Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2016:

Name of the enterprise / Nature of the interest

<table>
<thead>
<tr>
<th>Enterprise</th>
<th>Interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nothing to declare</td>
<td></td>
</tr>
</tbody>
</table>
Genes that enable cerebral metastasis of breast cancer

Rute Pedrosa, Department of Pathology
Erasmus MC – Rotterdam

No disclosures
Brain Metastasis - Introduction

- ~150,000 annually diagnosed cases of brain metastasis (BM)\(^1\)
  - 19% of cancer patients will develop complications from BM\(^2\):
    - 30% adults
    - 6-10% children
  - Autopsy: ~25% metastasis to the CNS
- Most common types of cancers that metastasize to the CNS\(^2\):
  - Lung (40-50%)
  - Melanoma (5-20%)
  - Breast (15-30%)
    - 10-16% stage IV patients
    - Triple-Negative and Her2+

\(^1\)Oncology (Williston Park) 2016; 30(10):923-33.
Pathogenesis of Brain Metastases

1) Tumour cell growth
2) Angiogenesis
3) Invasion
4) Intravasation
5) Survival in circulation
6) Arrest in new organ
7) Extravasation

Cross section of blood vessel
- Astrocyte
- Nucleus
- Endothelial cell
- Neuron
- Mitochondrion
- Pericyt
- Lumen of the capillary
- Basal membrane
- Astrocyte

Longitudinal section of blood vessel
- Blood
- Tight junction
- Endothelial cell
- Basal membrane
- Astrocyte
- Microglia
- Neuron

https://geneticliteracyproject.org
Aim of the study

To identify genes involved in the formation of cerebral metastasis of breast cancer.
What genes enable cerebral metastasis of breast cancer?

**Group A**

Women with brain metastasis + metastases to other organs  
\( n=13 \)

**Group B**

Women with metastases to other organs  
\( n=9 \)

**Selection for:**

- No adjuvant therapy
- ER negative
- # metastasis limited to 3 organs
**Methods**

- RNA isolation and quality control
- RNA expression profiles using Illumina WG-DASL microarrays

**Validation**

- RT-PCR
- IHC
- Functionality: BBB transgression model

**Samples of primary breast tumors**

**Identification of differentially expressed gene/s**

**Statistical analysis & Bioinformatics**
Data analysis

Independent Validation by RT-PCR

Metastasis to other organs

Metastasis to brain + other organs
## Data analysis

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Platform</th>
<th># of samples which metastasized to brain + other organs</th>
<th># of samples which metastasized to other organs</th>
<th>Total number of samples</th>
<th>P-value</th>
<th># of over-expressed probes in tumours with metastases to brain + other organs</th>
<th># of over-expressed probes in tumours with metastases to other organs</th>
<th>Total number of differentially expressed probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Illumina WG-DASL</td>
<td>13</td>
<td>9</td>
<td>22</td>
<td>0.001</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Illumina WG-DASL</td>
<td>13</td>
<td>9</td>
<td>22</td>
<td>0.01</td>
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<td>23</td>
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<tr>
<td>Breast cancer</td>
<td>Illumina WG-DASL</td>
<td>13</td>
<td>9</td>
<td>22</td>
<td>0.05</td>
<td>176</td>
<td>122</td>
<td>298</td>
</tr>
</tbody>
</table>

Additional validations for the differentially expressed genes.
Data analysis

Mets to brain + other organs  Mets to other organs

$p < 0.01 \ (n=55)$

Underexpressed genes  Overexpressed genes
Genes of interest

- **BOC**
  - *Cell adhesion associated, oncogene regulated*
  - Member of the immunoglobulin/fibronectin type III repeat family;
  - Component of a cell-surface receptor complex that mediates cell-cell interactions between muscle precursor cells;
  - Promotes myogenic differentiation

- **MAP2**
  - *Microtubule Associated Protein 2*
  - Microtubule assembly (neurogenesis)
  - Microtubule stabilization (crosslinking)
Validation by IHC

<table>
<thead>
<tr>
<th></th>
<th>BOC</th>
<th>MAP2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary breast cancer:</strong></td>
<td><img src="BOC_1.png" alt="Image" /></td>
<td><img src="MAP2_1.png" alt="Image" /></td>
</tr>
<tr>
<td>Brain metastasis</td>
<td><img src="BOC_2.png" alt="Image" /></td>
<td><img src="MAP2_2.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Primary breast cancer:</strong></td>
<td><img src="BOC_3.png" alt="Image" /></td>
<td><img src="MAP2_3.png" alt="Image" /></td>
</tr>
<tr>
<td>Metastases to other organs (no brain metastasis)</td>
<td><img src="BOC_4.png" alt="Image" /></td>
<td><img src="MAP2_4.png" alt="Image" /></td>
</tr>
<tr>
<td>samples</td>
<td>group</td>
<td># metastatic sites</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>1</td>
<td>brain</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>brain</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>brain</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>brain</td>
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</tr>
<tr>
<td>5</td>
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<td>8</td>
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<td>3</td>
</tr>
<tr>
<td>14</td>
<td>other organs</td>
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</tr>
<tr>
<td>15</td>
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<td>2</td>
</tr>
<tr>
<td>16</td>
<td>other organs</td>
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<td>17</td>
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<td>3</td>
</tr>
<tr>
<td>18</td>
<td>other organs</td>
<td>2</td>
</tr>
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</table>
## Validation by RT-PCR

<table>
<thead>
<tr>
<th></th>
<th>Discovery set</th>
<th>Extra validation set</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Metastasis to other organs</td>
<td>Metastasis to brain + other organs</td>
</tr>
<tr>
<td>Fresh-frozen</td>
<td>9</td>
<td>13</td>
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<tr>
<td>FFPE</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

**Relative Expression**

- **BOC**
- **MAP2**

### Bar Graph

- **p < 0.03**
- **p < 0.02**
- **p > 0.05**
Conclusions

<table>
<thead>
<tr>
<th></th>
<th>BOC</th>
<th>MAP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina WG-DASL</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>IHC</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>✓</td>
<td>✗</td>
</tr>
</tbody>
</table>

Ongoing steps…

- Functional studies to reveal the specific involvement of BOC and MAP2 in the formation of brain metastasis
- Validation of other differentially expressed genes
Thank you!

**Internal Oncology:**
- Vania de Weerd
- Anieta Sieuwerts
- Marcel Smid
- John Martens

**Pathology:**
- Marcel van der Weiden
- Dana Mustafa
- Johan M. Kros

**Patients**