer patients utilizing cell-free DNA (cfDNA) Streck BCT® tubes. Two tubes of blood are required for the PanTracer LBx assay.* ***2.*** *cfDNA is extracted from the collected peripheral blood samples.* ***3.*** *Sequencing libraries are constructed, and hybrid capture-based target enrichment is performed.* ***4.*** *Samples are sequenced to a read-depth of 35,000x. Sequencing data is analyzed to identify variants detected within individual samples.* ***5.*** *Reports are generated.*

Analytical validation

The analytical validation of the PanTracer LBx assay was performed in a CAP/CLIA-certified laboratory (Aliso Viejo, CA, USA) using a series of reference standards and clinical samples to characterize the assay's performance across variant classes. As part of the analytical validation process, the following key performance metrics were assessed: Limit of Detection (LOD), Limit of Blank (LOB), accuracy (analytical sensitivity and specificity), and precision.

Sensitivity: limit of detection (LOD)

LOD was assessed to determine the lowest detectable level of different variant classes that the test can still reliably detect. To assess the LOD of PanTracer LBx, reference material (SeraSeq® ctDNA Complete™ Mutation Mix) containing mutations at known variant allele frequencies (VAF), ranging from 0.1% to 2% was utilized. Samples were tested with two different inputs for the different VAF levels, 10 ng and 30 ng, across 10 replicates. The LOD95 values for SNVs and InDels, fusions, and CNVs were found to be 0.45% VAF, 0.50% VAF, and 1.205x respectively. LOD95 (LOD at 95% confidence) in this study is the lowest VAF that can be consistently detected in at least 95% of positive samples and is an industry standard used to benchmark assay sensitivity.

Specificity: limit of blank (LOB)

LOB was assessed to characterize the background, or "noise," in an assay; this helps prevent false-positive results from occurring. LOB was assessed using 21 healthy donor plasma samples. Specificity was calculated based on the number of false positives across the sequenced target regions. The specificity metrics for SNVs and InDels were 99.96%, and 100% for both fusions and CNVs.

Precision

Precision testing was conducted to evaluate the repeatability (intra-run precision) and reproducibility (inter-run precision) of the PanTracer LBx assay, including samples at two different VAF and input levels (4 samples in total). For evaluation of intra-run precision, each sample was processed on two runs in triplicate (n=6 for each sample, total 24 replicates). For evaluation of inter-run reproducibility, the samples were processed on six runs (n=6 for each sample, 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.

Analytical performance parameters		
LOD95	SNV/InDels	0.45% VAF
	Fusions	0.50% VAF
	CNVs	1.205x
LOB	SNV/InDels	99.96%
	Fusions	100%
	CNVs	100%
Precision	Intra-run	98.25%
	Inter-run	97.32%

Clinical performance

To assess the analytical sensitivity and specificity of PanTracer LBx, a total of 279 samples (Figure 2) from patients with advanced-stage cancers (stage III-IV) were sequenced, and the results were used for orthogonal comparison to those obtained with:

- InVisionFirst®–Lung, an amplicon-based, targeted liquid biopsy sequencing assay (N=146 samples), or
- One of four commercially available liquid CGP assays (N=133 samples). These samples were collected as part of a prospective multi-center protocol representing real-world community oncology testing patterns

Variant detection results were compared across platforms to evaluate sensitivity, specificity, and concordance. Orthogonal testing showed high concordance rates between the PanTracer LBx and the comparator assays, with an overall accuracy exceeding 99% across all variant types (Figure 3).

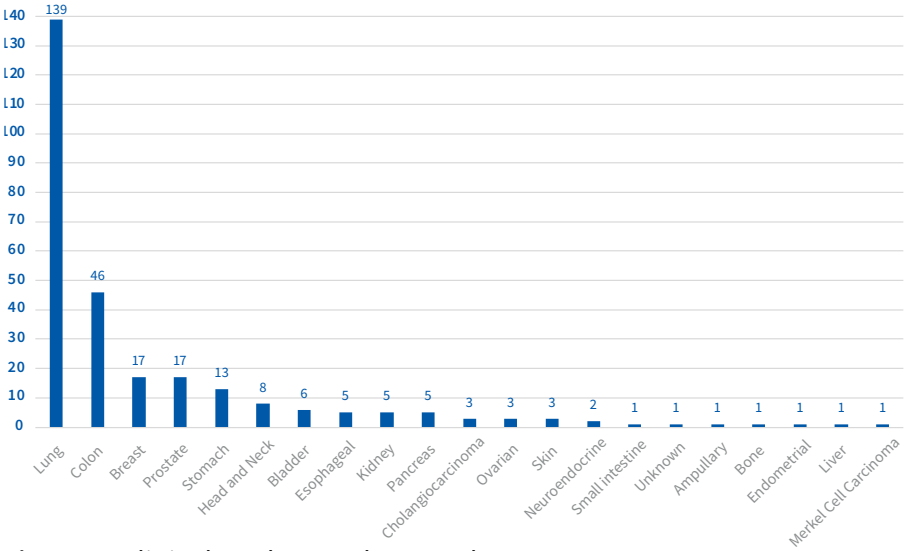

Figure 2: Clinical study sample count by cancer type

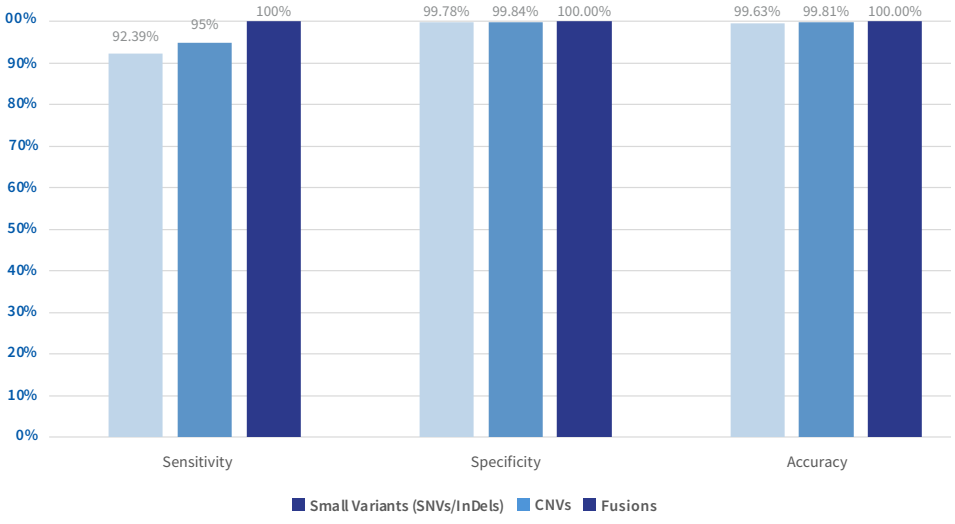

Figure 3: Sensitivity, specificity, and accuracy results for PanTracer LBx compared to five commercially available assays for SNVs, InDels, fusions, and CNVs.

Conclusion:

PanTracer LBx is a validated, comprehensive liquid biopsy test for advanced solid tumors, providing reliable detection of SNVs, InDels, CNVs, fusions, MSI, and bTMB from ctDNA. Analytical validation confirmed its high sensitivity and specificity across different variant types, and clinical studies showed strong agreement with leading commercial CGP platforms. By overcoming the limitations of tissue-based profiling, PanTracer LBx gives oncologists timely, actionable genomic insights, supporting optimal treatment decisions throughout cancer care.

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NeoGenomics, Inc. is a premier cancer diagnostics company specializing in cancer genetics testing and oncology data solutions. We offer one of the most comprehensive oncology-focused testing menus across the cancer continuum, serving oncologists, pathologists, hospital systems, academic centers, and pharmaceutical firms with innovative diagnostic and predictive testing to help them diagnose and treat cancer. Headquartered in Fort Myers, FL, NeoGenomics operates a network of CAP-accredited and CLIA-certified laboratories for full-service sample processing and analysis services throughout the US and a CAP-accredited full-service, sample-processing laboratory in Cambridge, England, United Kingdom.

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