Precision Medicine

LUNG CANCER:
Precision Medicine Strategy

Curtis Harris MD
Laboratory of Human Carcinogenesis
National Cancer Institute
NIH
Bethesda, Maryland
USA

Robles S and Harris CC  Lung Cancer 107:50-58, 2017
Precision medicine

“Tonight, I’m launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes — and to give all of us access to the personalized information we need to keep ourselves and our families healthier.”

— President Barack Obama, State of the Union Address, January 20, 2015

Disease classification

Towards Precision Medicine and a Molecular Taxonomic Classification of Disease

- Traditional Medicine
  - Lifestyle, medical & family history
  - Signs & Symptoms
  - Standard lab tests & imaging

- Precision Medicine
  - Multi-layered
  - Individual-centric
  - Interconnected

INFORMATION COMMONS
- Exposome
- Genome
- Transcriptome
- Microbiome
- Epigenome
- Metabolome
- Clinical Information
- Epidemiological data

- Lifestyle & family history
- Signs & Symptoms
- Standard lab tests & imaging
Research strategy
Lung cancer

Precision Medicine: LUNG CANCER

Report of the Surgeon General, 2014

Premature deaths caused by smoking and exposure to secondhand smoke, 1965-2014

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking-related cancers</td>
<td>6,587,000</td>
</tr>
<tr>
<td>Cardiovascular and metabolic diseases</td>
<td>7,787,000</td>
</tr>
<tr>
<td>Pulmonary diseases</td>
<td>3,804,000</td>
</tr>
<tr>
<td>Conditions related to pregnancy and birth</td>
<td>108,000</td>
</tr>
<tr>
<td>Residential fires</td>
<td>86,000</td>
</tr>
<tr>
<td>Lung cancers caused by exposure to secondhand smoke</td>
<td>263,000</td>
</tr>
<tr>
<td>Coronary heart disease caused by exposure to secondhand smoke</td>
<td>2,194,000</td>
</tr>
<tr>
<td>Total</td>
<td>20,830,000</td>
</tr>
</tbody>
</table>

Source: Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, unpublished data.

Cancer Statistics (ACS), 2014

Lung & Bronchus

Rate per 100,000 Population

Year of Diagnosis

Male

Female

Worldwide cancers

Major Worldwide Lethal Types of Human Cancer

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>(% of Total)</th>
<th>Mortality</th>
<th>Number of Deaths (Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>19.4%</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>9.1%</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>8.5%</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>6.0%</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>4.9%</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.0%</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>3.3%</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>55.1%</strong></td>
<td></td>
<td><strong>4.52 Million</strong></td>
</tr>
</tbody>
</table>

GLOBOCAN 2012: 8.2 million Cancer Deaths

www.cancerresearchuk.org
Geographical map

Smoking Prevalence and Cigarette Consumption in 187 Countries, 1980-2012

• Estimate Age-Standardized Prevalence of Daily Smoking in 2012 among Men

Deciles of prevalence were calculated for men and women combined.

Ng, M., et al., JAMA, 311(2): 183-192, 2015
Smoke exposure

“EXPOSURE TO SECONDHAND OR ‘ENVIRONMENTAL TOBACCO SMOKE IS CARCINOGENIC TO HUMANS”
(Host Factors in Human Carcinogenesis, IARC MONOGRAPH 183)

Some men have constitutions that are like wooded mountains running with springs, others like those with poor soil and little water, still others like land rich in pastures and marshes, and yet others like the bare, dry earth of the plain.

Hippocrates
Airs, Waters, Places

Hirayama, T., Cape Sunion, Greece, 1981
Never smokers

Molecular Epidemiology of Lung Cancer in Never Smokers

Hypothesis:
• Childhood exposure to second-hand smoke and genetic alterations in innate immunity increase lung cancer risk in never smoking adults

Conclusions:
• Parental secondhand smoke exposure during childhood is associated with dose-dependent increased lung cancer risk among never smokers in two cohorts.
  • Especially among those with an MBL-2 haplotype with a hyperactive innate immune system
  • Early age of onset of lung cancer

Susan Olivo-Marston

Collaboration with Jen Jin and Ping Yang, Mayo Clinic
A Precision Medicine Research Strategy

INFORMATION COMMONS

- Exposome
- Genome
- Metabolome
- Epigenome
- Transcriptome
- Microbiome
- Clinical Information
- Epidemiological data

INFORM

GUIDE

IMPROVE

Diagnosis

Health Outcomes

Treatment

Clinical Medicine

New Molecular Taxonomic Classification of Individual Patients

Biomedical Research

Prevention Research

Informed mechanistic studies

Target Identification

Molecular Mechanisms

Observational clinical studies

Modified: “Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease” (National Research Council 2011)
p53 Tumor Suppressor is at the Crossroads of the Exposome and Cancer Genome

External Environment
- Tobacco Smoke
- Infections
- Diet
- Food Toxins
- Radiation

Internal Environment
- Obesity
- Chronic Inflammation

Cancer Biomarkers of Risk and Prognosis

Cancer Prevention and Screening Strategies

Cancer Genome and Epigenome
- Genetic Susceptibility
- Driver Gene Targets

Understanding Carcinogenesis

Cancer Therapy

1Exposome coined by, Chris Wild, Int J Epidem., 41: 24, 2005

Chronic inflammation and infection can increase cancer risk.

<table>
<thead>
<tr>
<th>Inherited &gt; Acquired</th>
<th>Acquired &gt; Inherited</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disease</strong></td>
<td><strong>Disease Agent</strong></td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>Viral</td>
</tr>
<tr>
<td>Crohn's Disease</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td>Ulcerative Collitis</td>
<td>Hepatitis C</td>
</tr>
<tr>
<td>Familial Pancreatitis</td>
<td>Helicobacter Pylori</td>
</tr>
<tr>
<td></td>
<td>PID</td>
</tr>
<tr>
<td></td>
<td>Parasitic</td>
</tr>
<tr>
<td></td>
<td>S. hematobium</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
</tr>
<tr>
<td></td>
<td>Liver Fluken</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor Site</strong></td>
<td><strong>Tumor Site</strong></td>
</tr>
<tr>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>Colon</td>
<td>Liver</td>
</tr>
<tr>
<td>Colon</td>
<td>Gastric</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Ovary</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Global Impact**
- 2 million human cancers per year are related to infection.
- Other causes of inflammation are associated with many more cancers per year (e.g. smoking 6 million cancers/year).

*World Cancer Report, IARC, WHO, 2014*
Obesity is a chronic inflammatory disease

Cytokines

Increased Expression of Serum Pro-Inflammatory Cytokines is Associated with Lung Cancer Risk, Diagnosis and Survival

Increased Risk | Diagnosis | Poor Survival

↑ IL-8, ↑ CRP (EA)
↑ IL-1B & IL-10 (AA)
JNCI 103: 1112, 2011
J. Thoracic Oncol, 1494, 2014

↑ IL-8, ↑ IL-6 (AA, EA)
↑ IL-6 & -8 (AA, EA), ↑ MBL-2 (EA)
JNCI 103: 1112, 2011
CEBP: 215, 2010
JNCI 99: 1401, 2007
J. Thoracic Oncol, 1494, 2014
CEBP: 488, 2016

Health Disparity:
EA, European-American
AA, African-American
Collaboration: Ann Schwartz
Genome
NSCLC evolution

Knowledge of Non-Small-Cell Lung Cancer has Evolved Substantially in Recent Decades

1987

- KRAS: 25.1%
- None: 74.9%

2004

- KRAS: 15.3%
- EGFR: 20.0%

2014*

- EGFR: 32.2%
- KRAS: 11.3%
- MET ex14: 7.0%
- BRAF: 0.7%
- ALK fusion: 1.3%
- ROS1 fusion: 1.7%
- ERBB2: 1.7%
- HRAS: 0.4%
- NRAS: 0.4%
- MAP2K1: 0.4%
- RET fusion: 0.9%
- MET amp: 2.2%
- ERBB2 amp: 5.9%
- RIT1: 2.2%


NIH NATIONAL CANCER INSTITUTE
Chemical agents

Examples of Chemical Agents Causing Cancer

Human Exposure

A. Chimney Sweeper
   - Structure of Carcinogen: Benz[a]pyrene (BaP)
   - Disease: Scrotal cancer

B. Aspergillus flavus growing on corn
   - Structure of Carcinogen: Aflatoxin B₁
   - Disease: Liver cancer

C. Cigarettes
   - Structure of Carcinogens: Benzene, Formaldehyde, Acetaldehyde, NNK, 4-ABP, 1,3-Butadiene
   - Diseases: Lung and many other cancers

Loeb, L. and Harris, C.C., Cancer Res, 68: 6863-6872, 2008
Three decades

Three Decades (1970s, 1990s and 2010s) of Examples of Initial Seminal Advances in Exposure of Environmental Carcinogens Being Molecularly Linked to Mutagenesis

- Development of a Rapid Mutagenicity Testing of Chemical Carcinogens and Metabolic Activation of Chemical Carcinogens

- Discovery of TP53 Mutations Linked to Environmental Carcinogen Exposure

- Computational Analysis of Genome-Wide DNA Sequencing Data Identifies Exogenous and Endogenous Induced Mutations including those Caused by Chemical and Physical Carcinogens, Inflammation, DNA Repair Defects and Aging
### Exposome: Examples of Chemical and Physical Agents Causing Cancer and related TP53 Mutations in Human Cancer

<table>
<thead>
<tr>
<th>Carcinogen/ (exposure)</th>
<th>Target organ</th>
<th>Base substitution</th>
<th>Distinctive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1 (dietary contam.)</td>
<td>Liver</td>
<td>G to T</td>
<td>G to T</td>
</tr>
<tr>
<td>PAH (B[a]P) (smoking)</td>
<td>Lung</td>
<td>G to T with Strand bias</td>
<td>G to T with Strand bias</td>
</tr>
<tr>
<td>UV radiation (sunlight)</td>
<td>Skin</td>
<td>CC to TT</td>
<td>CC to TT</td>
</tr>
<tr>
<td>Aristolochic Acid (dietary contam.)</td>
<td>Urothelium</td>
<td>A to T with Strand bias</td>
<td>A to T with Strand bias</td>
</tr>
</tbody>
</table>

- Greenblatt, M......Harris, C.C., Cancer Res., 54: 4855-78, 1994
TP53 mutations

TP53 is the Gene most frequently Mutated in Cancer

TP53
Wild type as tumor suppressor
Mutant types
Loss of function as tumor suppressor
Gain of function as an oncogene

Genes most frequently mutated in various types of cancer in the TCGA Pan-Cancer study. Data were generated by analysis of the mutations released by Kandoth et al. Nature 2013.

Soussi & Wiman, 2015
TP53 functions

TP53 and its Functions Affect Multiple Layers of "-OMICS" Data in the Precision Medicine Paradigm

- Germline (e.g., R72P)
- Somatic (e.g., R249S)
- Mutations reflecting etiology (e.g., R249S)
- Cigarette smoke
  - Aflatoxin B1
  - Inflammation
- MicroRNA (e.g., miR-34)
- Cell cycle checkpoint
  - DNA repair
  - Apoptosis
- Integrity of mucosal barrier
- Prognosis
- Tumor metabolites
- Genomene
- Exposome
- Epigenome
- Transcriptome
- Metabolome
- Epidemiological Data

Three decades

Three Decades (1970s, 1990s and 2010s) of Examples of Initial Seminal Advances in Exposure of Environmental Carcinogens Being Molecurally Linked to Mutagenesis

• Development of a Rapid Mutagenicity Testing of Chemical Carcinogens and Metabolic Activation of Chemical Carcinogens
  • McCann J …… Ames BN; Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemicals. P.N.A.S 72:5135-39, 1975
  • Heidelberger, C. Chemical Carcinogenesis, Annual Rev Biochemistry 44:79-121, 1975

• Discovery of TP53 Mutations Linked to Environmental Carcinogen Exposure

• Computational Analysis of Genome-Wide DNA Sequencing Data Identifies Exogenous and Endogenous Induced Mutations including those Caused by Chemical and Physical Carcinogens, Inflammation, DNA Repair Defects and Aging
Somatic mutations
Carcinogens fingerprint

A Carcinogen’s Fingerprint in Human Lung Cancer DNA Can be Reproduced in Experimental Systems: Benzo(a)pyrene

Patient prognosis

Gain of Function (GOF) TP53 Mutations are Associated with Poor Prognosis of Lung Cancer Patients

- Other MISSENSE (n=81)
- TP53 LOF (n=64)
- TP53 WT (n=159)
- TP53 GOF (n=39)

Adjusted for patient age, race, sex, and smoking history, and tumor histology, and stage. Dataset of 352 surgical cases of NSCLC analyzed by TP53 capture sequencing.

Robles A and Harris CC, NCI-MD Case Control Study
Transcriptome
MicroRNA

MicroRNA and Cancer

- **MicroRNA**
  - Small non-coding RNAs that are evolutionarily conserved and regulate gene expression.
  - Protein output of hundreds of genes are repressed by each microRNA destabilizing mRNA and to a lesser extent inhibiting translation of mRNA.

- **Human Cancer**
  - MicroRNAs are differentially expressed in human cancers.
  - MicroRNAs can predict risk, diagnosis, prognosis and therapeutic outcome.
microRNA mechanisms
Hypothesis: MicroRNAs are Associated with Lung Cancer Diagnosis and Prognosis

- miRNAs profiles were significantly different both between primary lung cancers and corresponding non-cancerous lung tissues and among different histological types of lung cancer.

- Increased mir-21, mir-155 and mir-106b, and decreased let-7 were each associated with diagnosis and prognosis including stage 1 lung cancer.

let-7: confirmed studies by Takahashi and Slack

Yanaihara et al., Cancer Cell, 9:189, 2006
Upregulated microRNA

Commonly Up-Regulated microRNAs in Carcinomas

<table>
<thead>
<tr>
<th>miR</th>
<th>N</th>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>6</td>
<td>Breast Colon Lung Pancreas Prostate Stomach</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>5</td>
<td>Breast Colon Lung Pancreas Prostate</td>
</tr>
<tr>
<td>miR-191</td>
<td>5</td>
<td>Colon Lung Pancreas Prostate Stomach</td>
</tr>
<tr>
<td>miR-29b-2</td>
<td>4</td>
<td>Breast Colon Pancreas Prostate</td>
</tr>
<tr>
<td>miR-223</td>
<td>4</td>
<td>Colon Pancreas Prostate Stomach</td>
</tr>
<tr>
<td>miR-128b</td>
<td>3</td>
<td>Colon Lung Pancreas</td>
</tr>
<tr>
<td>miR-199a-1</td>
<td>3</td>
<td>Lung Pancreas Prostate</td>
</tr>
<tr>
<td>miR-24-1</td>
<td>3</td>
<td>Colon Pancreas Stomach</td>
</tr>
<tr>
<td>miR-24-2</td>
<td>3</td>
<td>Colon Pancreas Stomach</td>
</tr>
<tr>
<td>miR-146</td>
<td>3</td>
<td>Breast Pancreas Prostate</td>
</tr>
<tr>
<td>miR-155</td>
<td>3</td>
<td>Breast Colon Lung</td>
</tr>
</tbody>
</table>

- microRNAs shared by the signatures of six solid cancers*
- miR-21 is both up-regulated in 18 major cancers and a biomarker of poor survival in 14

*The list includes 21 commonly up-regulated microRNAs in three or more (N) types of solid cancers (p-value=2.5x10^-3).

Volina et al., PNAS 103: 1-5, 2006
Increased miR-21 expression
miR-21 expression

Increased miR-21 Expression is Associated with Poor Prognosis in Multiple Types of Cancers

- Lung Cancer
  - (Saito, M. et al., Clin Cancer Res., 2011)
  - p<0.001

- Colon Cancer
  - (Schetter et al., JAMA 2008)
  - p<0.0001

- Pancreatic Cancer
  - (Giovannetti, E., et al., Cancer Res. 2010)
  - p=0.03

- Breast Cancer
  - (Yan et al., RNA 2008)
  - p<0.001

- Melanoma
  - (Jiang et al., Acta Histochem., 2011)
  - p=0.028

- Head & Neck Cancer
  - (Avissar, et al., Carcinogenesis 2009)
  - p=0.034

- Tongue Cancer
  - (Li, J. et al., Clin Cancer Res. 2009)
  - p=0.008

- Astrocytoma
  - (Zhi, F. et al., Eur J Cancer 2010)
  - p=0.003

- Chronic Lymphocytic Leukemia
  - (Rossi, S. et al., Blood 2010)
  - p=0.004

- Gastric Cancer
  - (Jiang J. et al., PLoS ONE 2011)
  - p=0.004
miR-21

Examples of the Mechanistic Underpinning of miR-21 in Human Cancer

- Gene Amplification (Chr 17q23.2)
- Decreased Transcriptional Silencing
- EGFR
- IL-6
- IL-17
- IFN

Genotoxic stress (H₂O₂ or ionizing radiation)

miR-21

- TLR8

Inflammation

- Tumor Growth, Invasion and Metastasis
- Metalloproteinases (e.g., MMP-2 and -9)
- Focal Adhesion Kinase (FAK)
- Anchorage Independent Growth
- Migration

- TLR8

Cachexia

- Mismatch DNA Repair

References:

Seike .... Harris, PNAS 106: 12085-90, 2009
Schetter and Harris, Carcinogenesis 31: 37-49, 2010
He and Croce, PNAS 111: 4525-29, 2014
MiRNAs are Secreted by Cancer Cells in Exosomes and can Reach and Bind TLR8 Receptors in the Endosomes of Surrounding Immune and Muscle Cells

He, W.A., et al., PNAS, 111: 4525-9, 2014
4-gene classifier
Meta-Analysis of the 4-gene Classifier (↑ XPO1, BRCA1, HIF1α, ↓ DLC1) in 12 Independent Cohorts of Stage I ADC

- 883 Stage I Patients

4-gene in Stage IA

- Combined models included 9 datasets (n=817) with overall survival information

<table>
<thead>
<tr>
<th>Cohorts (n)</th>
<th>Stage I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse-free survival</td>
<td></td>
</tr>
<tr>
<td>Japan (149)</td>
<td>HR (95% CI) 2.19 (1.35, 3.56)</td>
</tr>
<tr>
<td>Lee (36)</td>
<td>HR (95% CI) 2.48 (1.18, 5.20)</td>
</tr>
<tr>
<td>Cancer-specific survival</td>
<td></td>
</tr>
<tr>
<td>US/Norway (67)</td>
<td>HR (95% CI) 2.71 (1.61, 4.55)</td>
</tr>
<tr>
<td>Overall survival</td>
<td></td>
</tr>
<tr>
<td>Directors (276)</td>
<td>HR (95% CI) 1.69 (1.26, 2.26)</td>
</tr>
<tr>
<td>Batthachjhee (76)</td>
<td>HR (95% CI) 1.61 (1.02, 2.53)</td>
</tr>
<tr>
<td>Tomida (79)</td>
<td>HR (95% CI) 2.18 (1.20, 3.97)</td>
</tr>
<tr>
<td>Botling (70)</td>
<td>HR (95% CI) 1.50 (0.98, 2.28)</td>
</tr>
<tr>
<td>Tang (87)</td>
<td>HR (95% CI) 1.94 (0.99, 3.78)</td>
</tr>
<tr>
<td>Rousseaux (81)</td>
<td>HR (95% CI) 1.61 (1.00, 2.58)</td>
</tr>
<tr>
<td>Matsuyama (52)</td>
<td>HR (95% CI) 2.23 (1.06, 4.70)</td>
</tr>
<tr>
<td>Wilkerson (62)</td>
<td>HR (95% CI) 1.97 (1.13, 3.44)</td>
</tr>
<tr>
<td>Bild (34)</td>
<td>HR (95% CI) 1.66 (0.87, 3.16)</td>
</tr>
<tr>
<td>Subtotal (817)</td>
<td>HR (95% CI) 1.73 (1.47, 2.02)</td>
</tr>
</tbody>
</table>

Okayama H, Schetter A ... Harris CC Cancer Epidemiol Biomarkers Prev 23:2884-94, 2014
Mechanistic cancer biomarkers

Mechanistic Cancer Biomarkers: Proof of Principle in Lung Adenocarcinoma

- Hypothesis: The combination of protein-coding genes that are mechanistically related to lung Adenocarcinoma and the non-coding mir-21 is a better prognostic classifier than either alone

Akagi et al, Cancer Res. 73: 3821-32, 2013
miR-21 and 4 coding genes
Non-coding RNA
Further Proof of Principle

- Colon Carcinoma
- Esophageal Adenocarcinoma
- Esophageal Squamous Cell Carcinoma
  Zhao et al Int J Cancer 132: 2901, 2013
- Lung Adenocarcinoma
- Breast Adenocarcinoma
Epigenome
DNA methylation

EPIGENOME: DNA METHYLATION

- **HYPOTHESIS:** An integrated biomarker classifier of stage I lung adenocarcinoma based on independent mRNA, microRNA and DNA methylation biomarkers, will further improve the prognostic classification

Robles a et al., J. Thoracic Oncology 10: 1037-48, 2015
Prognostic epigenetic signature

DNA Methylation in NSCLC is a Prognostic Epigenetic Signature

A Prognostic DNA Methylation Signature for Stage I Non–Small-Cell Lung Cancer

Juan Sanchoval, Jesus Mendez-Gonzalez, Ernest Nadal, Guoan Chen, F. Javier Carmona, Sergi Sayols, Sebastian Moran, Holger Heyn, Miguel Vizoso, Antonio Gomez, Montse Sanchez-Cespedes, Yussen Assenov, Fabian Müller, Christoph Beck, Miguel Taron, Josefin Mora, Lucia A. Muscarella, Triantafullis Liloglou, Michael Davies, Marina Pollan, Maria J. Pujades, Wenceslao Torre, Luis M. Montuenga, Elisabeth Brambilla, John K. Field, Luca Riz, Marco Lo Iacono, Giorgio V. Scaglioni, Rafael Rosell, David G. Beer, and Manel Esteller

(J Clin Oncol 2013;31(32):4140-7).

<table>
<thead>
<tr>
<th>DNA Methylation Signatures</th>
<th>Discovery Cohort (450K array)</th>
<th>Validation Cohort (pyrosequencing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 heat map stage 1</td>
<td>HR 2.35, 95% CI 1.29 to 4.28</td>
<td>HR 3.55, 95% CI 1.70 to 7.42</td>
</tr>
<tr>
<td>HIST1H4F</td>
<td>HR 2.46, 95% CI 1.25 to 4.85</td>
<td>HR 2.95, 95% CI 1.50 to 5.80</td>
</tr>
<tr>
<td>PCDHG86</td>
<td>HR 2.28, 95% CI 1.23 to 4.23</td>
<td>HR 2.71, 95% CI 1.36 to 5.38</td>
</tr>
<tr>
<td>NPBWR1</td>
<td>HR 2.14, 95% CI 1.18 to 3.87</td>
<td>HR 2.29, 95% CI 1.18 to 4.45</td>
</tr>
<tr>
<td>ALX1</td>
<td>HR 2.07, 95% CI 1.08 to 3.97</td>
<td>HR 2.03, 95% CI 1.09 to 3.81</td>
</tr>
<tr>
<td>HOX A9</td>
<td>HR 3.27, 95% CI 1.70 to 6.29</td>
<td>HR 3.24, 95% CI 1.61 to 6.54</td>
</tr>
<tr>
<td>0-1 X ≥ 2 hypermethylated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>genes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Survival curve

An Increasing Combined Score of Mechanistic Biomarkers Conferred Greater Risk for Poor Outcome in Stage 1 Lung Adenocarcinoma, and Within Stage 1A/1B Subgroup Analysis

Stage 1

- Cases were categorized according to the combined number of high values for HOXA9 methylation, miR-21 (Clin. Cancer Res. 2011;17:1875-82) and 4-gene signature (Cancer Res. 2013;73:3821-32) and HOXA9 promoter methylation(J. Thoracic Oncology 10: 1037-48, 2015).
- P values calculated by log-rank test for trend.
Microbiome
Microbiome and carcinogenesis

Bacterial and Viral Microbiome Promotes Carcinogenesis

H. pylori
- Gastric cancer

F. nucleatum
- Colon Cancer

Dysbiosis
- Multiple Cancers
- Cancer Therapy

HPV
- Cervical and Head and Neck Cancer

Genomic instability
- Cell polarity
- Dysregulated cell growth

Cell proliferation
- Prevention of apoptosis

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Microbiome, TP53 and lung cancer

Interaction between the Microbiome, Smoking and TP53 in Human Lung Cancer

- Certain taxa including Acidovorax were higher in both adenocarcinoma and squamous carcinoma (SCC) in smokers and former smokers than in non-smokers.

- A group of taxa were significantly associated with SCC, of which Acidovorax was enriched in smokers

- SCC cases with TP53 mutations had a higher abundance of the SCC-associated taxa, including Acidovorax

SCC-associated taxa are enriched in tumors with TP53 mutations, which establishes a microbiome-gene interaction in lung cancer tissue

Greathouse I ......Harris CC Genome Biology, 2018, August 24:19(1):123
Temperans challenge

A. temperans Challenge Accelerates Lung Tumorigenesis (Chenran Zhang and Natalia von Muhlinen)

- Mice challenged with A. temperans exhibit significantly larger tumors at week 13 post AdCre compared to L. gasseri or PBS.
Microbiome and immune modulation

- Various commensal microorganisms influence the local immunity.
- Intact commensal microbiota promotes lung cancer development through modulation of innate immune response and expression of cytokines such as IL17.
Metabolome

Precision Medicine

INFORMATION COMMONS

- Exposome
- Genome
- Transcriptome
- Epigenome
- Microbiome
- Metabolome
- Clinical Information
- Epidemiological data

KNOWLEDGE NETWORK

Individual Patients

Biomedical Research

Target Identification

Molecular Mechanisms

GUIDE

Prevention

Diagnosis

Treatment

Health Outcomes

Clinical Medicine

IMPROVE

Modifed: Toward Precision Medicine, National Research Council, National Academy of Science, 2011
Tumor Metabolism and Metabolome

- **Hypothesis**: Unbiased metabolomics will discover biomarkers associated with the risk, diagnosis, prognosis and therapeutic outcome of lung cancer.
Urinalysis

- Hippocrates tasted urine in the diagnosis of disease in his patients. (460-370, BC)

- Physiologist J. A. Armstrong writes, “From a liquid window through which physicians felt they could view the body’s inner workings, urine led to the beginnings of laboratory medicine…”
Creatine ribose

Creatine Ribose is Positively Correlated in Cancer and Urine

Lung Adenocarcinoma (targeted UPLC-MS/MS)
N = 48 (Tumor), 48 (Non Tumor)

\[ r = 0.6, p = 0.006 \]
Survival curve

**Lung Cancer Metabolome and Prognosis**

Combined Score (All cases)

- **Metabolites (urine)**
  - Creatine riboside
  - N-Acetyl Neuraminic acid
  - Cortisol sulfate
  - 561+

n=469

<table>
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<tr>
<th>High in</th>
<th>HR (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>in 1</td>
<td>1.10 (0.67-1.75)</td>
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<tr>
<td>in 2</td>
<td>1.36 (0.86-2.14)</td>
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<tr>
<td>in 3</td>
<td>1.84 (1.15-2.94)</td>
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<tr>
<td>in all</td>
<td>3.65 (2.34-6.00)</td>
<td>&lt;0.00001</td>
</tr>
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Mathe, E...Harris, CC, Cancer Res., 74: 3259-70, 2014
Low dose CT screening
Spiral CT scans
Low dose CT

Low-Dose Computed Tomography (LDCT)

- NLST reports benefits for high risk individuals (2011)
- CMS announces Medicare coverage (2015)
- USPSTF recommends LDCT (2013)
- Affordable Care Act mandates coverage (2015)

Individuals

- Lung Cancer Screening

- High risk: age 55-74, 30 pack-year history, quit within 15 years or current smoker
  - Need to identify high risk individuals outside criteria?
  - Need to identify individuals within the criteria and prioritize those with the greatest risk?
- 8.8 million Americans were LDCT-eligible (2010)
Unmet needs

**Early Stage Lung Cancer: Unmet needs**

- **At-risk individuals** (age 55-74, heavy smokers) → **LDCT scan** → **Clinical Diagnosis** → **Surgical Resection** → **Observation** (Stage I) → **Chemotherapy** (high-risk Stage IB) → **Adjuvant Chemo/Immunotherapy**

- **Identify individuals for priority screening**
- **Lung cancer Risk Screening Biomarkers**
- **Determine if a lesion observed in LDCT is likely to be cancer**
- **Early Diagnostic Biomarkers**
- **Identify patients at high risk of recurrence and candidates for Adjuvant Therapy**
- **Prognostic Biomarkers**
Precision medicine goals

A Goal of Precision Medicine is to Identify 25% of Stage I Lung Cancer Patients whose Cancer will recur

Stage I patients after curative surgery

25% of stage I patients recur and die of disease.

INFORMATION
- Exposome
- Genome
- Transcriptome
- Epigenome
- Microbiome
- Metabolome
- Clinical Information
- Epidemiological data

- Decrease False Positive Rate of 96.4%
- Decrease Financial Cost
- Improve Patient Care
- Guide Mechanistic Studies

Low risk
- Observe

High risk
- Adjuvant immuno- and chemo-therapy
- High risk patients who may have occult metastasis

Stage IA
- <3cm, N0
- Surgery

Stage IB
- 3-5cm, N0
- Surgery
Collaborators

COWORKERS AND COLLABORATORS

- Ichiro Akagi*
- Majda Haznadar*
- Teruhide Ishigame*
- Yasuyuki Kanke
- Ewy Mathe*
- Rintaro Noro*
- Hiro Okayama*
- Taka Oike*
- Ana Robles
- Amelia Parker
- Brid Ryan*
- Aaron Schetter*
- Nozumo Yanaihara*

- Bill Blot
- Carlo Croce
- Akihiko Gemma
- Frank Gonzalez
- Aage Haugen
- Yae Kanai
- Takashi Kohno
- Sharon Pine
- Ann Schwartz
- Masahiro Seike
- Peter Shields
- Kouya Shiraishi
- Jun Yokota

*Former Fellow