Breath testing identifies patients with lung cancer through the detection of ________ in their exhaled breath:

A. Tumor cells
B. Volatile organic compounds
C. DNA methylation
D. Micro RNAs
Lung Cancer Screening: Opportunity for Molecular Diagnosis? Biomolecular Markers in Breath Samples and Plasma Based Biomarkers

Jessica Donington, MD
NYU School of Medicine
Disclosures

- Research Funding For the NYU Thoracic oncology laboratory from NCI/NIH, DOD, CDC, Mensanna, Rosetta Genomics, SomaLogic, Celera, SourceMDx, Fujirebio, Pfizer, Response Genetics, Stephen Banner Lung Foundation,

- CDAs with Avantra, Caris Life Sciences, Integrated Diagnostics
The Early Detection Research Network

- Investigator-initiated infrastructure modeled after Cooperative Groups and established in 2000
- Collaborative and team science driven biomarker discovery, development and validation
- Mechanisms reward collaboration and team science
- Inclusive infrastructure that solicits extramural investigators through an Associate Membership Program
- Follows industrial/biotechnology standard practice for biomarker pipeline development
EDRN Collaborative Groups

https://edrn.nci.nih.gov/collaborative-groups
EDRN Structure Based on Biomarkers Development Pipeline
# EDRN Lung Collaborative Group

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Site(s)</th>
<th>Platform/Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Croce</td>
<td>OSU</td>
<td>MicroRNA and ultraconserved non-coding RNA</td>
</tr>
<tr>
<td>S. Dubinett</td>
<td>UCLA-Boston</td>
<td>Genomics, microRNA, Central/Peripheral Airways; Role of Inflammation and biomarkers; RNASeq</td>
</tr>
<tr>
<td>A. Spira</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Lenburg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Elashoff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Liebler</td>
<td>Vanderbilt</td>
<td>Mass spec, LCMRM; shotgun proteomics; plasma/tissue</td>
</tr>
<tr>
<td>D. Taab</td>
<td></td>
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</tr>
<tr>
<td>P. Massion</td>
<td>Vanderbilt</td>
<td>Case – control studies for diagnostic discovery; prospective CT imaging with collection of specimens; archive repository</td>
</tr>
<tr>
<td>H. Pass</td>
<td>North American Mesothelioma Consortium</td>
<td>Plasma/Tissue; Genomic, proteomic, glycomic, microRNA diagnosis and prognosis for mesothelioma; lung collaboration with W. Rom and industry</td>
</tr>
<tr>
<td>M. Huflejt</td>
<td></td>
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<tr>
<td>W. Rom</td>
<td>NYU</td>
<td>Ongoing screening (1143) and r/o lung cancer (1047) prospective cohorts; collaborations with industry/H.Pass</td>
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<tr>
<td>D. Sidransky</td>
<td>Hopkins</td>
<td>Epigenetic/methylation markers serum, plasma, sputum, BAL</td>
</tr>
<tr>
<td>S. Stass</td>
<td>U. Maryland</td>
<td>Analytic and Clinical Validation of lung biomarkers; microRNA for early detection/standardization of qPCR techniques</td>
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</table>
Biomarkers in the natural history of lung cancer

- BM of risk tobacco exposure
- Diagnostic BM
- BM of Response
- BM of Prognosis

- Screening Programs
- Lung Nodules
- Recurrence
- Prognosis

Disease non-measurable

Therapeutics
Biomarkers in the natural history of lung cancer

Disease non-measurable

BM of risk tobacco exposure

Lung Nodules

Diagnosis

BM of Response

Recurrence

BM of Prognosis

Prognosis

Screening Programs

Therapeutics

The NEW ENGLAND JOURNAL of MEDICINE

Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening

The National Lung Screening Trial Research Team

24% positive scans, 96% false positive rate
Biomarkers and CT Screening

- Genetic Risk Assessment
- Early Detection Diagnosis
- Prognostic Testing (Treatment Selection)
- Disease Progression
- Disease Recurrence

Pre-CT Test
- +ve: Evaluate for Biopsy (5-9 mm)
- -ve: Observation (<5 mm), Repeat Periodically

Post-CT Test
- Lung Nodule:
  - Observation (<5 mm)
  - Evaluate for Biopsy (5-9 mm)
  - Consider for Surgery (>10 mm)

Post-treatment Test

Modified from Charles Birse, Celera
# Early Detection Platforms

<table>
<thead>
<tr>
<th>Biomarker Source</th>
<th>Platform</th>
<th>Investigators</th>
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<tr>
<td>Airway Epithelium</td>
<td>Genomics</td>
<td>A. Spira</td>
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<tr>
<td>Sputum</td>
<td>Micro RNA</td>
<td>S. Stass</td>
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<td></td>
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<td>D. Sidransky</td>
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<tr>
<td></td>
<td>Proteomics</td>
<td>P. Maission, Somalogic, Integrated Diagnostics</td>
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<tr>
<td></td>
<td>Autoantibodies</td>
<td>Oncimmune</td>
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<tr>
<td>Exhaled Breath</td>
<td>Gas chromatography</td>
<td>Mensanna</td>
</tr>
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</table>
AIRWAY GENOMICS
Airway Genomics: Avi Spira, BU

• Can gene expression patterns in the RNA of large-airway epithelial cells provide insights into how individual smokers differ in their responses to cigarette smoke?

• Can such profiles identify smokers in whom the mutagenic effects of cigarettes has resulted in lung cancer presenting as a SPN?

Airway Genomics: Results

- 80 gene expression profile distinguishes smokers with cancer from those without (80% sensitive, 84% specific) and was independently validated in a 35 patient prospective set.
- 90% sensitivity for Stage I cancers.
- Combining bronchoscopic findings with the expression profile yielded 95% sensitivity and 95% NPV.
- Good for increasing yield and accuracy of bronchoscopy but NOT for large scale early detection.

Airway Genomics: nasal epithelium

• Determine relationship between smoking-related gene expression changes in nasal and bronchial epithelium
• Healthy 14 smokers and 13 nonsmokers

• 119 genes w/ similar changes in expression by smoking in bronchial and nasal epithelium
• 27 genes more dramatically affected in bronchial epithelium than nasal epithelium.
• Nasal epithelial gene expression may serve as a noninvasive surrogate to measure physiological responses to cigarette smoke and potentially early lung cancer diagnosis

**Objective:** Determine if altered miRNA expression in sputum is useful for early diagnosis of lung cancer.

**Methods:** RT-PCR performed on sputum from 23 pts with NSCLC and 17 cancer-free.

**Results:** Mir-21 expression was significantly higher in cancer pts (76.32 ± 9.79 vs. 62.24±3.82, p<0.0001).

Area under the ROC curve was 0.902 (70% sensitivity and 100% specificity).

Sensitivity significantly higher than with sputum cytology.

Xie Y, Lung Cancer, 2010
BIOMARKERS IN BLOOD
Objective: Measure plasma miRNAs expression which can;
   A. define risk for lung cancer development
   B. diagnose lung cancer in screen detected nodules
   C. define prognosis in screen-detected lung cancer.

Methods: Two large Italian lung cancer screening programs (diagnosis and validation)

Microfluidic cards determine top 100 miRNA profiles of pre-diagnostic (>1y) and diagnostic plasmas

Normalization, miRNAs expressed as ratios to each other and normalized to plasma from individuals that did not develop lung cancer.

Boeri M, PNAS, 2011.
RESULTS

(A) Risk: 16 ratios/15 miRNAs discriminate developing lung cancers, sensitivity 90%, specificity 80%
Validation: sensitivity 80%, specificity 90% (AUC-ROC = 0.85, p < 0.000)

(B) Diagnosis: 16 ratios/13 miRNAs classified lung cancer, sensitivity 84%, specificity 80%
Validation: sensitivity 75%, specificity 100% (AUC-ROC = 0.88, p < 0.0001)

(C) Prognosis: 10 ratios/9 miRNAs, identified poor prognosis sensitivity 100%, specificity 100%
Validation: sensitivity 80%, specificity 100%

Boeri M, PNAS, 2011.
**Objective:** Detection of aberrant DNA methylation of tumor suppressor genes in plasma of pts with CT detected abnormalities

**Methods:**

**Evaluation Set:**
- 24 disease-free individuals
- 13 individuals with lung cancer

**Independent Set:**
- 80 smokers with no nodules on CT scan
- 23 individuals w/ small solid or GGO on CT
- 70 patients with lung cancer

Bisulfite modified DNA from plasma; real time PCR with CT cutoffs

Methylation of five candidate tumor suppressor genes: (RarB, NISCH, B4GALT1, KIF1a, and DCC)

Epigenetics: Results

- 73% of cancer pts showed methylation of at least one of 4 genes compared to 28% of controls
- Progressive increase in methylation from control group with no CT abnormalities to patients with CT detected tumors

Epigenetics: Results

- Methylation also be related to smoking history
- Methylation of 1 of 4 genes highly specific for cancer diagnosis in never of light smokers

• **Objective:** Identify a serum based proteomic signature to identify cancers from at risk controls

• **Methods:** MALDI Mass spectrometry analysis of most abundant peptides in unfractionated serum from lung cancers pts & age, sex and smoking-matched controls
Proteomics: Pierre Massion and Daniel Liebler, Vanderbilt

- Seven peak proteomic signature
  - training set
    - overall accuracy of 78%
    - sensitivity of 67%
    - specificity of 89%
  - test set
    - overall accuracy of 73%
    - sensitivity of 58%
    - specificity of 86%

Massion P, JTO, 2007
Proteomics: Added value

- Builds on 7 feature proteomic (MALDI MS) signature which distinguished subjects with lung cancer from matched controls
- Hypothesized that the signature adds diagnostic value beyond clinical and radiographic information
- Two independent cohorts of prospectively collected pts with lung cancer and CT detected non-cancer nodules
  - Cohort A: Vanderbilt, 150 cancers/58 controls
  - Cohort B: Mayo, 25 cancers/37 controls

Pecot C, Cancer Epidemiol Biomarkers, 2012
Proteomics: Results, added value

• Pt in cohort A had greater pack years (61 vs. 33) and larger nodules (38 vs 23 mm) than cohort B

• Addition of 7 peak MALDI signature to clinical and radiographic data did not improve diagnosis in cohort A, but did provided added value in cohort B

Pecot C, Cancer Epidemiol Biomarkers, 2012
Proteomics: Results, added value

- In subgroup of 100 nodules between 5-20 mm, proteomic signature added significant value to clinical and radiographic data, increasing AUC from 0.67 to 0.72 (p<0.0001)

Pecot C, Cancer Epidemiol Biomarkers, 2012
INDUSTRIAL PARTNERS
Proteomics: Somalogic

**Somalogic**

- **Objective:** Identify and validate biomarkers that discriminate NSCLC from smokers

- **Methods:** 1326 samples form 4 independent biorepositories (NYU, RPMI, U of Pitt, BioServe)

- Included both CT-detected non-cancer nodules and smoking matched controls

- 2:1 training and validation

SomaLogic: Results

- 12 protein panel discriminates cancers from controls
- Training set:
  - 91% sensitivity
  - 84% specificity
- Validation set:
  - 89% sensitivity
  - 83% specificity

Proteomics: Integrated Diagnostics

- Molecular characterization of pulmonary nodules using shotgun proteomic analysis of tumors.
- 388 potential targets evaluated with MRM-MS and refined to a set of 13 classifier proteins for testing in the blood.
- 36 cooperative proteins
- 247 lung nodules, 4-30mm
- Training and testing algorithm

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Protein</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>LRP1</td>
<td>Prolow-density lipoprotein receptor-related protein 1</td>
<td>Secreted</td>
</tr>
<tr>
<td>BGH3</td>
<td>Transforming Growth factor beta induced protein 3</td>
<td>Secreted</td>
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<tr>
<td>COIA1</td>
<td>Collagen alpha-1(XVIII) chain</td>
<td>Secreted</td>
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<tr>
<td>TETN</td>
<td>Tetranection</td>
<td>Secreted</td>
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<tr>
<td>TSP1</td>
<td>Thrombospondin-1</td>
<td>Secreted</td>
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<tr>
<td>ALDOA</td>
<td>Fructose-bisphosphate aldolase A</td>
<td>Secreted</td>
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<tr>
<td>GRP78</td>
<td>78 kDa glucose-regulated protein</td>
<td>Endoplasmic reticulum</td>
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<tr>
<td>ISLR</td>
<td>Immunoglobulin superfamily containing leucine-rich repeat protein</td>
<td>Secreted</td>
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<tr>
<td>FRIL</td>
<td>Ferritin light chain</td>
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<td>LG3BP</td>
<td>Galectin-3-binding protein</td>
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<td>Peroxiredoxin-1</td>
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<tr>
<td>GSLG1</td>
<td>Golgi apparatus protein 1</td>
<td>Golgi membrane</td>
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A negative test implies a >2 fold decrease risk for cancer.

High NPV of the test would obviate biopsy in 1/4 patients with benign nodules.

<table>
<thead>
<tr>
<th></th>
<th>N=</th>
<th>Sens</th>
<th>Spec</th>
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<th>PPV</th>
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<tr>
<td>Discovery</td>
<td>143</td>
<td>82</td>
<td>66</td>
<td>95</td>
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<tr>
<td>Validation</td>
<td>104</td>
<td>71</td>
<td>44</td>
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<td>18</td>
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<tr>
<td>Validation 2</td>
<td>37</td>
<td>79</td>
<td>56</td>
<td>94</td>
<td>24</td>
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</tbody>
</table>

Autoantibodies: Oncimmune, Early CDT

- Early CDT-blood test that measures 7 autoantibodies (AAB) to lung cancer
- ELISA-based test performed at Oncimmune’s CLIA lab
- Extensive validation in case-control series
- Now clinically through limited release audit program

**CAGE, GBU 4–5, HER2, p53, c-myc, NY-ESO-1 and MUC1**

Oncimmune, Early CDT: Results 6 month Audit

Positive test associated w/ 5.4-fold increase in lung cancer

Jett J, Lung Cancer, 2014
Breath Testing: Mensanna

- Carcinogenesis results in induction of cytochrome P450 in lung cancer pts accelerates catabolism of volatile organic compounds
- Not classic biomarkers
  - Limited biologic significance
  - Abundance not effected by tumor mass
  - Abundance not reduced by tumor removal

Breath Testing: Mensanna

- Alveolar breath collected at primary facility and shipped to Mensanna
- Analyzed by gas chromatography and mass spectroscopy
- 404 pts, 193 lung cancer, 211 cancer free controls
- 2:2, training set: prediction set
- Weighted digital analysis

Breath Testing: Results

- 30 discriminating VOCs were identified
- Each subject given WDA discriminatory score based on VOC concentrations

Breath Testing: Results

• VOC weighted score discriminates cancers from both current and former smokers

• VOC weighted score identifies cancers regardless of stage

Conclusions

• The EDRN Lung Collaborative Group is charged with the discovery and validation of novel early detection, diagnostic, and prognostic biomarkers for lung cancer

• Companion molecular biomarkers should provide added value to CT screening efforts

• There is currently a gap between promise and product

• Introduction of greater numbers of at risk individuals should increase interest and resources for development and validation of lung cancer biomarkers
Breath testing identifies patients with lung cancer through the detection of ________ in their exhaled breath:

A. Tumor cells
B. Volatile organic compounds
C. DNA methylation
D. Micro RNAs