8533 **Poster Session**

Clinical validation of an activity-based enzyme assay for early stage lung cancer.

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Background: The USPSTF guidelines recommend annual LDCT scans for 15.5 million adults with a heavy smoking history. While LDCT can reduce deaths by 20%, screening compliance remains low. A blood test with clinically useful, cost effective performance could improve compliance and access when integrated with the standard of care. We describe here a lyophilized nanosensor system for detecting protease enzyme activity in sera with clinically useful diagnostic activity in early stage lung cancer. We evaluated the performance of the assay by examination of prospectively collected sera for detection of cancer in high risk patients. **Methods:** Sera were obtained in multiple independent studies to include pathologically confirmed, treatment naive lung cancer patients and LDCT confirmed negative individuals. Protease activity was measured on 18 different nanosensors built with protease targets mainly selective for members of the Matrix Metalloproteinase and Cathepsin families. Lyophilized plates were incubated with serum and enzyme activity was measured indirectly as a continuous variable by a fluorescent plate reader. A machine learning modeling tool (Emerge) was used to detect signal associated with a cancer "fingerprint" of protease activity. The analysis stratified allocation into training and testing sets of 250 samples each and reserved a third out of sample validation set (250 samples) for reporting. **Results:** 750 clinical samples included 30% lung cancers, 63% males, 91% smokers, and an average age of 63 years (SD=9). Cancer cases were distributed across stages I (41%), II (17%), III (20%) and IV (20%) with 5% unknown. Histological classification included 59% adenocarcinoma, 31% squamous cell and 11% other subtypes. Using an Emerge model with only nanosensor activity and gender as inputs, we evolved a balanced algorithm. The algorithm can be further modified to favor sensitivity or specificity depending on the application by applying model weighting factors. We report the performance observed in the 250 out of sample validation set at three points on this spectrum (Table). Among Stage I cancer samples, the balanced algorithm had an accuracy of 90% (26/29). **Conclusions:** Current LDCT tools show low compliance. We demonstrate clinical validity of a cost effective tool to detect lung cancer in support of LDCT screening. Based on a simple blood sample, the current test may predict early stage lung cancer with an accuracy of 90%. The performance suggests applications in LDCT compliance, post LDCT management, and eventually screening. A clinically validated version of this technology is being evaluated as a triage tool for LDCT screening. Research Sponsor: Hawkeye Bio, Inc.

	Specificity Max	Balanced	Sensitivity Max
Sensitivity:	62%	90%	97%
(95% CI)*	*(49-73%)*	*(80-96%)*	*(90-100%)*
Specificity:	86%	82%	54%
(95% CI)*	*(80-91%)*	*(76-88%)*	*(47-62%)*

* Clopper Pearson Exact Method.