Liquid Biopsy of Lung Cancer based on Extracellular Vesicles

Juan Pablo Hinestrosa[1], Harmeet Dhani[1], Gregor Schroeder[1], Jean M. Lewis[1], Heath I. Balcer[1], Larry Arakelyan[1], Razelle Kurzrock[2,3], and Paul R. Billings[1]

1. Biological Dynamics, Inc., San Diego, CA, USA, 2. Medical College of Wisconsin, Milwaukee, WI, USA; 3. WIN Consortium for Precision Medicine, Villejuif, France

Overview

Introduction

Liquid biopsy approaches for lung cancer are becoming increasingly common for diagnosis, prognosis, and management of the disease. Of the approximately 236K new lung cancer cases that will be diagnosed in 2023, over 70% of those will be regional or distant with diminished chances of curative resection. Therefore, highly sensitive and specific approaches for early detection are key for mortality reduction. Extracellular vesicles (EVs), a new class of blood-based cell-free biomarker, can be informative for the diagnosis of lung cancer at its earliest stages.

Methods

A case-control cohort of blood plasma samples from 143 pathologically confirmed lung cancer cases (stage I = 88, II = 41, III = 14; median age = 63 yrs.) and 491 controls (median age = 58 yrs) was used in this study. EVs were isolated using a proprietary technology and the EV protein cargo was analyzed via immunoassay.[1,2] A machine learning (ML) engine was employed to determine the most informative biomarkers and algorithm for differentiation between cases and controls in a detection-type setting.

Results

Using a stratified cross-validation approach, we found a biomarker signature that yielded an AUC of 0.971 (95% CI: 0.956 – 0.986) with an overall sensitivity of 91.6% (CI: 85.9% - 95.1%) at a specificity of 91.0% (CI: 88.2% - 93.3%). By stage, the following sensitivities were obtained: stage I: 93.2%, stage II: 87.8%, stage III: 92.9% at the 91% specificity threshold. The algorithm developed includes 13 EV-protein biomarkers using the adaptive boosted tree methodology.

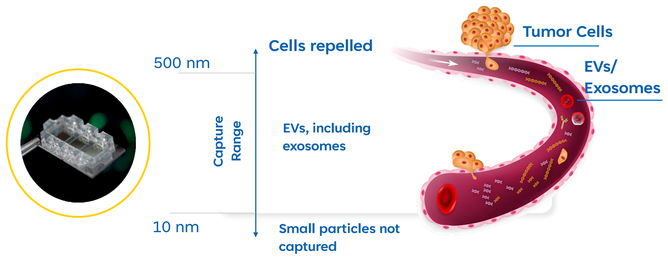

Figure 1. EVs/Exosome Isolation from Blood. The capture range for the ExoVerita® platform allows for the efficient isolation of EVs/Exosomes directly from blood while being unperturbed by large cellular material (> 500nm) or small analytes (<10nm). This yields a more refined isolation for the analytes of interest, e.g. EVs.

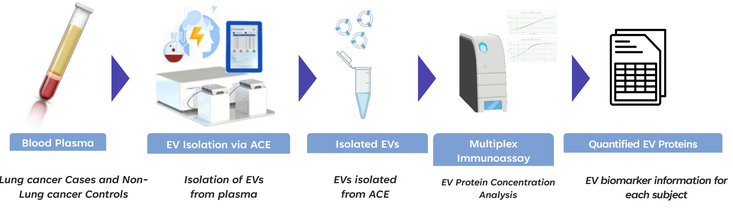

Figure 2. Data Acquisition Workflow. Cases and control samples are collected in K_2EDTA plasma and the EVs are isolated on the Verita™ Platform (Biological Dynamics, Inc.). The isolated EVs are tested for their EV protein concentration on a multiplex immunoassay commercial instrument. The quantified EV proteins are then arranged in relation to the subject information (stage, age, sex, etc)

Study Cohort

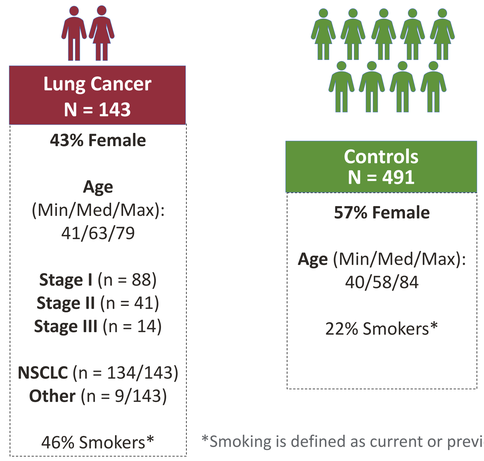

Figure 3. Study Cohort. The study cohort is composed of 143 Lung Cancer cases from stages I to III with non-small cell lung cancer (NSCLC) comprising 93.7% (n = 134/143), and 491 controls (no known cancer diagnoses or autoimmune diseases). All the lung cancer cases were reviewed by an independent pathologist for accuracy of staging.

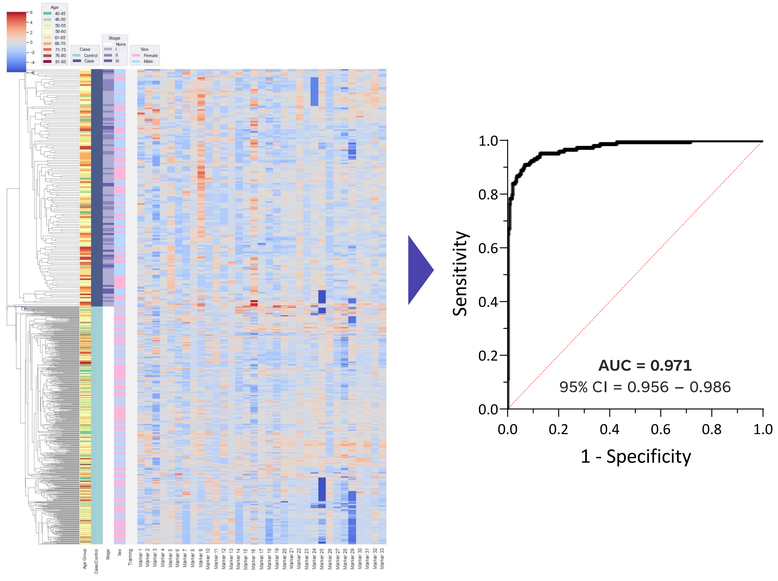

Figure 4. EV-Protein Biomarkers. A) Hierarchical clustering for the EV-protein biomarkers tested across the cases and controls. The data was transformed by dividing each observation by the mean of non-cancer observations within each individual EV biomarker and then applying a log2 transformation. B) Receiver Operating Characteristic curve for the ML algorithm, based on the adaptive boosted tree methodology, using the most informative biomarkers (13 EV proteins) for differentiation between lung cancer cases and controls. The AUC confidence intervals were calculated using the two-side Wilson approach.

DISCLAIMER: This is preliminary raw data from an ongoing study; we are currently evaluating the platform and assessing additional subjects as part of our test development. Change in performance, including performance degradation, is usually expected with both larger sample sizes and real-world settings.

Results

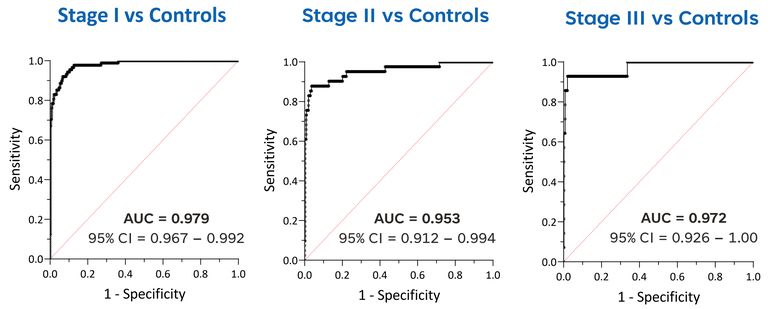

Figure 5. Receiver Operating Characteristic (ROC) Curves per stage. ROC curves and respective AUCs for comparison of stage I, II and III lung cancer cases to the control subjects using the ML model shown in Figure 4. The test performance is maintained at all stages. The AUC confidence intervals calculated using the two-side Wilson approach.

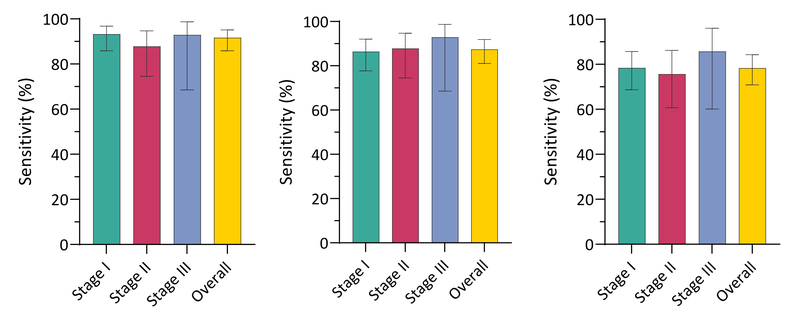

Figure 6. Lung Cancer Sensitivity at Multiple Specificities. Overall and per stage sensitivity at three specificity thresholds (91%, 95% and 99%) showing the ability of the assay to perform well at different diagnostic type settings, e.g high-risk cohorts. Further refinement and independent test sets will allow for locking down the final test specifications. The error bars represent the two-sided Wilson 95% confidence intervals.

Conclusions & Disclosures

Our pilot study demonstrates utility of a blood-based approach that can detect lung cancer at early stages, when treatment can be more effective. By combining multiple EV protein signatures, it is possible to achieve high sensitivity and specificity. Independently collected cohorts including confounding conditions, such as benign lung nodules and COPD, are being evaluated for validation purposes to improve performance in clinical settings.

Disclosures

This study was sponsored by Biological Dynamics, Inc.

References

1. Hinestrosa et al. *Front. Bioeng. Biotech.* **2020**, *8*, 581157
2. Hinestrosa et al. *Commun. Med.* **2022**, *2*, 29

Juan Pablo Hinestrosa, Ph.D.
(jp@biologicaldynamics.com)