Aspyre Clinical Test for Lung: Validation Of A Simple, Fast and Robust Method For Molecular Profiling Of Actionable Variants In Plasma

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Abstract

Molecular profiling of tumors is critical for patients with lung cancer to access personalized oncology therapeutics. We developed simplified genomic profiling technology to provide rapid (2-day TAT), accessible, and cost-effective diagnostics informing actionable genomic variants. Aspyre Clinical Test for Lung covers 114 variants in 11 genes (*ALK, BRAF, EGFR, ERBB2, KRAS, RET, ROS1, MET & NTRK1/2/3*) including single nucleotide variants (SNV), insertions, deletions (indel), and gene fusions from plasma-derived cfDNA and cfRNA simultaneously. Aspyre Clinical Test for Lung enables physicians to robustly and quickly act on this information to inform clinical management.

Assay specificity was tested using 60 healthy donor plasma samples. Assay sensitivity was determined using contrived samples in a 2-phase approach. Assay analytical accuracy and precision were assessed using NSCLC patient plasma extracts, healthy donor samples and contrived samples. We assessed the effects of common, potentially interfering substances on assay performance by spiking these into sample extracts.

The sensitivity of Aspyre Clinical Test for Lung exceeds target specifications. The technology is simple and fast using standard laboratory equipment (PCR and qPCR instruments) with cloud-based analysis. Aspyre Clinical Test for Lung has transformative potential for facilitating patient access to cost-effective, rapid, actionable molecular profiling of plasma.

Introduction

Comprehensive genomic testing of *EGFR, BRAF, ALK, RET, ROS1, ERBB2, KRAS, NTRK1, NTRK2, NTRK3* and *MET* is indicated in patients with NSCLC. The detection of abnormalities in these genes informs the use of targeted therapeutic agents. We have previously described development of a novel method, Aspyre, for rapid and low-cost detection of single nucleotide variants, insertions, deletions and complex events from DNA[1] and fusions and exon skipping from RNA[2], and validation of the clinical test for tissue samples derived from FFPE[3]. Here, we describe analytical validation of this assay for samples from plasma including testing of sensitivity (LoD95), specificity, analytical accuracy, analytical precision and resiliency to potential interfering substances.

Study Materials & Methods

Contrived reference samples Variant-specific DNA (SNVs, indels) and RNA (gene fusions, *MET* exon 14 skipping) oligonucleotides were manufactured by commercial suppliers, quantified by dPCR, and spiked into background wild-type DNA or RNA extracted from healthy donor samples.

Clinical samples NSCLC patient blood samples were obtained from commercial biobanks.

Ethical approval Institutional Review Board (IRB) or equivalent was obtained for sample use in diagnostics development by biobanks through collection sites. All data were de-identified so no patients could be identified by study personnel outside of the clinical trial site including the biobanks and the study authors.

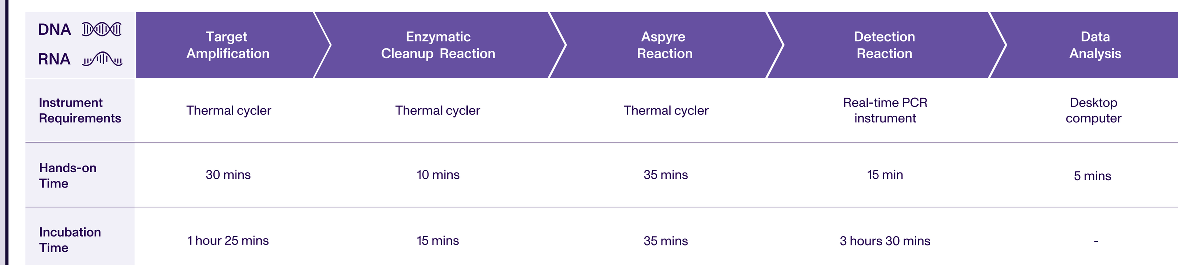
Nucleic acid extraction Nucleic acid from plasma was extracted using the Quick-cfDNA/cfRNA Serum & Plasma Kit (Zymo Research). Concentrations were determined by Qubit.

Aspyre Clinical Test for Lung 20 ng DNA and 42 ng RNA were analyzed at the Biofidelity Inc Laboratory, a CAP/CLIA site, using standardized protocols with simple thermocyclers and a QuantStudio 5 Real-Time PCR instrument (ThermoFisher Scientific). Data from real-time PCR instruments were downloaded and analyzed using custom cloud-based Aspyre Lab v1.3.1 software. All variant calling was blinded to results from orthogonal analyses..

Orthogonal testing DNA was sequenced through targeted capture and sequencing (Roche AVENIO ctDNA Targeted Assay, using Illumina NextSeq).

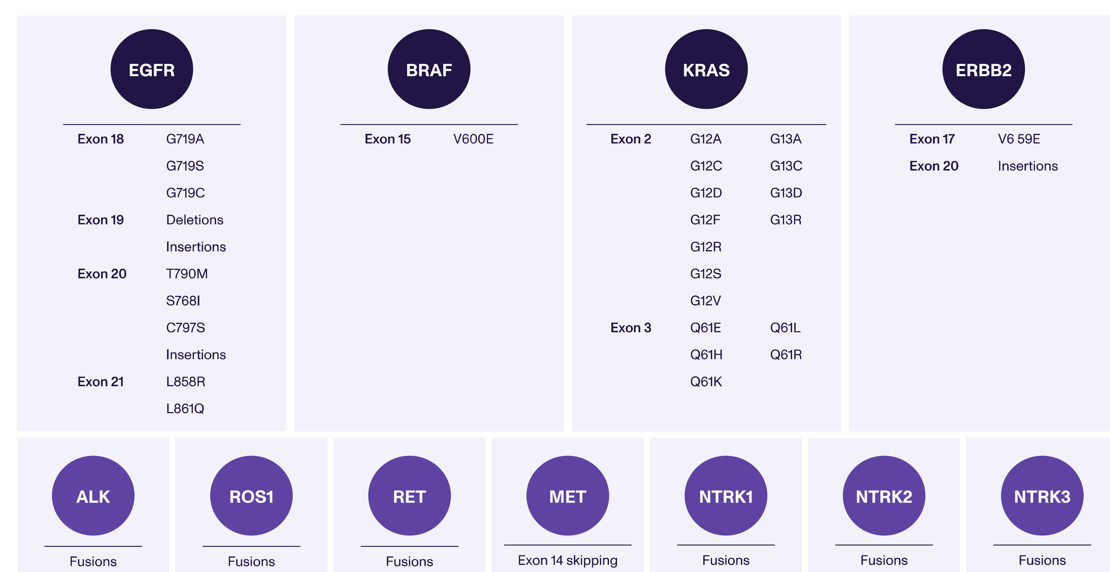
Interfering substances Low variant-containing contrived samples were spiked with hemoglobin and immunoglobulin to mimic potential passthrough of blood-based interferants through extraction process, before testing with Aspyre Clinical Test for Lung.

Aspyre Clinical Test for Lung Assay



DNA / RNA	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	5 mins
Incubation Time	1 hour 25 mins	15 mins	35 mins	3 hours 30 mins	-

The steps of the Aspyre Clinical Test for Lung assay workflow after nucleic acid extraction. The TAT from sample receipt to report return is completed at Biofidelity Inc Laboratory within 2 days.



Genes and variants covered by the Aspyre Clinical Test for Lung panel. A total of 114 genomic variants are assessed and aggregated as actionable variant calls where appropriate for treatment guidance e.g. *NTRK*-fusion positive call is aggregated from multiple potential fusion pairs.

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Aspyre
Clinical Test

Tissue

Lung Report

Patient Information	Specimen Details	Ordering Physician
Name: John Doe Date of Birth: 01 Jan 1960 Sex: Male	Specimen Type: FFPE Tissue Collection Date: 23 Aug 2023 Received Date: 26 Aug 2023 Report Date: 28 Aug 2023 Accession ID: BFI23243420 Block ID: B-23-1234	Name: Example report Institution: Test Oncology

Test Results Summary

Test result summary comments

Clinical Information and Indication for Testing

NSCLC - Non-Small Cell Lung Cancer

Biomarkers Identified

Biomarker	Variant Type	Associated Therapeutics*
BRAF exon 15 p.V600E	Missense	Dabrafenib + trametinib Dabrafenib Vemurafenib

*Bold text indicates guideline preferred therapeutics

Biomarkers Not Detected

The patient tested negative for targeted genomic variants in the following genes: ALK, EGFR, ERBB2, KRAS, MET, NTRK1/2/3, RET, ROS1

Result Interpretation

The Aspyre Clinical Test for Lung detected a clinically significant missense variant (p.V600E) in exon 15 of the BRAF gene. BRAF, an intracellular kinase, is frequently mutated in NSCLC; BRAF V600 is the most common activating mutation and a mutational hotspot within the protein kinase domain (IPR000719). BRAF is altered in 5.15% of NSCLC patients with BRAF V600E present in 1.37% of all NSCLC patients (https:// www.mycancergenome.org). Dabrafenib in combination with trametinib has evidence of efficacy for the treatment of patients with BRAF V600E mutant NSCLC (https://pubmed.ncbi.nlm.nih.gov/28919011/; https://pubmed.ncbi.nlm.nih.gov/27283860/). Single-agent dabrafenib and vemurafenib have also shown evidence of efficacy in patients with BRAF V600E under certain circumstances in NSCLC. Clinical correlation is REQUIRED.

EXAMPLE REPORT

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The Aspyre Clinical Test for Lung patient report. The two-page report includes a summary of patient and sample details, a summary of test results including biomarkers identified, and biomarkers not detected, and an interpretation of results.

Results

Sensitivity

Variant type	Gene variant – % VAF or copy number	Total positive/ total tests*
DNA		
SNV	*KRAS* exon 2 G12C COSM516 – 0.2	77/80
	EGFR exon 21 L858R COSM6224 – 0.3	
	EGFR exon 20 T790M COSM6240 – 0.4	
	BRAF exon 15 V600E COSM476 – 0.2	
Deletion	*EGFR* exon 19 E746-A750del COSM6223 – 0.2	60/60
Insertion	*ERBB2* exon 20 Y772-A775dup COSM20959 – 0.8	
	EGFR exon 20 A767-V769dup COSM12376 – 0.4	
RNA Fusions		
Fusion	*EML4-ALK* E13_A20 COSF408 – 6	119/120
	KIF5B-RET K15_R12 COSF1232 – 18	
	CD74-ROS1 C6_R36 COSF1200 – 6	
	TPM3-NTRK1 T8_N10 COSF1329 – 6	
	QKI-NTRK2 Q6_N16 COSF1446 – 6	
	ETV6-NTRK3 E5_N15 COSF571 – 6	
RNA Exon Skipping		
Exon skipping	*MET* exon 14 skipping – 100	17/20

LoD95 confirmation data. Results of testing 20 replicate contrived samples at levels pre-determined by an LoD estimation (range 0.2 - 1.6% VAF for SNV/indel, 6-48 copies for gene fusions and 100-200 copies for *MET* exon 14 skipping). Confirmed LoD95 by mutation class (SNV, indel, fusion and *MET* exon 14 skipping) were 0.25% VAF for SNV, 0.4% VAF for indel, 6 copies for fusions, and 100 copies for *MET* exon 14 skipping.
*Results were aggregated across the given variant class.

Specificity

Category	Number (n): Tested per Aspyre assay	Total Tested	Positive	False positive rate
Samples (DNA & RNA)	1	60	0	0
Nucleotide Variants	114	6840	0	0
Reportable Variants	71	4260	0	0
SNVs	26	1560	0	0
Indels + complex substitutions	31 + 20	3060	0	0
Fusions	36	2160	0	0
Exon Skipping	1	60	0	0

Negative variant calls from samples tested during AV. A single 'Reportable Variant' may cover multiple nucleotide variants where the associated therapeutics are identical: Aspyre Clinical Test for Lung tests for 114 variants, and outputs 71 potential calls. The expected-negative calls from all experiments within the assay validation (n = 26657) were combined to estimate a variant **false positive rate of 0.004% (CI95 0 - 0.02%)**

Analytical Precision

Level	Metric	Actual % (CI95, Clopper-Pearson)
Sample	PPA	100 (90-100)
	NPA	100 (94-100)
Variant	PPA	100 (95-100)
	NPA	100 (99.91-100)

Summary of analytical precision (repeatability and reproducibility) data. Shown are the positive and negative percent agreement values between runs of Aspyre Clinical Test for Lung (PPA/NPA), demonstrating 100% reproducibility (inter-run precision) and repeatability (intra-run precision). Samples were assayed in triplicate in four independent runs across four days by two operators using two real-time PCR instruments and two reagent lots

Results

Analytical Accuracy

Level	Metric	Actual % (CI95, Clopper-Pearson): DNA	RNA
Sample	PPA	100 (80-100)	88 (47-99.7)
	NPA	100 (91-100)	100 (91-100)
Variant	PPA	100 (80-100)	88 (47-99.7)
	NPA	100 (99.9-100)	100 (98-100)

Summary of analytical accuracy of Aspyre Clinical Test for Lung assessed using contrived and clinical samples. The PPA and NPA obtained across samples and variants for both clinical and contrived sample types and the associated 95% confidence intervals are shown. The study included 32 clinical samples and 7 variant-positive contrived samples for both DNA and RNA. The clinical cohort included 11 samples positive for variant detection by the orthogonal method.

Interfering Substances

Analyte	Variant	VAF/ Copies	Total positive / total replicates: control	hemoglobin	IgG
DNA	*KRAS* exon 2 G12C COSM516	0.4%	5/5	5/5	5/5
DNA	*EGFR* exon 19 E746-A750del COSM6223	0.4%	5/5	5/5	5/5
RNA	*EML4- ALK* E13_A20 COSM408	12 c	5/5	5/5	5/5
RNA	*CD74-ROS1* C6_R36 COSF1200	12 c	5/6*	4/5	5/5

Contaminants carried over from blood plasma extraction do not interfere with the Aspyre Clinical Test for Lung assay. Two DNA and two RNA contrived samples at twice the LoD95 were spiked with 1µg/µL hemoglobin or 150pg/µL immunoglobulin G (IgG) to mimic carryover from blood-derived substances that are not removed during sample extraction. Shown are the calls made compared to total Aspyre Clinical Test for Lung assay tests.
*1 false positive potentially due to cross-contamination

Discussion

Aspyre®

- Is a novel technology based on pyrophosphorolysis of oligonucleotide probes perfectly hybridized to sample-derived cfDNA or cDNA (from cfRNA) sequences.
- Combines the benefits of multi-gene testing with rapid TAT
- Has simple bioinformatics, and with easily interpretable clinical decision making (only actionable markers are tested)

In this study we demonstrate

- Aspyre Clinical Test for Lung has excellent analytical sensitivity, comparable to current NGS-based testing solutions with median LoD95:
  - 0.25 % VAF for SNV from DNA
  - 0.4 % VAF for indel from DNA
  - 6 copies for gene fusions from RNA
  - 100 copies *MET* exon 14 skipping from RNA.
- The assay showed 100% specificity in LoB studies with 1 false positive (or cross contamination) result out of 26657 calls made from all validation sample tests
- The assay is highly reproducible and repeatable across different operators, reagent lots, runs, days and instruments.
- No effects from carry over of interfering substances were detected.

Aspyre Clinical Test for Lung simplified genomic profiling informs fast, sensitive, cost-effective and actionable genomic data, and is transformative for cancer care

References

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