Poster ID: LB095

ASPYRE-Lung addresses critical gaps in NGS-based biomarker testing: robust variant calling from NGS QC fails

Elyse Shapiro[1], Ryan Evans[1], Elizabeth Gillon-Zhang[1], Julia Brown[1], Candace King[1], Cory Kiser[1], Mary Beth Rossi[1], Darren Davis[2], Michelle Taylor[2], Jennifer Clower[2], Jian Yang[2], Cullen Taylor[2], Robert Snyder[2], Wendy Levin[1]

1. Biofidelity Inc.*, Morrisville, North Carolina, USA
2. Precision for Medicine, Frederick, Maryland, USA

ABSTRACT

A major challenge with current Next Generation Sequencing (NGS)-based genomic testing of patients with Non-Small Cell Lung Cancer (NSCLC) is that as many as 25% of tissue samples fail to produce results due to quality control (QC) failure, leading to inadequate patient care (Hagemann *et al.*, 2015; Sadik *et al.*, 2022). ASPYRE® technology addresses the urgent need for robust, rapid and accessible diagnostics for actionable genomic biomarkers. The ASPYRE® -Lung panel covers 114 variants in 11 genes based on current clinical practice guidelines, with the ability to result challenging sample types.

In this study, we investigated an NSCLC patient sample set from Precision for Medicine, including a majority of biobanked specimens that had previously failed NGS QC, despite having sufficient clinical material, and for which genomic biomarker data were unavailable.

- Of the 94 patient samples that failed NGS QC, 98% of samples (92/94) passed ASPYRE-Lung QC and could inform patient care.
- In the 92 patient samples that passed ASPYRE-Lung QC, 47% (43/92) had a detectable variant identified by ASPYRE-Lung.
- All 26 samples that passed NGS QC also passed ASPYRE-Lung QC.
- 5 samples had inadequate remaining DNA for ASPYRE-Lung. Nevertheless, ASPYRE-Lung generated reportable data for 4/5 (80%) samples, detecting variants in 2/4 samples.
- ASPYRE-Lung has the potential to provide actionable clinical information on patient samples with insufficient quantity or quality for NGS testing

INTRODUCTION

Allele-Specific PYrophosphorolysis REaction (ASPYRE®) is a novel method for molecular testing of NSCLC biomarkers (Gray et al., 2022; Silva et al., 2021) that relies on the highly specific enzymatic degradation of probes hybridized with perfect complementarity to target DNA strands, through a reaction called pyrophosphorolysis. Built on the innovative ASPYRE technology, ASPYRE® -Lung is a targeted multi-gene panel that detects 114 actionable genomic biomarkers in 11 genes that have associated FDA-approved targeted therapeutics and well-established clinical utility in NSCLC. Here we demonstrated that ASPYRE-Lung LDT (run at Biofidelity's CLIA laboratory in North Carolina) can be utilized as a clinical testing solution for the significant number of patients samples that fail NGS, enabling more patients to benefit from targeted therapies.

MATERIALS & METHODS

Clinical samples. 120 formalin fixed paraffin embedded (FFPE) tissue samples were provided by Precision for Medicine's biobank. All samples were derived from NSCLC diagnosed patients.

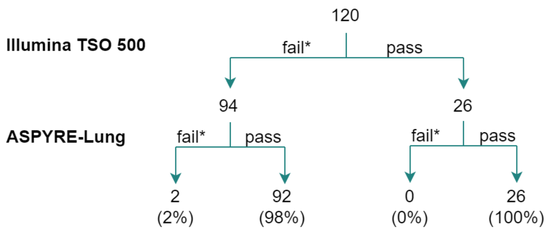
Nucleic acid extraction. DNA and RNA were manually extracted using the MagMAX™ FFPE DNA/RNA Ultra Kit.

Sequencing. Nucleic acid sequencing was performed at Precision for Medicine's CLIA laboratory in Houston, TX using the Illumina TruSight Oncology 500 (TSO 500) NGS assay.

ASPYRE. ASPYRE-Lung testing was performed at Biofidelity's CLIA laboratory in Morrisville, NC. The ASPYRE reaction was performed at a DNA input of 20 ng per reaction and an RNA input of 6 ng per reaction, except for 5 samples that were run at lower inputs, ranging from 4.25-14 ng DNA due to insufficient quantity.

Data analysis. Variant calls for samples and controls were generated by processing the raw data file generated by the Design and Analysis2 software (v2.6.0, Thermo Scientific) through the ASPYRELab software (v1.1.1).

Ethical approval. Institutional Review Board (IRB) approval was obtained for the use of samples in assay development by biobanks through participating collection sites in the country of origin.



*Assay fails include samples that failed either DNA analysis, RNA analysis, or both.

ASPYRE-Lung ASSAY

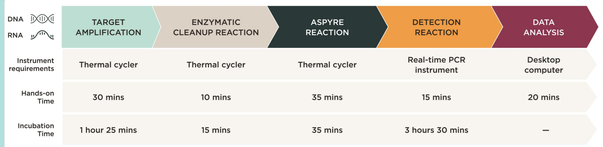


Figure 1 – General overview of the ASPYRE® -Lung workflow. Targets include single nucleotide variants (SNVs), insertions and deletions (indels), RNA fusions and splice variants in nucleic acid derived from FFPE.

→ **Simultaneous detection** of 114 DNA and RNA biomarkers

→ **Low input requirements:** 20 ng DNA & 6 ng RNA, 10% tumor cell content

→ **Turn-around time (TAT) of 2 days** from specimen to results

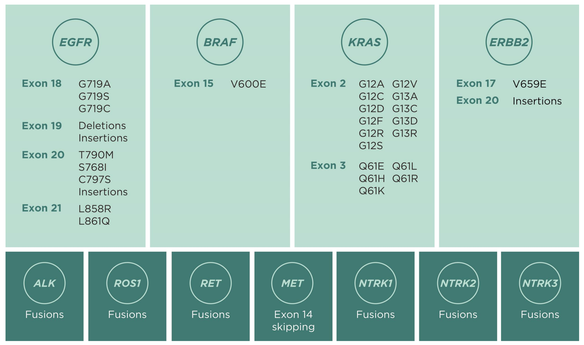


Figure 2 – ASPYRE-Lung's comprehensive lung panel identifies variants across guideline-recommended genes for NSCLC; 11 genes, 114 variants (77 DNA Mutations, 36 RNA fusions and one exon skipping event).

→ **No bioinformatic interpretation** required – easy to interpret results

→ **Clear, simple 2-page report** to rapidly inform patient decision-making

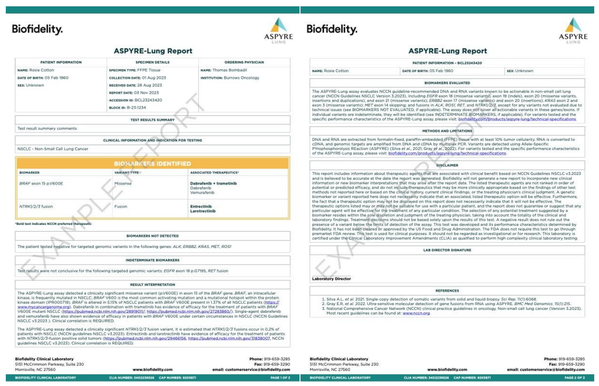
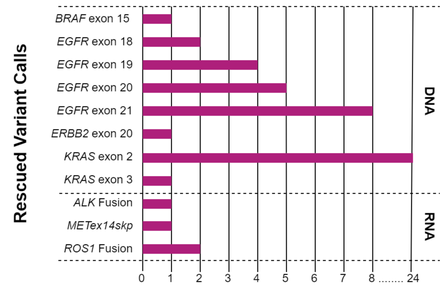


Figure 3 - The ASPYRE-Lung patient report. No user interpretation or further bioinformatics analysis is needed.

RESULTS

TSO 500 QC	% passing ASPYRE-Lung
Pass	100% (26/26)
Fail	98% (92/94)

Table 1: Cumulative QC Pass/Fail Results. Assay fails include samples that failed either DNA or RNA analysis, or both DNA and RNA analysis.



Gene	Exon	Variant	Number of Occurrences
BRAF	15	V600E	1
EGFR	18	G719C	1
		G719S	1
	19	E746_A750del	3
		L747_K754del	1
	20A	T790M	2
	20B	S768I	3
	21	L858R	7
		L861Q	1
ERBB2	20	Y772_A775dup	1
KRAS	2	G12A	1
		G12C	10
		G12D	5
		G12V	6
		G12R	1
		G13C	1
	3	Q61H	1

Figure 4 and Table 2: Actionable variants identified by ASPYRE-Lung in samples which failed NGS. ASPYRE-Lung variant calls are presented for samples that failed either DNA or RNA NGS analysis, or both DNA and RNA NGS analysis. Table 2 presents a detailed of identified DNA variants by exon number.

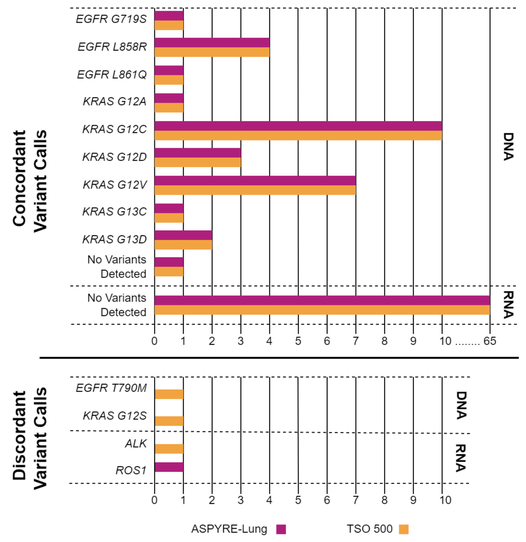


Figure 5: Concordance for Results Passing NGS QC. Concordance was evaluated based on targets present in both panels. Results are presented as the number of variant calls made in samples with passing DNA or RNA results.

SUMMARY

The key findings of this study include:

- ASPYRE-Lung was able to generate passing results for 98% (92/94) samples that failed TSO 500 sequencing QC.
- In the 92 NGS-failed samples rescued by ASPYRE-Lung, 47% (43/92) had a detectable variant identified by ASPYRE-Lung, consistent with the prevalence and distribution of variants expected in this patient population .
- For all 26 samples that passed NGS quality-control, ASPYRE-Lung generated reportable data for all 26 (100%), with a 96% concordance rate between ASPYRE and NGS results
- Of the 5 NGS QC-failure samples with inadequate DNA amounts for ASPYRE-Lung, ASPYRE-Lung was able to generate data on 4/5 (80%) samples and 2/4 had a detectable variant.

DISCUSSION

This study demonstrates that ASPYRE® -Lung is able to address critical gaps in NGS-based NSCLC biomarker testing; clinical reports covering guideline-recommended NSCLC genomic biomarkers were generated for 98% of samples that failed NGS QC. Additionally, ASPYRE-Lung has the potential to address tissue-limited (QNS) specimens due to ASPYRE's decreased tumor cell content and input requirements. Compared to NGS, ASPYRE-Lung has a high success rate, has a rapid TAT and is cost effective, making it suitable as a first-line testing option, or as a salvage test method for clinical samples that are either QNS or fail NGS QC, consistent with current practice guidelines.

ASPYRE-Lung is a transformative option in cancer care management, providing more patients with NSCLC actionable biomarker information, enabling all patients the potential to benefit from highly effective and better tolerated targeted therapies.

The ASPYRE-Lung workflow offers the potential for increased sensitivity of both DNA and RNA biomarker detection, less tumor cell content requirements compared to many NGS assays, many fewer steps than complex NGS-based testing, reduced bioinformatics requirements, and next-day TAT. Collectively, ASPYRE-Lung addresses the current multiple critical gaps in patient access to genomic testing, enabling more patients with NSCLC to benefit from highly active and well-tolerated targeted therapeutics.

ASPYRE-Lung REAGENTS (Research Use Only)

- Simultaneous analysis of DNA and RNA
- Comprehensive lung panel with NCCN guideline recommended biomarkers for NSCLC
- Runs on existing real time PCR instruments
- Straightforward implementation
- Reduced sample requirements
- Fast time to result

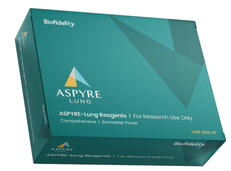


Figure 5: ASPYRE-Lung RUO Kit

REFERENCES

1. Hagemann *et al.* 2015. Clinical next-generation sequencing in patients with non-small cell lung cancer. *Cancer.* 121: 631-639
2. Sadik *et al.* 2022. Impact of clinical practice gaps on the implementation of personalized medicine in advanced non-small cell lung cancer. *JCO Precis Oncol.* 6: e2200246
3. Silva *et al*. 2021. Single-copy detection of somatic variants from solid and liquid biopsy. *Sci Rep*. 11(1):6068.
4. Gray *et al*. 2022. Ultra-sensitive molecular detection of gene fusions from RNA using ASPYRE. *BMC Med Genomics*. 15(1):215.

*All authors are employees of Biofidelity Inc and may have a financial interest including salary, equity, options, and intellectual property.