Final Progress Report

*Testing of ambroxol in the Thy1-αSyn mouse model of PD*

Marie-Francoise Chesselet, UCLA
Stephen Anderson and Robert Johnston, ExSAR

UCLA: Sudhakar Subramaniam, PhD
Garima Dutta, PhD
Chunni Zhu, PhD
Link Gaucher-PD

- GD patients: 5 fold higher risk for PD
- 1% of population carry $GBA$ mutations
- 5-30% of PD patients carry $GBA$ mutations
- 28% of LBDs patients carry $GBA$ mutations
Gaucher Disease Glucocerebrosidase and α-Synuclein Form a Bidirectional Pathogenic Loop in Synucleinopathies

Mazzulli et al., Cell, 2011
Pharmacological chaperones for Glucocerebrosidase (Gcase) stabilize GCase and increase its net-activity in the lysosome.

Pharmacological chaperones are designed to be selective, potent and reversible.
Amicus Therapeutics

At the Forefront of Therapies for Rare Diseases™
Neurotherapeutics, 2014

A GCase chaperone improves motor function in a mouse model of synucleinopathy

Franziska Richter DVM, PhD*, Sheila M. Fleming PhD*, Melanie Watson PhD*, Vincent Lemesre MS*, Lee Pellegrino BA†, Brian Ranes BMus†, Chunni Zhu MD, PhD*, Farzad Mortazavi PhD*, Caitlin K. Mulligan*, Pedrom C. Sioshansi*, Sindalana Hean*, Krystal De La Rosa*, Richie Khanna PhD†, John Flanagan PhD†, David J. Lockhart PhD†, Brandon A. Wustman PhD ‡, Sean W. Clark PhD† and Marie-Françoise Chesselet MD, PhD*
GCase chaperone (AT2101) in Thy1-aSyn mice

- Decreases alpha-synuclein neuronal levels
- Decreases small aggregates
- Decreases inflammation
- Improves motor function

*Supports efficacy of Gcase treatment in absence of Gaucher mutation*
Ambroxol as GCase chaperone

• Inhibits GCase in ER but not at the acidic pH of lysosomes
• Pharmacological chaperone in Gaucher cells
• Increases Gcase protein and activity in Gaucher patient cells
• Increases GCase levels in lysosomes
• Increases GCase in mouse neurospheres
• Up to 25mg/kg for up to 31 months in 3 Gaucher patients, improved neurological deficits without evidence of toxicity
Alpha-synuclein overexpressor (ASO= Thy1-aSYN)

• Eliezer Masliah, UCSD
• “Line 61”
• Over-expresses full length, human, wild-type, alpha-synuclein
• Broad over-expression in neurons
• Closer mimic to SPORADIC PD
Wild-type and Thy1-aSyn mice have similar GCase activity at baseline (28 days of age, naïve)
## Ambroxol Mouse Pharmacokinetics

**Bolus administration (oral gavage) to BDF1 male mice**  
Collection time points: 0.5, 1.0, 2, 8, & 24 hrs.  
Three doses: 30 mg/kg, 100 mg/kg, 300 mg/kg  
Three animals at each dose/time point (45 total)

<table>
<thead>
<tr>
<th>Plasma</th>
<th>30.0</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>0.46</td>
<td>0.80</td>
<td>3.4</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>AUC to Last (µg-hr/mL)</td>
<td>0.8</td>
<td>2.2</td>
<td>16.5</td>
</tr>
<tr>
<td>Last Time point</td>
<td>8</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>MRTINF (hr)</td>
<td>6.7</td>
<td>4.5</td>
<td>6.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brain</th>
<th>30.00</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (µg/g)</td>
<td>5.1</td>
<td>11.6</td>
<td>39.7</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>AUC to Last (µg-hr/g)</td>
<td>10.5</td>
<td>48.2</td>
<td>213</td>
</tr>
<tr>
<td>Last Time point</td>
<td>8</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>MRTINF (hr)</td>
<td>4.2</td>
<td>4.1</td>
<td>6.7</td>
</tr>
</tbody>
</table>

The pharmacokinetics indicate good brain uptake, indicating that blood-brain barrier penetration is not a problem. We also determined that ambroxol is not a substrate for human or mouse P-glycoprotein.

*ExSAR Confidential*
Ambroxol Mouse Pharmacokinetics (con’d)

Long term dosing via chow
Ambroxol was formulated with Teklad 7013 NIH-31 rodent chow.

Three doses: 50 mg/kg/day, 100 mg/kg/day, & 200 mg/kg/day
BDF1 mice (12 per dose) fed for seven days on each chow. (Survival bleeds on a subset verified that plasma levels had plateaued after five days.)
For each dose, six animals sacrificed at t=0 (morning) and t=8 hrs on Day 7.
Plasma and brain levels of ambroxol measured for each animal.

Brain Levels

<table>
<thead>
<tr>
<th>Dose</th>
<th>Time of sacrifice</th>
<th>Ambroxol level (AVG ± STD in ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg/kg/day</td>
<td>t = 0 hr</td>
<td>518 ± 154</td>
</tr>
<tr>
<td></td>
<td>t = 8 hr</td>
<td>81 ± 19</td>
</tr>
<tr>
<td>100 mg/kg/day</td>
<td>t = 0 hr</td>
<td>1256 ± 410</td>
</tr>
<tr>
<td></td>
<td>t = 8 hr</td>
<td>164 ± 56</td>
</tr>
<tr>
<td>200 mg/kg/day</td>
<td>t = 0 hr</td>
<td>2273 ± 483</td>
</tr>
<tr>
<td></td>
<td>t = 8 hr</td>
<td>483 ± 149</td>
</tr>
</tbody>
</table>

Note: Steady-state plasma levels (not shown) were approximately an order-of-magnitude lower than steady-state brain levels.
Study Design – Mouse enrollment

• 165 mice in total: WELL POWERED TO DETECT DRUG EFFECTS
• Groups
  – wild type-vehicle (n = 22)
  – wild type-ambroxol low dose - 50 mg/kg/day (n = 25)
  – wild type-ambroxol high dose - 150 mg/kg/day (n = 25)
  – Thy1-aSyn-vehicle (n = 34)
  – Thy1-aSyn-ambroxol low dose - 50 mg/kg/day (n = 30)
  – Thy1-aSyn-ambroxol high dose - 150 mg/kg/day (n = 33)
Ambroxol Study Timeline

Pre-treatment Motor test
- Beam
- Pole

Treatment begins (2m)

Post-1 (6m) Cognitive test
NOR

Post-1
Cognitive test (7m)
Ymaze
NPR

Motor test (7.25m)
- Beam
- Pole

Open field (7.25m)

Post-2
- Adhesive removal test (14m)

Post 2
- Open field (16m)
- Euthanasia

Treatment ends (16m)

Beam - Challenging beam test
Pole - Vertical pole test
NOR – Novel Object recognition test
Ymaze – Alternation test
NPR – Novel Place recognition test.
No change in chow consumption at 2-6m of age

All three diets
Ambroxol treatment had no effect on weight of the mice

One way ANOVA showed no significant treatment effects within wild type or Thy1-aSyn groups. Data are represented as the mean ± SEM; Wt-veh (N=22), Wt-low dose (N=25), Wt-high dose (N=25), Thy1-veh (N=34), Thy1-low dose (N=30), Thy1-high dose (N=33). (C) Figure showing the age of death and median age of death in each group mortalities.
Challenging Beam Test (2m and 7.25m)
No ambroxol treatment effect

A two-way ANOVA; Fisher’s LSD test to compare each Thy1-aSyn group to the corresponding wild type group and with Bonferonni correction to compare each dose of ambroxol to the corresponding vehicle group; Data are represented as the mean ± SEM;

Pre beam (A) Wt-veh (N=22), Wt-low dose (N=25), Wt-high dose (N=25), Thy1-veh (N=34), Thy1-low dose (N=29), Thy1-high dose (N=33); Post beam (B) Wt-veh (N=21), Wt-low dose (N=25), Wt-high dose (N=25), Thy1-veh (N=31), Thy1-low dose (N=28), Thy1-high dose (N=26), ***p<0.001 compared with wild type.
Moderate deficits in NPR but no ambroxol treatment effect

A two-way ANOVA; Fisher’s LSD test to compare each Thy1-aSyn group to the corresponding wild type group and with Bonferronni correction to compare each dose of ambroxol to the corresponding vehicle group; Data are represented as the mean ± SEM; Wt-veh (N=13-17), Wt-low dose (N=18-24), Wt-high dose (N=14-23), Thy1-veh (N=18-30), Thy1-low dose (N=15-26), Thy1-high dose (N=12-24), *p<0.05 compared with respective wild type group.
Ambroxol treatment did not alter PK-resistant alpha-synuclein aggregates in SN of Thy1-aSyn mice

One-way RM ANOVA followed by Bonferroni post tests. DL-dorsilateral, DM-dorsimedial, VL-ventrilateral and VM-ventrimedial. Data are represented as the mean ± SEM; Thy1-veh (N=21), Thy1-low dose (N=13), Thy1-high dose (N=14).
Lack of ambroxol effects in Thy1-aSyn mice

<table>
<thead>
<tr>
<th>Test</th>
<th>Genotype effect</th>
<th>Ambroxol effect (2-16 months treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MOTOR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenging Beam</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Vertical Pole</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Open field</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td><strong>COGNITION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novel place recognition</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Ymaze alternations</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td><strong>HISTOLOGY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha synuclein (SN)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>PK-resistant alpha synuclein aggregates (SN)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Microglial activation</td>
<td>N/A</td>
<td>NO</td>
</tr>
</tbody>
</table>
Comparison of AT2101 (Gcase chaperone) effects (Richter et al., 2014) to ambroxol effects

<table>
<thead>
<tr>
<th>Test</th>
<th>AT2101 Genotype effect</th>
<th>AT2101 effect (7day-on/7day-off from 1-5 months of age)</th>
<th>Ambroxol Genotype effect</th>
<th>Ambroxol effect (2-16 months treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTOR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Challenging Beam</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Vertical Pole</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Open field</td>
<td></td>
<td>YES</td>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>COGNITION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novel place recognition</td>
<td></td>
<td>YES</td>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>Ymaze alternations</td>
<td></td>
<td></td>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>Novel object recognition</td>
<td></td>
<td>NO</td>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>HISTOLOGY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha synuclein (SN)</td>
<td>YES</td>
<td>YES (alpha synuclein levels in dopaminergic neurons)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>PK-resistant alpha synuclein aggregates (SN)</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Microglial activation</td>
<td>YES</td>
<td>YES</td>
<td>N/A</td>
<td>NO</td>
</tr>
</tbody>
</table>
AT2101 increased GCase activity in brain 7 days after treatment end
Ambroxol at the given doses does NOT alter striatal/spleen GCase activity (16m)- Mahuran lab

A two-way ANOVA; Fisher’s LSD test to compare each Thy1-aSyn group to the corresponding wild type group and with Bonferonni correction to compare each dose of ambroxol to the corresponding vehicle group; Data are represented as the mean ± SEM; Wt-veh (N=16), Wt-low dose (N=23), Wt-high dose (N=19), Thy1-veh (N=21), Thy1-low dose (N=15), Thy1-high dose (N=17).
Kaplan Meier Survival Analysis – ALL mice in the study

Logrank Test
Chi square  1.334
df  2
P value  0.5132
P value summary  ns
Are the survival curves sig different?  No
Kaplan Meier Survival Analysis – Dead mice ONLY

Logrank Test
Chi square 6.898
df 2
P value **0.0318**
P value summary *
Are the survival curves sig different? Yes
Mice treated with high dose ambroxolol has an earlier age of death

- Deaths in all Thy1-aSyn mice might be related to handling and behavior testing stress
Lack of ambroxolol effect in Thy1-aSyn mice

- Good brain penetration
- No increase in striatal or peripheral Gcase activity
- Higher dose caused toxic effects specifically in tg mice
Final Progress Report

*Testing of ambroxol in the Thy1-αSyn mouse model of PD*

Marie-Francoise Chesselet, UCLA
Stephen Anderson and Robert Johnston, ExSAR

UCLA: Sudhakar Subramaniam, PhD
Garima Dutta, PhD
Chunni Zhu, PhD
Low dose (150 mg/day) long term treatment of Type 1 Gaucher patients with ambroxol

Pilot study using ambroxol as a pharmacological chaperone in type 1 Gaucher disease

Ari Zimran a, Gheona Altaescu b, Deborah Elstein a,*

a Gaucher Clinic, Shaare Zedek Medical Center, Hadassah Medical School, Hebrew University, Jerusalem, Israel
b Genetics Unit, Shaare Zedek Medical Center, Hadassah Medical School, Hebrew University, Jerusalem, Israel

ARTICLE INFO

Article history:
Submitted 24 September 2012
Available online 22 October 2012
(Communicated by A. Zimran, M.D., 24 September 2012)

Keywords:
Gaucher disease
Glucocerebrosidase activity
Ambroxol
Pharmacological chaperones
Enzyme replacement therapy

ABSTRACT

The purpose of this pilot was to assess the tolerability and efficacy of ambroxol as a pharmacological chaperone in patients with symptomatic, type 1 Gaucher disease who present with measurable disease parameters but are not receiving enzyme replacement therapy (ERT) in order to provide proof of concept and/or ascertain the suitability of ambroxol for a larger clinical trial. The Israeli Ministry of Health Form 29c was employed to prescribe ambroxol for off-label use. Twelve patients were dispensed 2 capsules of 75 mg of ambroxol daily for 6 months. There were 8 females (66.7%). Mean age at entry was 41.1 (range: 24–63) years. Mean body weight at entry was 66.4 (range: 46.5–100) kg. One patient withdrew because of a hypersensitivity reaction, one because of elective splenectomy. No patient experienced clinically relevant deterioration in disease parameters measured. One patient achieved a robust response relative to baseline: +16.2% hemoglobin; +32.9% platelets; −2.8% liver volume; and −14.4% spleen volume. Three patients, including the above one, elected to continue on ambroxol for a further 6 months; hemoglobin levels and liver volumes were relatively stable, but platelet counts further increased in the above patient (+52.6% from baseline) and spleen volumes decreased further in all three patients (−6.4%, −18.6%, and −23.4% from baseline). Thus, ambroxol may be a safe option for Gaucher disease patients with potential disease-specific efficacy and should be expanded into a clinical trial using higher doses and placebo-controlled design.

© 2012 Elsevier Inc. All rights reserved.
ABX is inhibitory at neutral (ER) but not at acidic (Lysosomal) pH

• Importance of pH

ABX is a very poor binder to GCase at the pH of lysosomes. Thus, it binds to GCase in the ER, but releases and does not inhibit in lysosomes (the cellular destination).
Treatment with either ABX or IFG increases both the activity and protein levels in mutant N370S/N370S Gaucher patient cells.

G. Maegawa
ABX and IFG Increase GCase levels in Lysosomes

**GCC Lamp-1 Merge**

- **GCC**
  - ABX
  - IFG

- **Lamp-1**
  - DMSO
  - ABX 60 µM
  - IFG 30 µM

- **Merge**
  - DMSO
  - ABX 60 µM
  - IFG 30 µM

*Gcc (N370S/N370S)*

- **GCC**
  - 5 days
  - ABX 60 µM
  - IFG 30 µM

B. Rigat
ABX Also Increases GCase Activity in Normal Mouse Neurospheres (from PPS cells)

New: Single experiment at a single dose of 10 µM IFG and 30 µM ABX and 2fggp (a covalent inhibitor of GCase); Will need to do a dose-response for IFG
High dose (up to 25 mg/kg/day) long term treatment of Type 3 Gaucher patients with ambroxol. (Abstract for the Lysosomal Disease WORLD Symposium, February, 2013)

<table>
<thead>
<tr>
<th>Title</th>
<th>Chaperone therapy for neuronopathic Gaucher disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1) Division of Child Neurology, Tottori University Faculty of Medicine</td>
</tr>
<tr>
<td></td>
<td>2) Matsue Medical Center</td>
</tr>
<tr>
<td></td>
<td>3) Shizuoka Institute for Epilepsy and Neurological Disorders</td>
</tr>
<tr>
<td></td>
<td>4) International University of Health and Welfare Graduate School</td>
</tr>
<tr>
<td></td>
<td>Aya Narita¹, Shinji Itamura³, Kentarou Shirai¹, Norika Kubota², Rumiko Takayama³, Yukitoshi Takahashi³, Yoshihiro Maegaki¹, Yoshiyuki Suzuki⁴, Kousaku Ohno³</td>
</tr>
</tbody>
</table>

Background] Chaperone therapy is expected to make contributions to alleviating neurological symptoms in lysosomal storage disorders, since small molecules can cross the blood-brain barrier. Gaucher disease (GD) is characterized by a deficiency of the lysosomal enzyme, glucocerebrosidase (GBA). Ambroxol (ABX), a commonly-used expectorant, has been reported to have chaperone activity on mutant GBA. We aimed to investigate the effect of ABX on neurological manifestations of GD.

[Methods] We enrolled 3 patients with Type 3 GD. The genotypes were N188S/G193W and N188S/?. Before inclusion in the study, written informed consent was obtained and the study was approved by the institutional review board. They received ABX combined with ERT. **ABX treatment began with 3 mg/kg/day, with doses escalating over time to 25 mg/kg/day.** The evaluation of efficacy included changes in neurological or neurophysiological assessments, activities of daily living (ADLs), hematological and clinical laboratory assessments, and safety evaluations.

[Results] **The median (range) exposure to ABX was 12 (12-31) months.** No clinical or biochemical adverse effects were found. ABX enhanced GBA activity in lymphocytes to normal levels with a serum concentration of more than 1 μM. ABX was detected in the cerebrospinal fluid, at approximately 14% of serum levels. As ABX dose increased, persistent myoclonus and seizures were ameliorated, and oculomotor deficits were improved. Improvement of the neurological symptoms also correlated with recovery of the patients’ ADLs.

[Conclusions] ABX improves or stabilizes neurological symptoms in Type 3 GD. Combination therapy with high-dose ABX and ERT may be beneficial for neuronopathic GD patients with specific mutations, and the early clinical application should be explored.
Thy-1 aSyn mice:

PRE-MANIFEST Phase (1-12 months):
Inflammation
Alpha-synuclein aggregates
Early non-motor deficits
Pre-manifest motor deficits

MANIFEST Phase (from 14 months):
Loss of dopamine in striatum
L-dopa reversible motor deficits
The pharmacological chaperone AT2101 reduces alpha-synuclein in dopaminergic neurons.
# Study Plan

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>1.75m</th>
<th>2m</th>
<th>4m</th>
<th>6m</th>
<th>8m</th>
<th>10m</th>
<th>12m</th>
<th>14m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer to facility</td>
<td></td>
<td>☐</td>
<td>☐</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-drug test (1.75m)</td>
<td>☐</td>
<td>☐</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (2m)</td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post1 testing (6m)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor &amp; Cognitive</td>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post2 test (14m) Motor</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Euthanasia</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Timeline (Nov 2012 - Oct 2014)

<table>
<thead>
<tr>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov Dec</td>
<td>Jan Feb Mar Apr May</td>
<td>Jun Jul Aug Sep Dec</td>
</tr>
<tr>
<td>mice assignment</td>
<td>Drug Treatment</td>
<td></td>
</tr>
<tr>
<td>pre-motor testing</td>
<td>pre-motor data analysis</td>
<td></td>
</tr>
<tr>
<td>Post1 testing</td>
<td>Post1 data analysis</td>
<td>Post2 testing</td>
</tr>
<tr>
<td>Post2 testing</td>
<td></td>
<td>Post2 data analysis</td>
</tr>
<tr>
<td>Euthanasia &amp; Tissue processing</td>
<td>Biochem. &amp; Pathol. analysis</td>
<td></td>
</tr>
</tbody>
</table>
Mortalities

- There were mortalities both in Thy1-aSyn (6) and wild-type mice (2) in different treatment groups which seems to be of other causes such as genital mass (GM) or swollen bladder (SB) and is unrelated to the treatment
- **Mortalities per group:**
  1. Thy1-aSyn- green diet – 2 (GM, unknown)
  2. Thy1-aSyn- yellow diet – 1 (GM)
  3. Thy1-aSyn- red diet – 3 (SB, GM, GM)
  4. Wild-type- green diet – 2 (unknown, unknown)
  5. Wild-type- yellow diet - 0
  6. Wild-type- red diet - 0
Progress and adjustments

• **Mice assignment: Complete**
  – Nov 2012 – Mar 2013 (includes mice for a pilot study to measure brain ambroxol levels in Thy1-aSyn mice)

• **Pre-treatment motor testing: Complete**
  – Challenging beam, vertical pole
  – analysis is ongoing: 30% completed

• **Post1 (6-7m) cognitive testing: Ongoing**
  – Ymaze alternations, Novel place recognition, Novel object recognition
  – 40% testing complete

• **Post1 (7.25m*) motor testing: Ongoing**
  – Challenging beam, vertical pole, Open field
  – 25% testing complete
  – *Motor testing was postponed to 7.25m since recent studies showed it is ideal to perform cognitive tests prior to motor testing
Progress

• **Post2 motor testing: Starts late Oct 2013**
  – Adhesive removal test, Challenging beam, Open field
  – Analysis begins Dec 2013

• **Euthanasia and tissue processing: Nov 2013 – May 2014**

• **Biochemical and pathological analysis : Jun - Oct 2014**
  – After tissue from all the mice are collected
  – Biochemical: striatal dopamine levels, ambroxol levels and evaluation of GCase activity
  – Pathology by immunohistochemistry:
    – alpha-synuclein (+/- proteinase K) in substantia nigra
    – quantification of alpha-synuclein aggregates in substantia nigra
    – microglial marker Iba-1 in striatum and substantia nigra
    – quantification of activated microglia in striatum and substantia nigra
    – synaptophysin in striatum
    – tyrosine hydroxylase in striatum
ABX and IFG Reduce GCase levels in the ER

<table>
<thead>
<tr>
<th>Condition</th>
<th>Gcc (N370S/N370S)</th>
<th>PDI= ER marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td><img src="image1" alt="Gcc" /></td>
<td><img src="image2" alt="PDI" /></td>
</tr>
<tr>
<td>5 days</td>
<td>(Gcc signal enhanced)</td>
<td></td>
</tr>
<tr>
<td><strong>ABX</strong></td>
<td><img src="image1" alt="Gcc" /></td>
<td><img src="image2" alt="PDI" /></td>
</tr>
<tr>
<td>60 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IFG</strong></td>
<td><img src="image1" alt="Gcc" /></td>
<td><img src="image2" alt="PDI" /></td>
</tr>
<tr>
<td>30 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Rigat
Ambroxol significantly reduces glucosylceramide (GC) levels in treated mutant lymphoblasts.

Note: both ambroxol and isofagomine were used at 20 µM.