**Disclosure Information**

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

<table>
<thead>
<tr>
<th>Name of the enterprise / Nature of the interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nothing to declare</td>
</tr>
</tbody>
</table>
T lymphocytes induce the expression of GBP1 and facilitate brain metastasis of breast cancer

Rute Pedrosa, Department of Pathology
Erasmus MC - Rotterdam

No disclosures
Aim of the study

To identify specific pathways involved in the formation of cerebral metastasis of breast cancer.
Which specific pathways are involved in 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

Samples of primary breast tumors

RNA isolation and quality control

RNA expression profiles using Illumina WG-DASL microarrays

Statistical analysis & Bioinformatics

Identification of differentially expressed gene/s

Validation

- RT-PCR
- IHC
- Functionality: BBB transgression model
## Data analysis

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Platform</th>
<th># of samples which metastasized to brain</th>
<th># of samples which metastasized to other organs but brain</th>
<th>Total number of samples</th>
<th>P-value</th>
<th># of over-expressed probes in tumours with metastases to brain</th>
<th># of over-expressed probes in tumours with metastases to other organs</th>
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</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>
<td>32</td>
<td>23</td>
<td>55</td>
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<td>22</td>
<td>0.05</td>
<td>176</td>
<td>122</td>
<td>298</td>
</tr>
</tbody>
</table>

**Pathway analysis**
### Ingenuity pathway analysis (IPA) results

<table>
<thead>
<tr>
<th>Pathway</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication between innate and adaptive of the immune system</td>
<td>1.65E-08</td>
</tr>
<tr>
<td>Antigen presenting pathway</td>
<td>1.44E-06</td>
</tr>
<tr>
<td>Type I Diabetes mellitus signaling</td>
<td>4.71E-06</td>
</tr>
<tr>
<td>T-helper cell differentiation</td>
<td>7.1E-06</td>
</tr>
</tbody>
</table>
IPA results (cont.)

- **Up-regulated in primary breast that metastasized to brain.**
- **Up-regulated in primary breast that metastasized to other organs.**
IHC of T lymphocytes markers

<table>
<thead>
<tr>
<th>CD3</th>
<th>Primary breast cancer: Brain metastasis</th>
<th>Primary breast cancer: Metastases to other organs (no brain metastasis)</th>
</tr>
</thead>
</table>
# Tumor tissue: IHC results

<table>
<thead>
<tr>
<th>Samples</th>
<th>Metastatic site</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>brain</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>brain</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>brain</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>brain</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>brain</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>brain</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td>10</td>
<td>other organs</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>other organs</td>
<td>1</td>
<td>0</td>
<td>0</td>
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Functional studies: BBB transgression model

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

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

**In vitro Blood Brain Barrier (BBB) model**
- 3 μm pores membrane
- Insert
- HUVEC cells
- Human Astrocytes

Erasmus MC Cancer Institute
T lymphocytes: What role do they play?

Breast cancer cell line

\[ \text{T lymphocytes} \]

5-day co-culture

Green tracker

Overnight
BBB transgression model: results

MDA-MB-231 only (control)

MDA-MB-231 co-cultured with T lymphocytes

MDA-MB-231 cultured with conditioned media of T lymphocytes

Breast cancer cell line; T lymphocytes
BBB transgression model: quantitative results

# of cells that succeeded transgression of BBB

$p = 0.01$

Control  T lymphocytes co-culture  Culture with conditioned medium of T lymphocytes

MDA-MB-231
How do T lymphocytes change the ability of breast cancer cells in crossing the BBB?

MDA-MB-231 only (control) protein extraction

7 up-regulated proteins

MDA-MB-231 co-cultured with T lymphocytes protein extraction

11 up-regulated proteins

Proteomics measurement (LC-MS)
How do T lymphocytes change the ability of breast cancer cells in crossing the BBB?

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Proteomics measurement (LC-MS)

11 up-regulated proteins

MDA-MB-231 co-cultured with T lymphocytes

GBP1↑

(Guanylate Binding Protein 1)
## IHC of GBP1

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<th>GBP1</th>
<th>Brain metastasis</th>
<th>Metastases to other organs (no brain metastasis)</th>
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**Primary breast cancer:**
- Brain metastasis
- Metastases to other organs (no brain metastasis)
Human primary breast cancer samples that developed metastasis to the brain and to other organs

Select the protein that was expressed at the RNA level

Performing mass spectrometry measurements using three breast cancer cell lines before and after incubating them with T cells.

Confirm by applying an in vitro functional studies. Using three breast cancer cell lines and a BBB model

RNA-expression profiles

Pathway analysis

Select the most significant pathway

Confirm by IHC
Conclusions

- T cell response facilitate brain metastasis of ER- breast cancers
- T lymphocytes induce the expression of GBP1
- GBP1 is one of the responsible proteins for changing the ability of breast cancer cells in crossing the BBB
Thank you!

Internal Oncology:
Vania de Weerd
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Tumor Immunology:
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Mandy van Brakel
Reno Debets

Proteomics:
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Lennard Dekker
Theo Luider

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

Patients