Poster ID: 3252

Simplified genomic profiling using Aspyre Lung solves real-life challenges with limited and poor-quality tissue

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Aspyre® Lung

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ABSTRACT

Introduction Advanced NSCLC treatment guidelines recommend testing patients for genomic biomarkers to guide the use of over 30 FDA-approved targeted therapies. Challenges in NSCLC molecular testing include the need for assessment of multiple variants in both DNA and RNA, sample quality and quantity, cost, and clinical need for rapid turn-around time for timely treatment initiation (recommended <14 days). Aspyre Lung (Tissue or Blood) is a targeted genomic profiling assay which informs on 114 actionable and prognostic biomarkers across 11 first-line genes (*ALK*, *BRAF*, *EGFR*, *ERBB2*, *KRAS*, *MET*, *RET*, *ROS1*, *NTRK1/2/3*) in patients with NSCLC. The assay uses standard PCR and real-time PCR equipment and interrogates nucleic acid directly without costly sequencing in a highly sensitive and cost-effective manner, enabling rapid and accurate clinical decision making.

Methods We present results from an initial cohort of clinical samples sent to Biofidelity Inc. Laboratory (a CAP/CLIA site). De-identified demographic and clinical data were analyzed including biopsy type, pathology diagnosis, tumor content, % necrosis, turnaround time, and variant identified. 177 clinical samples were analyzed, with retrospective, research and non-NSCLC samples excluded prior to analysis.

Results 177 samples were eligible for inclusion. Diagnoses included adenocarcinoma (n=90), squamous cell carcinoma (n=34), unspecified NSCLC (n=49) and other (n=4). 20/177 samples were quantity not sufficient for one or more of tumor (n=11), DNA (n=6) or RNA (n=9); however, 177/177 samples passed internal assay performance checks for both DNA and RNA analysis (100%). Median tumor content was 40% (range 2-95%). Overall, 85 (47.8 %) of samples were positive for a variant, including SNV in *BRAF*, *EGFR*, and *KRAS*, *EGFR* exon 19 deletions and exon 20 insertions, insertions in *ERBB2*, *MET* exon 14 skipping and gene fusions involving *ALK*, *RET* and *ROS1*. QNS samples yielded nine variant calls of which seven were actionable. Of the samples with associated staging information, 32 were Stages I-IIIA (with 22 variant calls, 68.8%) and 30 were IIIB-IV (with 18 variant calls, 60.0%). 93.2% of samples were reported within the target two-day turnaround time (median 2, range 1-4).

Conclusion Aspyre Lung (Tissue) has a high assay success rate and proven rapid 2-day turnaround time, suitable as a cost-effective method for testing in the first-line setting or at relapse, or for samples that are scant or of low quality, and can provide a large fraction of NSCLC patients with actionable biomarker information. Aspyre Lung offers a new paradigm for informing cancer care management and enables more patients with NSCLC to benefit from effective and better tolerated targeted therapies.

Aspyre Lung WORKFLOW

	Target Amplification	Enzymatic Cleanup Reaction	Aspyre Reaction	Detection Reaction	Data Analysis
Instrument Requirements	Thermal cycler	Thermal cycler	Thermal cycler	Real-time PCR instrument	Desktop computer
Hands-on Time	30 mins	10 mins	35 mins	15 min	20 mins
Incubation Time	1 hour 25 mins	15 mins	35 mins	3 hours 30 mins	-

The steps of the Aspyre Lung assay workflow after nucleic acid extraction. Typical TAT from sample receipt to final results is 2 days.

STUDY MATERIALS & METHODS

Sample selection 200 tissue samples were initially considered for inclusion. The following were excluded: samples cancelled during workflow (7 no tissue, 6 stained with incompatible dyes, 5 no tumor, and 2 sent in error that were not NSCLC), samples received for proficiency testing, samples received as part of a retrospective trial, research and development samples.

Ethics statement and patient consent All patients who submitted samples for clinical decision-making signed informed consent allowing de-identified data to be used for research purposes.

Biofidelity (Inc) laboratory workflow FFPE tissue samples (blocks or slides) submitted to the laboratory for analysis were accessioned, starting the turnaround time clock. Laboratory-validated SOPs were followed as described in (Evans 2024). Briefly, blocks were sectioned (5 µm, ≤ 9 slides), and a slide H&E stained for review by an on-call pathologist (% tumor, % necrosis, tissue and region of interest areas, and confirmation of a diagnosis consistent with NSCLC). DNA and RNA from acceptable specimens were extracted separately and quantified. Standard nucleic acid inputs of 20 ng DNA and 6 ng RNA were used wherever possible; low input levels of 5 ng DNA and 1.5 ng RNA have also been validated. Samples with nucleic acid inputs of lower than these were marked with a QNS tag for the appropriate nucleic acid.

Aspyre Lung runs and data analysis Aspyre Lung reactions were run as described in (Evans 2024) at Biofidelity Inc, a CAP/CLIA site, with full adherence to SOPs by trained personnel. Data were downloaded from QuantStudio 5 RealTime 384 well PCR System instruments (ThermoFisher), exported via DA2 software. Raw Data CSV were input to cloud-based custom Aspyre Lab software, which takes fluorescence real-time PCR data, performs checks and normalizations, and outputs variant calls (Palmer 2025). A second operator performed double-checks after data analysis to ensure validity. Patient data were combined with assay run data to create a clinical report (Figure 1).

Data curation Laboratory data were downloaded and compiled. Where discrepancies or missing entries were flagged, original documentation was inspected to resolve ambiguities if possible. In particular 26 samples lacked an assigned diagnosis; for these, pathology data were inspected to ensure consistency with NSCLC, and a diagnosis of NSCLC, not otherwise specified (NOS) listed.

ASSAY DETAILS

Aspyre Lung targets 114 genomic variants across 11 genes.

Table 1: Genes and variants detected by Aspyre Lung. Variants in *EGFR, BRAF, KRAS* and *ERBB2* are analysed using DNA, and gene fusions and *MET* exon 14 skipping from RNA.

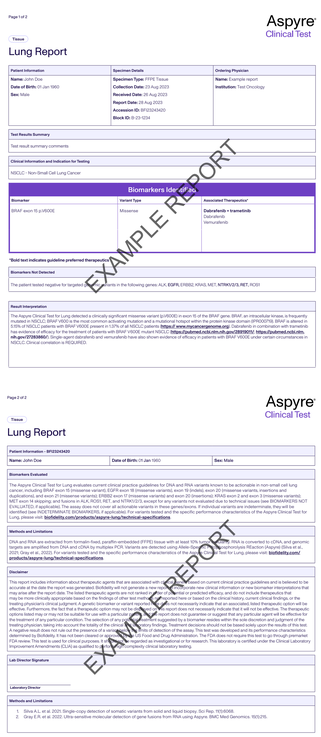

Figure 1: The Aspyre Lung patient report. The two-page report includes a summary of patient and sample details, and test results including biomarkers identified, biomarkers not detected and indeterminate biomarkers, in addition to an interpretation of results.

Variable		No. Patients (n= 177)
Age (years)	Mean (SD)	67.3 (10.9)
	Median	68
	Range	28-94
Sex	Female	91 (51.4%)
	Male	86 (48.6%)
Ethnicity	Hispanic/latino	2
	Not Hispanic/latino	49
	Not specified	126
Race	White	72
	Black or African American	11
	Asian	3
	Other	1
	Unknown /not stated	90
Pathology diagnosis	Adenocarcinoma	90 (50.8%)
	Squamous cell carcinoma	34 (19.2%)
	NSCLC NOS	49 (27.7%)
	Other NSCLC: • Adenosquamous carcinoma, • invasive mucinous adenocarcinoma, • sarcomatoid carcinoma, • spindle cell carcinoma	 1 1 1 1
Stage at diagnosis	Stage I	12
	Stage II	11
	Stage IIIA, IIIB, NOS	9, 4, 7
	Stage IV	26
	Staging Unknown	108
Tissue Specimen type	Core needle biopsy	36
	Fine needle aspirate	35
	Cryobiopsy	24
	Resection/excision	23
	Transbronchial biopsy	18
	Cytology cell block	7
	Unknown	34

Table 2: Demographic and clinical data for patients with samples analyzed in this study. Not all data categories were supplied for all samples submitted. NOS – not otherwise specified

Metric	Variables		
	Tumor content (%)	Necrosis (%)	Turnaround time (days)
Mean (SD)	43.5 (26.2)	5.89 (15.0)	2.04 (0.4) [2 days 57 min]
Median	40	0	2
Range	2 - 95	0 - 90	1 - 4

Table 3: Laboratory results from all samples analyzed. Shown are the mean, median and range of characteristics of samples received at Biofidelity (n=177). Excluded are samples that were from retrospective pilots, research samples, cancelled runs, or non-NSCLC.

RESULTS

Gene	Variant	Pathology diagnosis		
		Adenocarcinoma (n)	Squamous cell carcinoma (n)	NSCLC, NOS (n)
DNA				
BRAF	exon 15 p.V600E	1		2
EGFR	exon 18 p.G719A	1		
	exon 19 p.E746_A750del	3		1
	exon 19 p.E746_T751delinsA			1
	exon 19 p.L747_A750delinsP	1		
	exon 19 p.L747_P753delinsS	1		
	exon 19 p.L747_T751del	1		
	exon 20 p.A763_Y764insFQEA	1		
	exon 21 p.L858R	9		
ERBB2	exon 20 p.Y772_A775dup	3		
KRAS	exon 2 p.G12A	1		1
	exon 2 p.G12C	15	1	4
	exon 2 p.G12D	4		2
	exon 2 p.G12R	2	1	
	exon 2 p.G12S	2		
	exon 2 p.G12V	10		1
	exon 2 p.G13C	3		
	exon 3 p.Q61H	2		
	exon 3 p.Q61L			1
RNA				
MET	exon 14 skipping			2
ALK	fusion	1		3
RET	fusion		1	
ROS1	fusion	1		
DNA & RNA				
None		28	31	31
Total variant positive		62 (68.9%)	3 (8.8%)	18 (36.7%)
Total overall		90	34	49

Table 4: Variant calls from all patient samples analyzed, stratified by resectable (I-IIIA) vs metastatic (IIIB-IV) NSCLC. Samples at Stage III without further classification are noted separately (III NOS). Samples were included in the analysis if staging information was provided (n=69). Samples with no variant calls which included any QNS tag are indicated separately; samples that included a QNS tag with a positive variant call are integrated into the variant call data.

Figure 2: Shown are the variants identified in all clinical samples analyzed. Although Aspyre Lung has identified samples with two or more variants previously (Herlihy 2024, Evans 2024), all positive samples in this data set had a single variant call.

Patient	Date of samples	Diagnosis	Stage	Sample site	Variant identified
A	2 Aug 2024	Adenocarcinoma	NK	Hip, left, reamings	*EGFR* p.L858R
	7 Aug 2024			Lumbar spine, L2, left, biopsy	*EGFR* p.L858R
B	2 Aug 2024	Adenocarcinoma	IV	Right upper lobe lung nodule	*KRAS* p.G12V
	19 Aug 2024			Left upper lobe lung ground glass opacity	None
C	20 Dec 2024	NSCLC, NOS	IIB	Lymph node EBUS FNA	*BRAF* p.V600E
	20 Dec 2024			Lymph node EBUS FNA	*BRAF* p.V600E
D	4 Mar 2025	Adenocarcinoma	III	Left upper lobe nodule	*KRAS* p.G12V
	4 Mar 2025			Right middle lobe nodule	*KRAS* p.G12R

Table 5: Pairs of samples sent from four patients. Two pairs were sent together, and two pairs were sent within a month of each other. All pairs were from different sites within the patient. The sites and variants identified from each sample are shown.

Gene	Variant	Stages I-IIIA, Number of samples	Stage III NOS, Number of samples	Stages IIIB-IV, Number of samples
BRAF	p.V600E	2		
EGFR	p.G719A			1
	p.E746_A750del			2
	p.L747_A750delinsP	1		
	p.L747_T751del			1
	p.L858R	3		2
ERBB2	p.Y772_A775dup			2
KRAS	p.G12A	1		
	p.G12C	5	2	2
	p.G12D	3		3
	p.G12R		1	
	p.G12V	3	1	2
	p.G13C	1		
	p.Q61H	2		
MET	Exon 14 skipping			1
ALK	Fusion	1		1
RET	Fusion			1
None	None	9	3	11
None & QNS	Tumor, DNA or RNA (or combination)	1		1
Total	Any	32	7	30

Table 6: Breakdown of variants identified in patient samples stratified by diagnosis. Excluded from this table are the four patients whose diagnosis fell outside of these categories.

DISCUSSION

Aspyre Lung has been validated for use with challenging samples with tumor content as low as 10% and necrosis as high as 90%, and resulted in successful clinical reporting across the samples reported here. Variants were also identified in samples with 1-5% tumor cell content, including BRAF p.V600E, EGFR p.L858R, KRAS p.G12C, EGFR exon 19 deletion and ALK fusion.

7/43 (16%) of patients with early or locally advanced NSCLC (I-IIIA) received confirmation of EGFR or ALK variants that render them ineligible for neoadjuvant immune-chemotherapy.

A median turnaround time of 2 days from sample receipt to clinical report was achieved, with 93.2% of samples meeting this metric.

Tissue samples were from multiple centers across the US. To date, no sample meeting primary acceptance criteria has failed QC for processing (defined through internal run controls). Demographics of patients mirror those from the general US NSCLC population by age, gender, pathologic diagnoses and biopsy types.

Variants identified across all cases match those from previous surveys of large-scale cohorts (Friedlaender 2024), indicating that Aspyre Lung captures a proportionate representation of the spectrum of driver variants of NSCLC. 143/177 specimen types were known; 113/143 (79%) were obtained through needle-based biopsies or minimally invasive methods with consequently limited tissue.

SUMMARY

Delays in molecular testing due to assay failure and long TAT often push time to treatment decisions to beyond recommended limits, risking avoidable disease progression and missing the opportunity for curative intent.

Aspyre Lung offers a robust, accessible, cost-effective method for simplified genomic profiling giving patients the actionable biomarker data needed for timely clinical decision-making.

Aspyre Lung is also available for research use in kit format; details available from customersupport@biofidelity.com

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Data from this study are currently being prepared for publication.

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