Performance Evaluation of an Ultra-Sensitive Assay for NSCLC Biomarker Testing

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INTRODUCTION

There are over 30 FDA-approved targeted therapies for NSCLC, but biomarker testing is first required. Most tissue biopsies are core needle biopsies or fine needle aspirates, with limited tissue level and quality, and low tumor content, rendering them unsuitable for many NGS assays. Tissue biopsies may not be feasible for some patients, due to frailty or prioritization of other tests, favoring liquid biopsy as a non-invasive alternative.

To address this need, CellCarta has collaborated with Biofidelity to validate the Aspyre Lung technology as a clinical trial assay for patient management (Diels et al., submitted for ESMO 2025). Aspyre Lung is a sensitive, PCR-based assay, for DNA/RNA from FFPE or cfDNA/cfRNA from liquid biopsy samples, detecting 114 somatic variants across 11 first line-recommended genes (EGFR, BRAF, KRAS, ERBB2, ALK, ROS1, RET, MET, NTRK1-3). In this poster is presented assay performance data from very low input material (down to 5 ng DNA / 1.5 ng RNA from FFPE and to 5 ng cfDNA / 6 ng cfRNA from blood respectively), demonstrating that Aspyre Lung fills clinical practice gaps as a fast, cost-effective and highly sensitive assay for management of NSCLC patients in clinical trials.



Figure 1. Genes and variants covered by Aspyre Lung. A total of 114 genomic variants are assessed and aggregated as actionable variant calls where appropriate for treatment guidance / patient management

METHODS

- **Methods:** Aspyre combines multiplex PCR with allele-specific pyrophosphorolysis (PPL) and rolling circle amplification (RCA). Aspyre Lung was performed according to the manufacturer's IFU.
- **Samples:** FFPE NSCLC tissue blocks and cfDNA/cfRNA from blood (healthy donors and NSCLC patients); contrived reference samples created with known variants.
- **Recommended Input Levels:**
  - FFPE: 20 ng DNA / 6 ng RNA
  - Blood: 20 ng cfDNA / 42 ng cfRNA
- **Low Input Levels:**
  - FFPE: 5 ng DNA / 1.5 ng RNA
  - Blood: 5 ng cfDNA / 6 ng cfRNA
- **Data Analysis:** Variant calling was done using cloud-based Aspyre Lab software.
- **Performance Metrics Evaluated:**
  - Limit of Detection (LoD95)
  - Specificity (Limit of Blank, LoB)
  - Analytical Accuracy
  - Analytical Precision

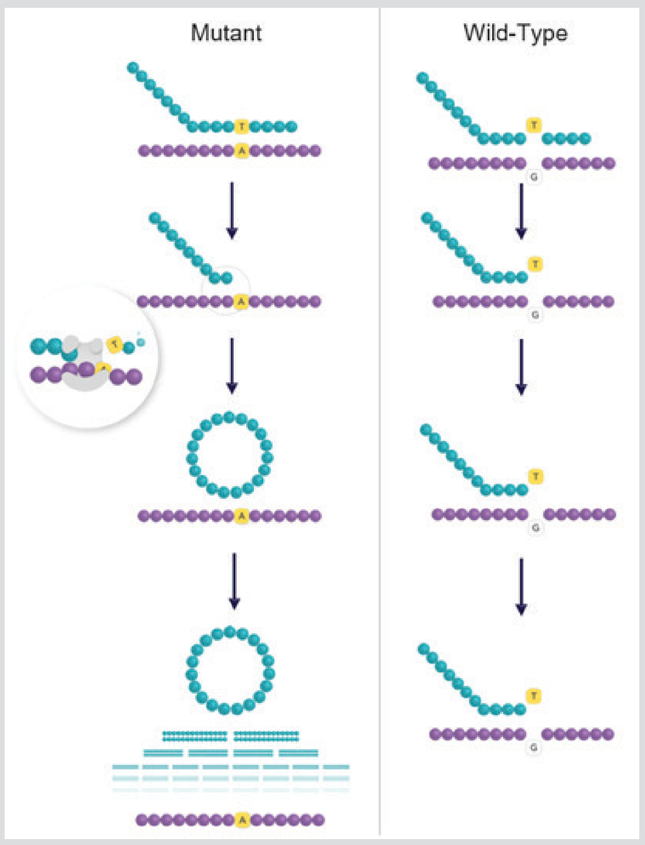


Figure 2. Aspyre technology. Aspyre probes hybridize to both wild-type (WT) and mutant (MT) DNA but are mismatched to WT molecules. Reverse polymerization specifically digests only perfectly matched probes annealed to mutated DNA. Those annealed to WT DNA are not digested due to mismatch. Digested probes from MT DNA are released and circularized by ligation, while the non-digested probes remain double stranded to WT DNA. Circularized probes from MT DNA are isothermally amplified and detected using fluorescent dye(s), Probes that hybridized to WT molecules are not amplified.

RESULTS

FFPE Tissue (Low Input Validation)

- **Sensitivity (LoD95):**
  - SNVs/Indels: ≤ 3% VAF
  - Fusions: ≤ 100 copies
  - MET exon 14 skipping: ≤ 200 copies
- **Specificity:** 0% false positives across >6,000 variant calls
- **Accuracy:** 100% Positive and Negative Percent Agreement against orthogonal NGS data from 29 NSCLC samples
- **Precision:** 100% concordance across operators, reagent lots, instruments, and days

Variant type	Gene, Exon, Protein Variant, COSMIC ID	Total positive/total tests**
DNA		
SNV	*KRAS* exon 2 G12C COSM516	218/220
	EGFR exon 21 L858R COSM6224	
	EGFR exon 20 T790M COSM6240*	
	BRAF exon 15 V600E COSM476	
	EGFR exon 18 G719A COSM6239*	
	EGFR exon 18 G719S COSM6252*	
	EGFR exon 20 C797S COSM6493937*	
	EGFR exon 20 C797S COSM5945664 *	
Deletion	*EGFR* exon 19 E746_A750del COSM6223	80/80
Insertion	*ERBB2* exon 20 Y772_A775dup COSM20959	
	ERBB2 exon 20 H773dup COSM12377**	
	EGFR exon 20 A767_V769dup COSM12376	
RNA Fusions		
Fusion	*EML-ALK* E13_A20 COSF408	119/120
	KIF5B-RET K15_R12 COSF1232	
	CD74-ROS1 C6_R34 COSF1200	
	TPM3-NTRK1 T8_N10 COSF1329	
	QKI-NTRK2 Q6_N16 COSF1446	
	ETV6-NTRK3 E5_N15 COSF571	
RNA Exon Skipping		
Exon skipping	*MET*	20/20

Figure 3. LoD95 confirmation data on FFPE samples. LoD95 at low input was estimated for 6 DNA variants (marked with *) at a range between range 3-10% VAF. Results were then confirmed by testing 20 replicates of freshly prepared contrived samples. Variants whose LoD95 was not estimated beforehand were run at 20 replicates at their respective LoD95 determined for the initial standard-input Aspyre Clinical Test for Lung FFPE validation (3% for indels, 100 copies for gene fusions and 200 copies for MET exon 14 skipping). Confirmed LoD95 by mutation class were ≤3% VAF for SNV and Indel, ≤ 100 copies for fusions, and ≤ 200 copies for MET exon 14 skipping.

** Results were aggregated across the given variant class.

Liquid Biopsy (Low Input Validation)

- **Sensitivity (LoD95):**
  - SNVs/Indels: ≤ 0.8% VAF
  - Fusions: 6 copies
  - MET exon 14 skipping: 100 copies
- **Specificity:** 0% false positives across >26,000 variant-level tests
- **Accuracy:**
  - DNA: 100% PPA & NPA
  - RNA: 88% PPA (due to RNA availability, not test performance)
- **Precision:** 100% inter- and intra-run reproducibility

Nucleotide Variant	Reported Variant	Variant Type	Standard Input LOD	Low Input LOD
COSM516	KRAS exon 2 p.G12C	SNV	0.2%	0.4%
COSM6224	EGFR exon 21 p.L858R		0.3%	0.8%
COSM6240	EGFR exon 20 p.T790M		0.4%	1.6%
COSM476	BRAF exon 15 p.V600E		0.2%	0.4%
COSM6223	EGFR exon 19 p.E746_A750del	Deletion	0.2%	0.4%
COSM20959	ERBB2 exon 20 p.Y772_A775dup	Insertion	0.8%	1.2%
COSM12376	EGFR exon 20 p.A767_V769dup		0.4%	0.8%
Median DNA			**0.3%**	**0.8%**
COSF408 (EML4-ALK_E13-A20)	ALK Fusion	RNA Fusion	6c	6c
COSF1200 (CD74ROS1_C6-R34)	ROS1 Fusion		6c	6c
COSF1329 (TPM3-NTRK1_T8-N10)	NTRK Fusion		6c	6c
COSF1446 (QKI-NTRK2_Q6-N16)	NTRK Fusion		6c	6c*
COSF571 (ETV6-NTRK3_E5-N15)	NTRK Fusion		6c	6c
COSF1232 (KIF5B-RET_K15-R12)	RET Fusion		18c	18c
Median RNA Fusion			**6c**	**6c**
COSM2931 (p.L982_D1028del)	MET exon 14 skipping	Exon skipping	100c	100c

*<individual verification threshold, but confirmed with variant class and no impact to class median.

Figure 4. LoD95 confirmation data on blood samples. This table shows that, at the reduced input of 5 ng DNA and 6 ng RNA, the median DNA variant LoD was confirmed at 0.8%, the median RNA fusion LoD was confirmed at 6 copies, and MET exon 14 lskipping LoD was confirmed at 100 copies. These median LoDs, along with the individual variant LoDs are shown alongside the LoDs from the standard input Aspyre Lung Blood validation. The lower input reduced the median sensitivity of DNA variants approximately 2-fold, but is still within the original validation's acceptance criteria.

Limit of Blank (LoB) Validation at CellCarta

An independent LoB study was conducted at CellCarta using 30 FFPE tonsil tissue specimens from cancer-free donors. DNA (20 ng) and RNA (6 ng) were extracted and tested in duplicate across 5 days using two reagent lots.

- **Total Tests:** 60 (30 DNA + 30 RNA)
- **Results:**
  - 0 positive calls detected across all 114 variant targets
  - Observed LoB: 0%
- **Clopper-Pearson 95% CI:** 0–6% per sample; 0–0.09% across all reportable variants

These results independently confirm the high specificity of Aspyre Lung with no false positives, validating its use in clinical trial settings where analytical stringency is essential.

Performance Characteristic	FFPE		Pass/Fail
	Acceptance Criteria	Observed	
LOB	Observed proportion boundary - 60 measurements: >90%	0% FPR 100% observed proportion	PASS

Figure 5. LoB confirmation at CellCarta on FFPE samples

DISCUSSION

NGS failure rates due to low input material exceed 25% in NSCLC tissue testing. The Aspyre assay achieves equivalent or better sensitivity and specificity at drastically reduced nucleic acid input, enabling actionable results from limited, degraded or low-quality specimens.

Aspyre's rapid turnaround, lack of need for complex bioinformatics, and compatibility with standard PCR instruments make it suitable for widespread clinical deployment and decentralized trial settings.

CONCLUSION

The Aspyre Lung provides accurate, rapid, and highly sensitive detection of actionable NSCLC variants from both FFPE and blood samples—even at low input. This enables access to biomarker testing for a greater proportion of patients, especially where tissue is limited or liquid biopsy is the only option.

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