Blavatnik Pitch 2021:

Increasing Blood-Brain Barrier Permeability by blocking Netrin1-Unc5B signaling
1. The problem

- Blood-brain barrier (BBB) integrity is crucial to normal life and is tightly controlled.
- The BBB prevents drug delivery into the diseased brain.
- BBB opening to allow transit of therapeutic agents (drugs, nanobodies, etc.) valuable for treating a variety of indications (brain cancer & metastasis, neurological diseases).
2. CNS drug delivery market

- BBB prevents development of >99% of potential drugs. **Economic burden**

- Less than 1% of drug companies have a BBB drug targeting program

- CNS drug delivery market size is expected to reach **USD 4.6 billion by 2026**

- Anti-Unc5B mediated CNS drug delivery has enormous profit potential

**Competition:**

1. focused ultrasound with microbubbles (focused BBB opening, could be combined with anti-Unc5B)
2. Mfsd2a Blocking AB under development at Harvard (opens BBB to transcytosis)

Pardridge WM, 2020, Frontiers in Aging Neurosci 11, 373
3. The product: Monoclonal AB that blocks Netrin binding to Unc5B

Delivery: Intravenous injection, low dose (10mg/kg)

<table>
<thead>
<tr>
<th>Anti-Unc5B-3</th>
<th>Human Unc5B</th>
<th>Rat Unc5B</th>
</tr>
</thead>
<tbody>
<tr>
<td>KD = 1.29nM</td>
<td>KD = 1.34nM</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IP: Unc5B (R&amp;D)</th>
<th>IgG</th>
<th>CTRL anti-Unc5B-1</th>
<th>Anti-Unc5B-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netrin1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Robo4</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Flr2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Protein expression (a.u.)

- Netrin1-IP (/Unc5B-IP)
- Robo4-IP (/Unc5B-IP)
- Flr2-IP (/Unc5B-IP)

Blocks binding of Netrin1 to Unc5B

- Netrin1-IP (/Unc5B-IP): p = 0.0079
- Robo4-IP (/Unc5B-IP): NS
- Flr2-IP (/Unc5B-IP): NS
Anti-Unc5B controls permeability in the brain

The effect lasts for a few hours

but not in other organs

Adult WT

Antibodies (i.v., 10mg/kg, 1h)

Cadaverine (i.v., 30min)


g (cadaverine) / mg (brain)

p = 0.0079

CTRL IgG Anti-Unc5B-3

Adult WT

Antibodies (i.v., 10mg/kg, 8h)

Cadaverine (i.v., 30min)


g (cadaverine) / mg (organ)

Lung

Heart

Kidney

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

ng (cadaverine) / mg (organ)

Lung

Heart

Kidney

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

ng (cadaverine) / mg (organ)

Lung

Heart

Kidney

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

ng (cadaverine) / mg (organ)

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ng (cadaverine) / mg (organ)

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CTRL IgG Anti-Unc5B-3

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ng (cadaverine) / mg (organ)

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ng (cadaverine) / mg (organ)

Lung

Heart

Kidney

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

CTRL IgG Anti-Unc5B-3

ng (cadaverine) / mg (organ)
4. Therapeutic utility: delivery of bioactive molecules and chemotherapeutics

**10kDa Dextran**
- Unc5B<sup>fl/fl</sup> vs CTRL IgG: p = 0.0317
- Anti-Unc5B-3 vs CTRL IgG: p = 0.0079

**40kDa Dextran**
- Unc5B<sup>fl/fl</sup> vs CTRL IgG: p < 0.0001
- Anti-Unc5B-3 vs CTRL IgG: p = 0.0079

**70kDa dextran**
- Unc5B<sup>fl/fl</sup> vs CTRL IgG: NS
- Anti-Unc5B-3 vs CTRL IgG: NS

**Nanobodies**
- p = 0.0012

**BDNF (brain)**
- p = 0.0006

**BDNF (plasma)**
- NS

**pTyr-IP (/Trk-B-IP)**
- p = 0.0079

**Protein expression (a.u.)**
- p = 0.0012

**Caspase3 positive cell in tumor**
- DMSO
- Vincristine
- p=0.0219
5. The mechanism: Unc5B and Ntn1 maintain WNT signaling at the BBB

- Nuclear β-catenin
- BBB gene expression
- Claudin5 upregulation
- PLVAP downregulation

Anti-Unc5B-3 inhibits Wnt
Wnt-β-catenin overactivation
Rescues BBB leak in Unc5B kos

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Image showing the mechanism with nodes labeled Unc5B, Netrin, and the effect on gene expression. Diagrams illustrate the relationship between Unc5B and Wnt signaling components.
6. Utilization of Blavatnik funds:

Transient BBB opening in non-human primates

Monitored by MRI + anti-Unc5B following Gadolinium delivery

Determine safety and efficacy

Outsourced to Charles River or equivalent company

Estimated budget 300.000$

7. The team

Anne Eichmann, PhD
Ensign Professor of Medicine (Cardiology)
Professor of Cellular and Molecular Physiology

Kevin Boye, PhD
Postdoctoral associate

Luiz Henrique Geraldo, PhD
Postdoctoral associate