Potential Blood-Based Biomarkers for the Early Detection of Lung Cancer

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Prevent Cancer Meeting
Dialogue for action on cancer screening and prevention
Disclosures

• I am an unpaid advisor to Oncimmune, Integrated Diagnostics, Ajinomoto, Natera, Optellum, Veracyte and Nucleix.

• I am the PI on studies at VUMC sponsored by Ajinomoto and Veracyte. The sponsors do not support my salary.
RATIONALE: Early Detection of lung cancer saves lives

• Saves lives; >10 x more than non-surgical therapeutics
• Increases the chances for successful treatment
• Reduces cost of cancer care
• Results in the decline in U.S. cancer deaths
• Enjoys only 15% of cancer research funds

Rationale for the use of biomarkers in the CT screening era

- Saved by Screening: 12,000
- NLST criteria: 26.7% of population covered: 42,700
- All died of lung cancer
- Other risks:
  - Smokers quit > 15 years ago
  - Younger
  - Familial
  - COPD
  - Radon
  - Diesel
  - Viral
  - ... 160,000

Ma, Cancer 2013
Molecular biomarkers in lung cancer management

Atwater, Ann Transl Med 2016
A 3 pronged approach

Aim 1. Structural Imaging
- Radiomics
- Volume Growth

Non-invasive evaluation of IPNs

Aim 2. Molecular Biomarkers
- Vandy signature
- Oncimmune signature
- Integrated Diagnostics signature

Deliverables:
- Improved diagnostic accuracy of IPNs, reduced benign bx rate
- Reproducible volumetric analysis
- Validation of promising blood biomarkers
Lung cancer

Granuloma

Lung cancer

Granuloma
## Diagnostic biomarkers for lung cancer

<table>
<thead>
<tr>
<th>Candidates</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Phase 3</th>
<th>Phase 4</th>
<th>Phase 5</th>
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<tbody>
<tr>
<td></td>
<td>Discovery, Prediction</td>
<td>Assay validation</td>
<td>Retro-longitudinal</td>
<td>Prospective screening</td>
<td>Cancer Control</td>
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<td><strong>SERUM/PLASMA</strong></td>
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<td>VOCs</td>
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</table>
Criteria for clinical use of biomarkers

- Analytical Validation of the assay
- Validates in an Independent cohort
- Causes a Stage shift
- Reduces false positive
- Adds value or change management
- Cost effectiveness
- Cancer Control

Cost & time

Innovation & biology

Atwater et al. Seminars in Respiratory and Critical Care Medicine, 2016
Sensitivity Matters

A: ELISA
B: 100% vasc permeability
C: not shed by healthy cells
D: shedding is 1000x nl
E: not shed in nl and improved 1,000 x assay sensitivity from baseline
F: not shed in nl and improved 100,000 assay sensitivity from baseline

Hori Gambhir STM 2011
ctDNA: Outstanding specificity
We still lack sensitivity!

- Variable input 1-20 ng of cell free DNA
- Detection of a mutation in < 1: 100,000 cells-- One diploid cell= 6.6pg
- Size of ctDNA is around 166 bp (nucleosome + linker)
- 1 ng of DNA fragment of 166 bp = 5x10^9 molecules
- ctDNA is 0.01% to >90% fraction of circulating DNA
- CAPP seq detects 2.5 ctDNA molecules in 10^6 -- Safe-Seq has sens 9 in 10^6
- Detection limits of mutant allele fractions in ctDNA is 0.1-0.01%, or ~<1 mutant template molecular in 1 mL of plasma
- Need coverage depth as high as 10,000× to uncover rare ctDNA mutations. Coverage depth competes with sequencing errors

Seifert et al. CCR 2016, Cohen et al. Science 2018
CTCs We lack sensitivity! Ilie, PLoS One 2014

Murat Karabacak et al.
STM, 2013.

Dhar et al.
Renier et al.
Precision oncology 2017
Thoracic Imaging Repository- XNAT Radiomics

209 nodules; 221 radiomics features (7 components)

Funding
U01 CA196405

Publications
Maldonado AJRCCM 2015
Cytometry B Clin Cytom. 2017
Diehn, Nature Med 2014

Shared Resources
Genomic Sciences
Quantitative Sciences
Flow/Mass Cytometry core
Innovative Translational Research
CYFRA 21-1

• Fragment of Cytokeratin 19.
• CYFRA 21-1 epitope is a polypeptide, which is most likely released following cell death (Stieber et al, 1993; Sheard et al, 2002).
• Serum fragments of cytokeratin-19 can be detected using anti-CYFRA 21-1 antibody (Pujol et al, 1993).
• Patients with nonmalignant disease showed almost no elevation of serum CYFRA 21-1, except in cases of cirrhosis, renal failure, or infectious lung disease
• Stability data demonstrated that CYFRA 21-1 is stable in serum for a minimum of 48 h at ambient temperatures and 14 days at 4 °C.
Compensated Backscatter Light Interferometry (CBSI)

Mix-and-Read Free-Solution Assay

- Determine if probe produces quantifiable signal.
  - Calibration done using spiked serum samples.
  - Non-specific binding tested by titrating antibody with serum.
Daily Standards of Spiked Serum Ensures Accurate CYRFA Quantification

- Black line = Pooled calibration curve
- Grey area = 95% confidence interval
- Colored dots = Protein standards run on each day
- Minimum of 6 replicates at each concentration
# Patients Characteristics

<table>
<thead>
<tr>
<th>Assembled cohorts</th>
<th>Preliminary data</th>
<th>VUMC n=225</th>
<th></th>
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<tbody>
<tr>
<td><strong>Patients</strong></td>
<td>ADC (%)</td>
<td>SCC (%)</td>
<td>SCLC (%)</td>
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<td></td>
<td>N=45</td>
<td>N=44</td>
<td>N=61</td>
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<td><strong>Age ± SD</strong></td>
<td>65.2 ± 8.0</td>
<td>65.8 ± 7.8</td>
<td>63.9 ± 8.9</td>
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<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Male</td>
<td>26 (58)</td>
<td>29 (56)</td>
<td>36 (59)</td>
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<tr>
<td>Female</td>
<td>19 (42)</td>
<td>15 (34)</td>
<td>25 (41)</td>
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<td><strong>Nodule Size (cm) ± SD</strong></td>
<td>2.7 ± 1.7</td>
<td>2.7 ± 2.0</td>
<td>3.6 ± 2.6</td>
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<td><strong>Smoking Status</strong></td>
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<td>Never Smoker</td>
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<td>0 (0)</td>
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<td>Ex-Smoker</td>
<td>26 (58)</td>
<td>23 (52)</td>
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<td>Current Smoker</td>
<td>19 (42)</td>
<td>21 (48)</td>
<td>40 (65)</td>
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<td><strong>Pack Years ± SD</strong></td>
<td>50.1 ± 31.3</td>
<td>53.9 ± 23.5</td>
<td>63.7 ± 32.8</td>
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<td><strong>Cancer Path Stages</strong></td>
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<td>IIB-IV</td>
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<td>Limited</td>
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<td>Extensive</td>
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<td>Adenocarcinoma</td>
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<td>Other malignant</td>
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<td><strong>Benign Histologies</strong></td>
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<td>TB</td>
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<td>Fungal Infection</td>
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<td>Bacterial Infection</td>
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<td>Inflammation</td>
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<td>Fibrosis</td>
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<td>Hamartoma</td>
<td>2 (3)</td>
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</table>
Results from Vanderbilt 225 Patients Cohort

- Normal Adeno SCC Small Cell
- Determined Cyfra Concentration (ng/ml)
- ELISA LOQ (3 ng/ml)
- BSI LOQ (40 pg/ml)
Validation cohort Vandy 2  IPNs 6-30 mm
Validation of CYFRA 21-1 IPNs 6-30 mm

<table>
<thead>
<tr>
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<th>CYFRA 21.1- BSI</th>
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<tr>
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<td>Cancer Prev</td>
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<tr>
<td>Nodule size mm</td>
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Vandy 1 dataset
AUC 0.93

Vandy 2 dataset
AUC 0.79
Patient Provider report

Probability of cancer before the Biomarker test

- Patient A: 34% chance of cancer, 11% chance of no cancer
- Patient B: 62% chance of cancer, 80% chance of no cancer

Probability of cancer after the Biomarker test

- Patient A: 34% chance of cancer, 62% chance of no cancer
- Patient B: 62% chance of cancer, 87% chance of no cancer

Intermediate probability for cancer

- 3 mo f/u CT
- 3 mo f/u CT or PET CT or tissue bx
- Tissue biopsy
Biomarker driven trial for the management of IPNs

T 0 consent

T 15 days visit patients-providers are given results

Chest CT IPN 6-30 mm
Pretest Prob 15-80%

Test
protein BM CYFRA 21-1 miRNA Radiomics FDG-PET

Posttest Prob >80%

Posttest Prob 15-80%

Posttest Prob <15%

Accuracy Invasive biopsies Early stage Futile surgery Stage Shift Cost Effectiveness

SOC

SOC

SOC

T 3 mo outcomes

Survival Cost Effectiveness

T 0 Blood Images PRO

T 15 days visit patients-providers are given results

T 3 mo outcomes

T 2 years outcomes

T 2 years Blood Images PRO
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•Patients