AACR: 5350

External user testing of Biofidelity's ASPYRE-Lung® assay demonstrates breakthrough capabilities and ease of use

Paulina Powałowska[1], Nicola D Potts[1], Brandon A Smith[2], Kala F Schilter[2], Honey V Reddi[2], Lan Beppu[3], Jerald Radich[3], Charles Massie[4], Quentin Vicentini[5], Tom Brown Jr.[5], Robert J Osborne[1,6], Barnaby Balmforth[1,6]

1 – Biofidelity Ltd. Cambridge Science Park, Cambridge, UK. 2 - Medical College of Wisconsin, Milwaukee, WI, USA. 3 – Fred Hutchinson Cancer Research Centre, Seattle, WA, USA. 4 – CRUK, Hutchison MRC Research Centre, Cambridge, UK. 5 – ATDBio Ltd. Oxford Science Park, Oxford, UK. 6 – Biofidelity Inc. Research Triangle Park, NC, USA.

Biomarker screening in NSCLC

More than 20 targeted therapies for non-small cell lung cancer (NSCLC) have been FDA approved[1], offering better outcomes for patients with fewer side effects. Multi-gene biomarker testing is required for optimal patient care, but <50% of US patients receive it[2].

Biofidelity's ASPYRE-Lung® assay is designed to solve this problem by enabling simple adoption of ultra-sensitive biomarker testing worldwide, using existing instrumentation, staff & IT infrastructure.

ASPYRE-Lung covers all NCCN-recommended genes, with **114 clinically actionable biomarkers** across 11 genes, including 77 **DNA** variants (substitutions and indels), and 37 **RNA** variants (fusions and exon skipping) with a **fast turn around time**.

ASPYRE LUNG

>> Simple >> Fast >> Low Cost >>

Current barriers to biomarker testing

Next-generation sequencing (NGS) is currently the only viable multi-gene biomarker testing solution, but has significant limitations:

- Complex workflows & analytics, requiring skilled staff & automation
- High costs of implementation & execution
- Slow turnaround times (typically 2-5 weeks)
- Required to batch large numbers of samples
- High sample-input requirements
- Poor reimbursement

This has resulted in centralization of testing in large labs, further limiting access & increasing turnaround times

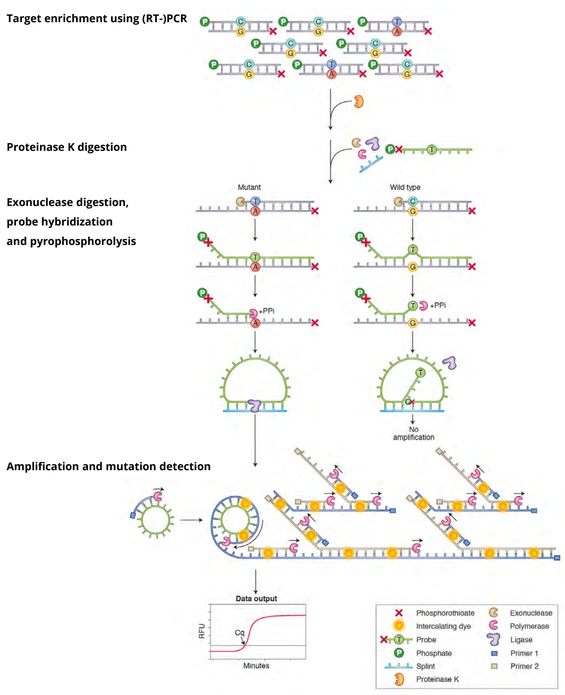
Simple concept design

Biofidelity developed **ASPYRE-Lung®** to overcome barriers to access:

- **Simple to adopt** using instrumentation already in >95% of labs
- **Fast turn around time -** nucleic acid to result in same day
- **Low cost** & no requirements for sample batch size
- Parallel **DNA & RNA** analysis across 11 genes
- **Plasma or tissue** analysis in a single assay
- Analysis requiring **no specialist bioinformatic support**

The ASPYRE assay

Schematic overview of ASPYRE technology workflow



Adapted from Silva et al. 2021[3]

DNA & RNA in parallel methodology:

External users were provided with DNA liquid biopsy reference samples containing multiple mutations, and single mutation RNA reference samples. All samples were run in-house to provide comparison data to test site outputs.

Reference samples for:

DNA

SeraCare ctDNA Mutation Mix v2 containing mutations in *BRAF, EGFR, ERBB2* and *KRAS* at 0.25% variant allele fraction (VAF).

RNA

Synthetic RNA oligonucleotides containing fusions for *RET, ROS1* and *NRTK1* at a mean of 6 copies in background of 1ng lung RNA.

ASPYRE-Lung assay results:

Simultaneous identification of 114 mutations from DNA and RNA material in a single assay demonstrating breakthrough capability

Sensitivity

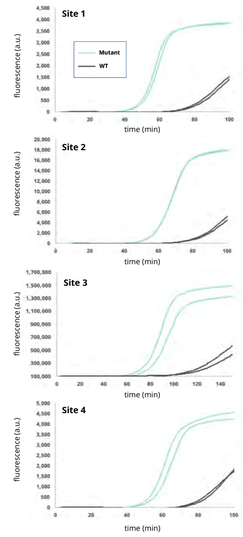
All test sites correctly identified nine mutations at either 0.25% VAF (DNA) or 6 copies (RNA), consistent with or exceeding the performance of NGS or direct single-mutation detecting assays.

Two mutations were not called, appearing as false negatives (FN) from all sites in this testing round. Subsequent improvements to the assay probes resolved all these FN signals. Further development and testing has also improved mutation calling robustness.

Specificity

No false positive (FP) results were observed. Cross-talk between two variants was observed, but the analysis tool enabled this to be corrected, resulting in no impact on specificity.

100% concordance was observed between in-house results and all test sites.



Fluorescence signals observed for the detection of EGFR L858R mutation at 0.25% allele fraction across multiple sites. Different scaling of y-axis is due to use of different instrument manufacturers/models and is normalized during analysis, so does not affect variant calling

Ease of Use

Four sites were able to **independently** on-board the assay using **existing equipment & personnel**.

Reagents & instructions for use (IFU) were supplied, with no additional training. Each site established the assay and produced **results within one day**.

Turnaround time, with a **single operator** performing the work, was less than one day. Operators ranged from low-experience Molecular Technologists to PhD scientist and required no further training.

Hands-on time was approximately 1.5 hours divided across four steps.

Feedback process and improvements

Feedback on assay format and IFU was received from all four testing sites in the form of questionnaires and open discussion.

Revisions to the assay have been subsequently incorporated to further improve simplicity & ease of adoption:

- Improved usability, e.g. minimum pipetting volume adjusted
- Clearer instructions for use
- Improved reagent stability and shipping conditions
- Improved assay & software analysis, reduced FP and FN rates

Conclusions

ASPYRE-Lung® is a break-through technology developed to enable all laboratories to offer high-sensitivity NCCN guideline-compliant comprehensive testing for NSCLC patients.

The assay can detect low allele fractions of SNVs, indels, fusions & exon skipping variants with simultaneous DNA & RNA analysis.

Ease of adoption is key to broad access. All sites in this study were able to set up & complete the assay workflow in < 1 day using existing instruments and personnel.

Detection concordance between all four sites & in-house testing:

- All sites detected variants at 0.25% allele fraction using 20ng input DNA & 6 copies using 1ng input RNA.
- Two false negatives & one cross-talk between variants observed; subsequent development has resolved these findings.

Thanks

Biofidelity Ltd extends our thanks to the collaborating partners who undertook alpha testing of our first generation ASPYRE-Lung product: MCW- USA, Fred Hutchinson CRC – USA, CRUK – UK, ATDBio – UK.

References

[1]Tan AC, Tan DSW.
Targeted Therapies for Lung Cancer Patients With Oncogenic Driver Molecular Alterations
J. Clin Oncol. 2022 Feb 20;40(6):611-625

[2]Robert NJ, Nwokeji ED, Espirito JL, Chen L, Karhade M, Evangelist MC, Spira AI, Neubauer MA, Bullock SA, Coleman RL.
On behalf of MYLUNG Consortium Collaborators: The U.S. Oncology Network and sponsors
Biomarker tissue journey among patients (pts) with untreated metastatic non-small cell lung cancer (mNSCLC) in the U.S. Oncology Network community practices.
Journal of Clinical Oncology 2021 39:15 (suppl): 9004

[3]Silva AL, Powalowska PK, Stolarek M, Gray ER, Palmer RN, Herman B, Frayling CA, Balmforth BW.
Single-copy detection of somatic variants from solid and liquid biopsy.
Sci Rep. 2021 Mar 16;11(1):6068.