Identification of Driver Mutations in Transbronchial Needle Aspirates of Suspicious Lung Nodules Concurrent with Diagnostic Bronchoscopy

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INTRODUCTION

Lung cancer remains the number one cause of cancer-related mortality in the United States for both men and women. While overall 5-year survival rates remain poor, the development of therapies that target genetic alterations in some lung cancers has created new and better treatment options for those patients, particularly those with advanced disease. The National Comprehensive Cancer Network (NCCN) Guidelines[1] currently recommend testing for multiple targets for which therapies are available, including *EGFR, BRAF, HER2, KRAS, ALK, ROS1, NTRK1/2/3, MET* exon 14 skipping and *RET,* using comprehensive molecular profiling for all patients with advanced or metastatic disease with adenocarcinoma, large cell carcinoma, and non-small cell lung cancer (NSCLC) not otherwise specified. The Guidelines also recommend consideration of testing in patients with squamous cell carcinoma.

Patients with NSCLC and nodal disease will typically have both diagnosis and stage determined by cytology obtained by transbronchial needle aspiration (TBNA). The ability to detect the NCCN-recommended alterations with accuracy in small cytology samples is therefore crucial. These minimally invasive approaches to obtain tissue present a potential limitation for molecular testing — the possibility that insufficient tissue remains for full molecular testing due to tissue depletion after necessary staining and immunohistochemistry, as well as PCR for targeted molecular testing performed at many institutions. The Guidelines specifically recommend strategies for optimizing tumor yield to mitigate this risk but also recommend repeat biopsy when the yield is inadequate. Studies show that as many as 13% of patients may need repeat sampling,[2] leading to increased costs and delays in time to appropriate therapy.

Collection of an additional dedicated biopsy sample for molecular testing at the time of a diagnostic and staging bronchoscopy could enable earlier identification of appropriate molecular targets, reducing the likelihood of insufficient tissue while improving the time to results. We show the feasibility of detecting NCCN-recommended alterations by comprehensive genomic profiling (CGP) of samples obtained with TBNA obtained during the initial bronchoscopy, using targeted DNA and whole-transcriptome RNA sequencing in a panel of more than 50 genes designed specifically to meet biomarker testing needs of lung cancer patients now and in the future.

METHODS

- **Patient samples.** TBNAs were taken during the patient's diagnostic bronchoscopy and collected into RNA protect under an IRB-approved collection study. Samples were transported to Veracyte and frozen at -80°C until extraction.
- **Nucleic Acid Extraction and QC.** TBNAs were extracted using the Qiagen AllPrep Micro Kit according to manufacturer's instructions. DNA was quantitated using PicoGreen (Promega). RNA was quantitated using Quantifluor (Promega). Fluorescence for both PicoGreen and Quantifluor was read on a Tecan Microplate reader. RNA Integrity was determined using the Agilent 2100 BioAnalyzer.
- **NGS assays.** Percepta Genomic Atlas assay is a combination of targeted AmpliSeq Focus DNA and whole exome TruSeq RNA Exome next generation sequencing (NGS) (Illumina) comprising a comprehensive gene panel including *EGFR, ALK, ROS1, BRAF, KRAS, NTRK1/2/3, MET, RET,* and *HER2*. AmpliSeq Focus Assay was run with 10 ng of DNA according to the manufacturer's instructions and sequenced using the MiniSeq (Illumina). TruSeq RNA Exome was run using 50 ng RNA with a custom Hamilton-automated implementation of the assay and sequenced on the NextSeq 500/550. TruSight Oncology 500 (TSO500, Illumina) was run with 40 ng of DNA according to the manufacturer's instructions and sequenced on the NextSeq 500/550 (Illumina).

TABLE 1.
Subject Demographics

Histopathology Diagnosis	Subjects	Mean Age	Male	Female	TBNAs
NSCLC-Adeno	7	71	4	3	12
NSCLC-SCC	5	74	4	1	8
NSCLC	4	67	0	3	6
SCLC	3	65	1	2	6
Metastatic Leiomyosarcoma	1	61	0	1	2
Benign	5	53	2	3	9

RESULTS

FIGURE 1.
Percepta Genomic Atlas informs treatment decision at the time of diagnosis.

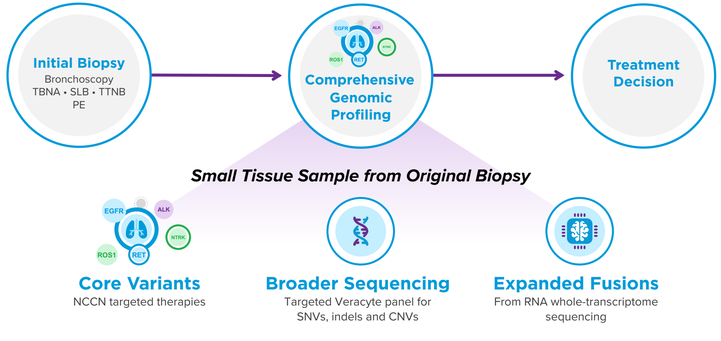
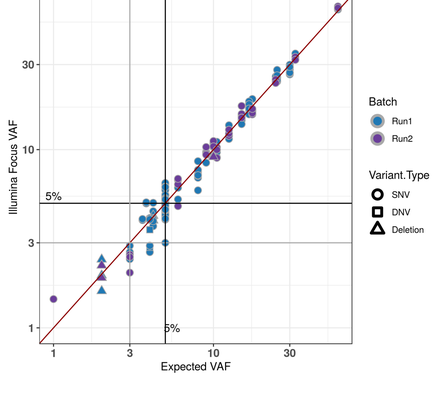


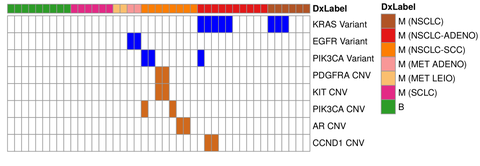
FIGURE 2.
PGA is highly reproducible for detecting nucleotide variants in DNA.



Illumina AmpliSeq Focus detection of HD701 and HD827 control variants (Horizon Discovery). X-axis is the expected Variant Allele Frequency (VAF) from the Horizon Certificate of Analysis and the y-axis is the VAF detected by Illumina AmpliSeq Focus. Shown are the results of 2 plates – blue fill = run 1, purple fill = run 2. Circle point shape = SNV, square point shape = DNV, and Triangle point shape = indel. Dark horizonal and vertical lines are = 5% VAF, grey horizontal and vertical lines are 3% VAF.

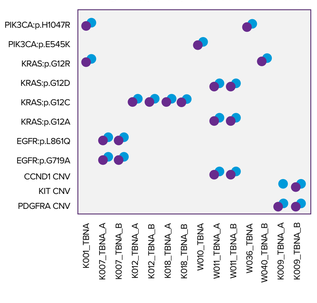
RESULTS (CONT'D.)

FIGURE 3.
DNA variants are detected in small bronchoscopy samples taken during diagnostic workup.



The first row shows the Diagnostic Histopathology Label – M NSCLC (Brown), NSCLC-Adeno (red), NSCLC-SCC (orange), Metastatic Adeno (salmon), Metastatic Leiomyosarcoma (light orange), SCLC (pink), Benign (green). The waterfall plot shows positive alterations (rows) and samples (columns). SNVs (blue) and Copy Number Variants (CNV) (orange) were detected in the TBNAs.

FIGURE 4.
Percepta Genomic Atlas can detect variants within bronchoscopy samples.



Variants identified in bronchoscopy samples. Each row is a variant or CNV, each column is a sample. Purple Circles indicate positive for Percepta Genomic Atlas, Blue circles indicate positive for reference NGS Assay. >95% agreement between Percepta Genomic Atlas and reference NGS assay.

CONCLUSIONS

- The Percepta Genomic Atlas NGS assay is being developed to specifically address the challenge of providing timely, comprehensive genomic profiling from small lung cancer biopsy samples using DNA and RNA sequencing.
- >95% of all TBNA had sufficient RNA and DNA to run through Percepta Genomic Atlas. 100% of subjects had sufficient nucleic acid from at least 1 TBNA.
- Clinically actionable gene alterations were detected in TBNAs collected into RNA protect at the time of diagnostic bronchoscopy.
- Early identification of these molecular alterations could decrease the need for additional procedures due to inadequate tissue and lead to earlier appropriate therapeutic decisions.

References

1. NCCN Guidelines, version 4.2021, Non-Small Cell Lung Cancer
2. Lim C, et al. *Ann Oncol* 2015;26 (7):1415-1421.

Disclosures

Joshua Babiarz: Veracyte, Inc. Employee