Targeting M1 muscarinic and sigma-1 receptors in Alzheimer's disease

Reversal of pathological hallmarks and associated cognitive dysfunction in McGill-R-Thy1-APP rats

Hélène Hall, PharmD, PhD
Postdoctoral fellow | Cuello lab
McGill university
Montreal, Canada
## Faculty Disclosure

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Honoraria/ Expenses</th>
<th>Consulting/ Advisory Board</th>
<th>Funded Research</th>
<th>Royalties/ Patent</th>
<th>Stock Options</th>
<th>Ownership/ Equity Position</th>
<th>Employee</th>
<th>Other (please specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- No, nothing to disclose
- Yes, please specify:

## Off-Label Product Use

Will you be presenting or referencing off-label or investigational use of a therapeutic product?

- No
- Yes, please specify:
Stimulation of M1 muscarinic receptors

- reduces amyloid pathology
- decreases tau hyperphosphorylation
AF710B (aka ANAVEX 3-71): a novel M1/sigma-1 agonist

**AF710B drug (aka ANAVEX 3-71)**

- Allosteric modulator of M1 muscarinic receptor: modulation of the APP processing  
  (Nitsch et al, 1992, Science; Caccamo et al, 2006, Neuron)

- Agonist of sigma 1 receptor: enhanced cognition, neuroprotection  
  (Senda et al, 1996, Eur J Pharmacol, Meunier et al, 2006, J Pharmacol Exp Ther.)

**In 3xtg mice:**

- AF710B attenuates cognitive deficits
- Reduces AD-like hallmarks  
  (amyloid pathology, tau hyperphosphorylation, inflammation)
McGill-R-Thy1-APP rats: a model of AD-like amyloid pathology

- Express human APP, carrying mutations associated with familial AD, under Thy1 promoter
- One single transgene insertion per allele, minimal genetic charge

**Pre-plaque** (early): Accumulation of intraneuronal Aβ

- 1 week
- 6 months

**Post-plaque** (late, >9mo): Deposition of Aβ into extracellular plaques

- 15 months
- 20 months

Changes over time:

- Cognitive decline
- Inflammation
- Oxidative stress
- Impaired LTP
- Hypometabolism
- Hippocampal shrinkage
- Mitochondrial dysfunction

Hélène Hall | McGill University | Cuello lab

Leon *et al.*, 2010, JAD 20(1)-113

Wilson *et al.*, 2016 Cerebral Cortex

Wilson *et al.*, 2016 Cerebral Cortex

Leon *et al.*, 2010, JAD 20(1)-113
Experimental design

- Long-term treatment of McGill transgenic rats with AF710B, at an advanced stage of pathology.
AF710B fully reverts the cognitive deficit in aged McGill tg rats

- **Novel Object Recognition**
  - Based on spontaneous differential exploration of familiar and new objects
  - Rodents will naturally spend more time exploring novel objects over familiar objects

![Diagram showing novel object recognition](image-url)

Wilson *et al.*, 2017 submitted
AF710B fully reverts the cognitive deficit in aged McGill tg rats

**Novel Object Recognition**

- Based on spontaneous differential exploration of familiar and new objects
- Rodents will naturally spend more time exploring novel objects over familiar objects

![Diagram showing novel object recognition experiment](image)

**Discrimination Index**

<table>
<thead>
<tr>
<th>wt</th>
<th>tg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Hall et al, 2017, in preparation
AF710B fully reverts the cognitive deficit in aged McGill tg rats

- Morris Water Maze

  - 5 days of training to locate hidden platform
  - 4 trials/day
  - Probe trial on day 6

**Acquisition phase**

- Figure showing latency to reach platform over days for wt vehicle and tg vehicle groups.

Hall et al, 2017, in preparation
AF710B fully reverts the cognitive deficit in aged McGill tg rats

- **Morris Water Maze**

  ✓ 5 days of training to locate hidden platform
  ✓ 4 trials/day
  ✓ Probe trial on day 6

![Diagram of Morris Water Maze](image1)

Acquisition phase

*\( p<0.05, **p<0.01, ***p<0.001; \) two-way ANOVA

![Bar chart of latency to reach platform](image2)

Probe test

![Bar chart of number of zone crossings](image3)

Performance on trial 4 - day 5

![Graph of latency to reach platform](image4)

Representative swim paths

Hall et al, 2017, in preparation
AF710B reduces AD-like amyloid pathology in McGill tg rats

- **Decrease in Aβ immunoreactivity (McSA1 staining)**

- **Decrease in mature plaques (ThioS+)**

- **Decrease in cortical Aβ42 (ELISA)**

- **Increase in CSF Aβ42**

*Hall et al, 2017, in preparation*
AF710B reduces inflammation in McGill tg rats

Early and late inflammation in McGill rats

- Increase in cytokines and chemokines

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>wt 4 months</th>
<th>tg 4 months</th>
<th>tg 13 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>IFNγ</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>IL-1α</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IL-12</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-13</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IL-2</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IL-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-10</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-17α</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>wt 4 months</th>
<th>tg 4 months</th>
<th>tg 13 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL1</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>GMCSF</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IP-10</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Rantes</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Resistin</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Hanelz et al, 2014, Neurobiol Aging
35(10)-2249
AF710B reduces inflammation in McGill tg rats

**Early and late inflammation in McGill rats**

- Increase in cytokines and chemokines

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>wt 4 months</th>
<th>tg 4 months</th>
<th>tg 13 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>IFNγ</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>IL-1α</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-1β</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-8</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IL-12</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>IL-13</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IL-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-10</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TGF-β</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-17α</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemokines</th>
<th>wt 4 months</th>
<th>tg 4 months</th>
<th>tg 13 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL5</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CXCL1</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IP-10</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Rantes</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Resistin</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Hanzel *et al.*, 2014, Neurobiol Aging 35(10)-2249

**AF710B has anti-inflammatory properties**

- Microglia recruitment to Aβ-burdened neurons in CA1

- Hippocampal levels of Iba1

- Cortical IL 10 mRNA

*AF710B reduces inflammation in McGill tg rats*

*p<0.05, **p<0.01; two-way ANOVA*

Hall *et al.*, 2017, in preparation
AF710B has synaptogenic properties

- **At the postsynaptic level**
  AF710B restores synaptic spines

- **At the presynaptic level**
  AF710B restores synaptophysin levels

---

- *p<0.05; two-way ANOVA

---

Fisher *et al*., Neurodegener Dis 16(1-2)-95
AF710B has synaptogenic properties

➢ **At the postsynaptic level**
AF710B restores synaptic spines

Fisher et al, Neurodegener Dis 16(1-2)-95

➢ **At the presynaptic level**
AF710B restores synaptophysin levels

Hall et al, 2017, in preparation

**Synaptophysin mRNA levels**

**Synaptophysin protein levels**

Iulita, Bistue et al, 2017, submitted
AF710B (a dual M1/Sigma-1 agonist) has long-lasting procognitive effects at the late stages of the amyloid neuropathology in McGill-R-Thy1-APP rats.

- **Disease-modifying effects:**
  - Decrease in AD-like amyloid pathology
  - Anti-inflammatory effect
  - Synaptogenic activity

- Effects maintained following a 5-week interruption in the treatment, suggesting true disease-modifying properties.
Acknowledgements

Dr A. Claudio Cuello
Adriana Ducatenzeiler
Dr M. Florencia Iulita, PhD
Lisi Flores Aguilar
Palma Gubert
Dr Mark Hancock

Richard and Edith Strauss Canada Foundation

Dr Abraham Fisher

ANAVEX is a trademark of Anavex Life Sciences