Princess Margaret Cancer Centre’s Experiences with Blood-based and Breath-based Analyses of Mesotheliomas

Geoffrey Liu, MD FRCPC FISPE
Professor of Medicine, Epidemiology, Medical Biophysics, Pharmacology and Toxicology, Institute of Medical Science
Alan B. Brown Chair in Molecular Genomics
Senior Clinician Scientist, Princess Margaret Cancer Centre
University Health Network, Dalla Lana School of Public Health, University of Toronto
Disclosures

Honoraria, Advisory Boards
• Pfizer
• Novartis
• Takeda
• Roche
• Abbvie
• Merck
• Bristol Myers Squibb
• AstraZeneca
• Bayer

Speaking Engagements
• EMD Serono
• AstraZeneca

Research Grants
• CIHR
• CCSRI
• NCI (US) and NIDCR (US)
• AstraZeneca
• Takeda
Biomarker Studies

• Surrogate seromarker of bulk of disease (monitoring)
• Diagnostic seromarker
• Prognostic seromarker
• Etiological serobiomarker
• New and emerging breath biomarkers

• Serial plasma/serum/whole blood collection for ~150 mesothelioma patients
• Pre-treatment blood sampling for ~50 more mesotheliomas
• One time collection (any time point) for ~80 more mesotheliomas
• One time or serial sampling for ~500 asbestos exposed individuals with negative CT scans for mesothelioma (screened participants)
Rationale: Mesothelioma growth patterns
Mesothelin

- tumour differentiation antigen
- normally present on the mesothelial cells
- highly expressed in several human cancers including malignant mesothelioma, pancreatic, ovarian and lung adenocarcinoma.

![Diagram of mesothelin processing and localization](image)
Mesothelin as a surrogate of bulk disease
Correlations between sMRP and blinded descriptive assessment

Correlations between sMRP and modified RECIST response criteria

Correlations between sMRP and standard RECIST response criteria
Diagnostic Biomarkers can be helpful

Benign

Malignant
Fibulin-3

- secreted glycoprotein, one of a family
- AKA: EGF-containing fibulin-like extracellular matrix protein 1, EFEMP1
- elongated structure with many calcium-binding sites (tandem arrays of epidermal growth factor-like domains)
- overlapping binding sites for several basement-membrane proteins, tropoelastin, fibrillin, fibronectin and proteoglycans.
Fibulin-3 as a Blood and Effusion Biomarker for Pleural Mesothelioma

Harvey I. Pass, M.D., Stephen M. Levin, M.D., Michael R. Harbut, M.D., Jonathan Melamed, M.D., Luis Chiriboga, Ph.D., Jessica Donington, M.D., Margaret Huflejt, Ph.D., Michele Carbone, M.D., Ph.D., David Chia, Ph.D., Lee Goodglick, Ph.D., Gary E. Goodman, M.D., Mark D. Thornquist, Ph.D., Geoffrey Liu, M.D., Marc de Perrot, M.D., Ming-Sound Tsao, M.D., and Chandra Goparaju, Ph.D.

Departments of Cardiothoracic Surgery (H.I.P., J.D., M.H., C.G.) and Pathology (J.M., L.C.), New York University Langone Medical Center, and the Department of Preventive Medicine, Mount Sinai School of Medicine (S.M.L.) — both in New York; the National Center for Vermiculite and Asbestos-Related Cancers, Karmanos Cancer Institute, Detroit (M.R.H.); University of Hawaii Cancer Center, Honolulu (M.C.); the Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles (D.C., L.G.); Swedish Cancer Institute (G.E.G.) and Fred Hutchinson Cancer Research Center (M.D.T.) — both in Seattle; and Princess Margaret Hospital, University Health Network and University of Toronto, Toronto (G.L., M.P., M.-S.T.)
B  Asbestos Exposure versus Stage I or II Mesothelioma

D  Blinded Fibulin-3 Validation Trial

Princess Margaret data
Mesothelioma has variable prognosis dependent on tumour, stage, clinical, and treatment characteristics.
Plasma Biomarker Enrichment of Clinical Prognostic Indices in Malignant Pleural Mesothelioma

Harvey I. Pass, MD*, #, Chandra Goparaju, PhD*, Osvaldo Espin-Garcia, MMath+, Jessica Donington, MD*, Michele Carbone, MD®, Devalben Patel, BSc+, Zhuo Chen, PhD+, Ronald Feld, MD+, John Cho, MD+, Shirish Gadgeel, MD^, Antoinette Wozniak, MD^, Abraham Chachoua, MD^, Natasha Leighl, MD^, Ming-Sound Tsao, MD^, Marc de Perrot, MD^, Wei Xu, PhD^, and Geoffrey Liu, M.D^  

*NYU Langone Medical Center, New York, NY 10016  
^Karmanos Cancer Institute, Wayne State University, Detroit Michigan, 48201  
®University of Hawaii Cancer Center, Honolulu, Hawaii 96813  
+Princess Margaret Cancer Centre, Ontario Cancer Institute, University Health Network and University of Toronto, Toronto, ON
## Discovery (NYU/KCI) Cohort

<table>
<thead>
<tr>
<th>Prognostic variables</th>
<th>EORTC CPI</th>
<th>CALGB CPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPI alone (for log-osteopontin analysis), c-index (95% CI)</td>
<td>0.649 (0.59–0.70)</td>
<td>0.641 (0.59–0.69)</td>
</tr>
<tr>
<td>CPI alone (for log-mesothelin analysis), c-index (95% CI)</td>
<td>0.645 (0.59–0.70)</td>
<td>0.640 (0.59–0.69)</td>
</tr>
<tr>
<td>CPI + log-osteopontin, c-index (95% CI)</td>
<td>0.767 (0.71–0.82)</td>
<td>0.763 (0.71–0.81)</td>
</tr>
<tr>
<td>CPI + log-mesothelin, c-index (95% CI)</td>
<td>0.692 (0.63–0.76)</td>
<td>0.724 (0.66–0.79)</td>
</tr>
</tbody>
</table>

**Improvement in Harrell’s c-indices when adding log-osteopontin**

<table>
<thead>
<tr>
<th>Prognostic variables</th>
<th>EORTC CPI</th>
<th>CALGB CPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement in Harrell’s c-indices when adding log-osteopontin</td>
<td>0.118 (0.10–0.18)</td>
<td>0.122 (0.11–0.18)</td>
</tr>
<tr>
<td>Improvement in Harrell’s c-indices when adding log-mesothelin</td>
<td>0.045 (0.03–0.11)</td>
<td>0.084 (0.06–0.13)</td>
</tr>
</tbody>
</table>

## Validation (PMCC) Cohort

<table>
<thead>
<tr>
<th>Prognostic variables</th>
<th>EORTC CPI</th>
<th>CALGB CPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPI alone, c-index (95% CI)</td>
<td>0.596 (0.55–0.64)</td>
<td>0.602 (0.54–0.66)</td>
</tr>
<tr>
<td>CPI + log-osteopontin, c-index (95% CI)</td>
<td>0.811 (0.76–0.86)</td>
<td>0.781 (0.73–0.83)</td>
</tr>
<tr>
<td>CPI + log-mesothelin, c-index (95% CI)</td>
<td>0.650 (0.58–0.72)</td>
<td>0.649 (0.58–0.71)</td>
</tr>
</tbody>
</table>

**Improvement in Harrell’s c-indices when adding log-osteopontin**

<table>
<thead>
<tr>
<th>Prognostic variables</th>
<th>EORTC CPI</th>
<th>CALGB CPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement in Harrell’s c-indices when adding log-osteopontin</td>
<td>0.216 (0.20–0.26)</td>
<td>0.179 (0.16–0.23)</td>
</tr>
<tr>
<td>Improvement in Harrell’s c-indices when adding log-mesothelin</td>
<td>0.054 (0.03–0.12)</td>
<td>0.047 (0.03–0.10)</td>
</tr>
</tbody>
</table>
Figure 1. Visual inspection of model fit curves evaluating tertiles of the risk score generated from the pooled prognostic model
Germline Biomarker: SMARCA2/BRM functional polymorphisms

RESEARCH ARTICLE

Association of two BRM promoter polymorphisms and smoking status with malignant pleural mesothelioma risk and prognosis

Min Joon Lee, Nathan Kuehne, Katrina Hueniken, Shermi Liang, Sudhir Rai, Hadas Sorotsky, Michael Herman, Daniel Shepshelovich, Jeffrey Bruce, Mindy Liang, Devalben Patel, Dangxiao Cheng, Zhuo Chen, Lawson Eng, M. Catherine Brown, John Cho, Natasha B. Leighl, Marc de Perrot, David Reisman, Wei Xu, Penelope A. Bradbury, Geoffrey Liu

First published: 29 July 2019 | https://doi.org/10.1002/mc.23088
SMARCA2/BRM is the ATP-engine of the SWI/SNF complex that drives chromatin remodeling; Loss of its function is associated with worse outcomes, as SMARCA controls many oncogenesis functions.
Two functional promoter SMARCA2/BRM polymorphisms are functional by altering binding of a MEF2D-HDAC complex that represses SMARCA2 gene expression.

Liu et al, Oncogene, 2011
These two SMARCA2 promoter polymorphisms regulate SMARCA gene expression, which alters cellular proliferation through multiple mechanisms.

Liu et al, Oncogene, 2011
Chromatin Immunoprecipitation and Luciferase promoter swap experiments across multiple cell lines supports MEF2/HDAC/skeletal binding and control of gene expression BRM polymorphisms.

Lee et al., Mol Carcinogenesis, 2019; Liu et al., CCR, 2017
EMSA and DAPA analyses support the differential binding of the two SMARCA polymorphisms to MEF2/HDAC9 and scaffolding proteins

Lee et al, Mol Carcinogenesis, 2019

A. Oligonucleotide probes for BRM -1321

<table>
<thead>
<tr>
<th>Probe Description</th>
<th>Abbreviation</th>
<th>Probe Sequence and Percent Homology to MEF2 binding site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>W</td>
<td>5'-CTCAGGAGCAAG<strong>TTT</strong>AAAGTCAAGCATC-3' 70% homology</td>
</tr>
<tr>
<td>Variant</td>
<td>V</td>
<td>5'-CTCAGGAGCAAG<strong>TTT</strong>AAAGTCAAGCATC-3' 100% homology</td>
</tr>
<tr>
<td>Variant left tandem sequence replaced</td>
<td>VSR</td>
<td>5'-CTCAGGAGCAAGCCGC<strong>TTT</strong>AAAGTCAAGCATC-3' 60% homology</td>
</tr>
<tr>
<td>Variant right tandem sequence replaced</td>
<td>V3R</td>
<td>5'-CTCAGGAGCAAGCCGC<strong>TTT</strong>AAAGTCAAGCATC-3' 60% homology</td>
</tr>
</tbody>
</table>

B. Oligonucleotide probes for BRM -741

<table>
<thead>
<tr>
<th>Probe Description</th>
<th>Abbreviation</th>
<th>Probe Sequence and Percent Homology to MEF2 binding site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>W</td>
<td>5'-CCCTTTTCATTTTTTACCTGGAAT-3' 90% homology</td>
</tr>
<tr>
<td>Variant</td>
<td>V</td>
<td>5'-CCCTTTTCATTTTTTACCTGGAAT-3' 90% homology</td>
</tr>
<tr>
<td>Variant left tandem sequence replaced</td>
<td>VSR</td>
<td>5'-CCCTTTTCGCGCGTATTTTTTACCTGGAAT-3' 80% homology</td>
</tr>
<tr>
<td>Variant right tandem sequence replaced</td>
<td>V3R</td>
<td>5'-CCCTTTTCGCGCGTATTTTTTACCTGGAAT-3' 90% homology</td>
</tr>
<tr>
<td>Variant middle tandem sequence replaced</td>
<td>VR</td>
<td>5'-CCCTTTTCGCGCGTATTTTTTACCTGGAAT-3' 90% homology</td>
</tr>
<tr>
<td>Variant middle sequence replaced, double length</td>
<td>VDR</td>
<td>5'-CCCTTTTCGCGCGTATTTTTTACCTGGAAT-3' 80% homology</td>
</tr>
</tbody>
</table>

C. DNA affinity precipitation analysis (DAPA) in the UMCC-9 cell line

D. BRM mRNA (by qPCR) fold changes during knockdown of YWHAs by anti-YWHA shRNAi

Electrophoretic Mobility Shift Assay
SMARCA2 Polymorphisms are associated with mesothelioma prognosis

### BRM Polymorphisms and MPM Overall Survival

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Total N (Multivariable)</th>
<th>Genotype</th>
<th>%</th>
<th>Univariable HR (95% CI)</th>
<th>p-value</th>
<th>Multivariable aHR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRM-741</strong></td>
<td>258 (253)</td>
<td>Wild type</td>
<td>29%</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygote</td>
<td>45%</td>
<td>1.26 (0.9-1.8)</td>
<td>0.18</td>
<td>1.47 (1.0-2.1)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygote</td>
<td>26%</td>
<td>2.21 (1.5-3.2)</td>
<td>&lt;0.001</td>
<td>2.71 (1.8-4.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BRM-1321</strong></td>
<td>256 (251)</td>
<td>Wild type</td>
<td>31%</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heterozygote</td>
<td>48%</td>
<td>0.93 (0.7-1.3)</td>
<td>0.68</td>
<td>1.19 (0.8-1.7)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homozygote</td>
<td>21%</td>
<td>2.02 (1.4-3.0)</td>
<td>&lt;0.001</td>
<td>2.69 (1.8-4.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Both BRM-741 and BRM-1321</strong></td>
<td>256 (251)</td>
<td>Double Wild type (DWT)</td>
<td>19%</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No homozygotes but not DWT</td>
<td>50%</td>
<td>0.83 (0.6-1.2)</td>
<td>0.33</td>
<td>1.04 (0.7-1.5)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One homozygous variant</td>
<td>16%</td>
<td>1.18 (0.8-1.9)</td>
<td>0.477</td>
<td>1.64 (1.0-2.7)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Double homozygous variants</td>
<td>16%</td>
<td>2.46 (1.6-3.9)</td>
<td>&lt;0.001</td>
<td>3.18 (2.0-5.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
SMARCA2 polymorphisms and differential risk by smoking status

a new risk factor for never-smokers with mesothelioma?
A protective factor in smokers?

<table>
<thead>
<tr>
<th>Variable</th>
<th>BRM Polymorphisms and MPM Risk</th>
<th></th>
<th>Ever-Smokers</th>
<th>Never-Smokers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N (%)</td>
<td>adjusted OR$^1$ (95% CI)</td>
<td>p-value</td>
<td>N (%)</td>
</tr>
<tr>
<td>Total N=1054</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td></td>
<td>191 (26%)</td>
<td>Reference</td>
<td></td>
<td>86 (27%)</td>
</tr>
<tr>
<td>Heterozygote</td>
<td></td>
<td>356 (49%)</td>
<td>0.69 (0.4-1.1)</td>
<td>0.11</td>
<td>139 (43%)</td>
</tr>
<tr>
<td>Homozygote</td>
<td></td>
<td>183 (25%)</td>
<td>0.28 (0.2-0.5) &lt;0.001</td>
<td></td>
<td>99 (31%)</td>
</tr>
<tr>
<td>Total N=1046</td>
<td></td>
<td>725 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td></td>
<td>263 (36%)</td>
<td>Reference</td>
<td></td>
<td>100 (31%)</td>
</tr>
<tr>
<td>Heterozygote</td>
<td></td>
<td>338 (47%)</td>
<td>1.26 (0.8-2.0)</td>
<td>0.29</td>
<td>135 (42%)</td>
</tr>
<tr>
<td>Homozygote</td>
<td></td>
<td>124 (17%)</td>
<td>0.26 (0.1-0.6) <strong>0.002</strong></td>
<td></td>
<td>86 (27%)</td>
</tr>
<tr>
<td>Total N=1040</td>
<td></td>
<td>721 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double Wild type (DWt)</td>
<td></td>
<td>131 (18%)</td>
<td>Reference</td>
<td></td>
<td>53 (17%)</td>
</tr>
<tr>
<td>No homozygotes but not DWt</td>
<td></td>
<td>360 (50%)</td>
<td>0.90 (0.5-1.5)</td>
<td>0.69</td>
<td>138 (43%)</td>
</tr>
<tr>
<td>One homozygous variant</td>
<td></td>
<td>156 (22%)</td>
<td>0.32 (0.2-0.7) <strong>0.002</strong></td>
<td></td>
<td>72 (23%)</td>
</tr>
<tr>
<td>Double homozygous variants</td>
<td></td>
<td>74 (10%)</td>
<td>0.18 (0.1-0.6) <strong>0.004</strong></td>
<td></td>
<td>56 (18%)</td>
</tr>
</tbody>
</table>
SMARCA2 polymorphisms and differential risk by smoking status: a new risk factor for never-smokers with mesothelioma? A protective factor in smokers?
Blood-based biomarkers

• Risk factors and biology: BRM
• Diagnosis and diagnostic supplement: Fibulin-3
• Prognosis: Osteopontin and BRM germline genetics
• Monitoring: Mesothelin
• Future: BAP1 through liquid biopsies?

Now onto something new: Breathomics
“Fetor Oris”
- From ancient times
- Potential sign of abscess
- Dental care
- DDx by Pliny

• Pseudomonas aeruginosa
• Grape-like smell
“Direct release” hypothesis

- Secondary stomach cancer in the lungs

Blood-breath equilibrium hypothesis

- Diagram showing secondary stomach cancer in the lungs
- Detecting Cancer from HN, Esoph, Stomach, Lung

- Alcohol Leaving Blood Into Breath
- ETOH in Blood
- ETOH in Breath
- Alveolus
- Deoxygenated blood from pulmonary artery
- Air
- To Breath Machine
Dogs as Proof-of-Principle

- lung cancer
- melanoma
- breast cancer
- bladder cancer
- prostate cancer
- Colorectal cancer
Dogs as Proof-of-Principle

Quality control

Scale up issues

- lung cancer
- melanoma
- breast cancer
- bladder cancer
- prostate cancer
- Colorectal cancer
Dogs as Proof of Principle

- Lung cancer
- Melanoma
- Breast cancer
- Bladder cancer
- Prostate cancer
- Colorectal cancer

Scale up issues

Proof-of-Principle
Figure 7. (A) Blow up version of the SpinoNose 2.0. (B) SpinoNose (1), Communication Unit (2) and power adaptor (3).
oxidising ambient

electron depletion at surface and grain boundaries
↓
high resistance

reducing ambient

electron rich surface and grain boundaries
↓
low resistance

Scheme of the reaction between CO and adsorbed oxygen (O-ad) on the SnO₂ surface
Lung cancer pilot
Mesothelioma
(pending funding)
Technology Overview

Picomole Breath Analysis
Comprised of Three Components:
- Breath Sampler
- Breath Analyzer
- Breath Software

Other technologies: Picomole

Breath Analyzer

The breath sample is loaded into the analyzer and heated to release the VOCs into the infrared spectroscopy cavity-ringdown chamber where the absorption properties of the VOCs are measured by lasers.

VOC Composite Absorption Spectrum Output

*representative and not actual disease spectrum
Biomarker research can provide new information about biology and ALSO help with all aspects of mesothelioma care.
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Stef Schouwenburg
Dr. Erin Stewart

Dr. Penelope Bradbury
Clinical Research Team
Dr. Sabine Schmid
Kristen Dietrich
Michael Herman
Ashraf Altesha

Clinicians
Dr. John Cho
Dr. Natasha Leighl
Dr. Marc De Perrot
Dr. Adrian Sacher

External Collaborators
Dr. Harvey Pass
Dr. David Reisman
Dr. Maitland van der Zee
Kim Blackwood

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- Imperial Oil Foundation
- International Association of Heat and Frost Insulators and Asbestos Workers Local 95 of Ontario
- International Association of Heat and Frost Insulators and Asbestos Workers (U.S.)
- International Brotherhood of Boilermakers Local 128
- I.U.O.E. Local 793
- Loretta’s Legacy Foundation
- Master Insulators Association of Ontario
- Mechanical Contractors Association Toronto
- Mechanical Industry Advisory Committee (MIAC)
- Motley Rice LLC
- Ontario Pipe Trades Council
- Ontario Sheet Metal Workers and Roofers Conference Inc.
- Sarnia Occupational Health Clinic for Ontario Workers
- United Association of Journeymen & Apprentices Local 67
- United Association of Plumbing and Pipe Fitters Local 46
- Many others