- Drug discovery through identification of common denominators
- Broad impact
A Common Denominator

- Congenital diseases
- Variable disease onset
- Secondary insults modulate disease

- Oxidative stress
- Mitochondrial defects
- ER stress
- Misfolded proteins
- Inflammation

Lysosomal dysfunction → Drugs
A Broader Impact

Rare Diseases

- Congenital diseases
- Variable disease onset
- Secondary insults modulate disease

Oxidative stress
Mitochondrial defects
ER stress
Misfolded proteins
Inflammation

Lysosomal dysfunction

Neurodegenerative diseases

- Genetic predisposition
- Variable disease onset
- Secondary insults contribute to disease

Drugs
Cellular endpoints
- Proliferation
- Differentiation
- Survival
- Neurite outgrowth

Drug screen
- FDA approved drug screen
- Confirm candidate “hits”
- Verification in relevant human iPSC cells

Genetic
- Disease phenotype
- Clinical history
- Identification of new mutations
- Cohort access

Clinical application
- Identification of clinical trial cohorts
- Analysis of carriers
- Relevant “recovery” readouts

Structural Analysis
- Identify BBB permeable drug
- Test in animal model
- Test for off target effects
A Specific Example

Krabbe Disease
- Lysosomal storage disease
- Known mutation in GALC
- Heterozygosity associated with mild phenotype

Drug Screen
- Identify candidates
- Confirm cellular rescue
- Effect on lysosome
- Test outcome in iPSCs
- Identify harmful drugs.

Clinical application
- Identification of clinical trial cohorts
- Clinical history of patients receiving the “bad drug”
- Analysis of carriers?

Oligodendrocyte Progenitors
- Proliferation
- Differentiation
- Lysosomal Function

Structural Analysis
- Identified BBB permeable drugs
- Test in animal model (Twi)
- No reported CNS toxicity
Krabbe Disease:

- Severe, progressive loss of myelin & neurodegeneration
- Enzymatic deficiencies (mutations) in galactocerebrosidase (GALC)
- Accumulation of the toxic lipid *Psychosine (Psy)*
- *Twitcher* mouse: a pathologically/genetically authentic murine model
A Specific Example….

**Oligodendrocyte Progenitors**
- Proliferation
- Differentiation (Lysosomal Function)

**Krabbe Disease**
- Lysosomal storage disease
- Known mutation in GALC
- Heterozygosity associated with mild phenotype

**Drug Screen**
- Identify candidates
- Confirm cellular rescue
- Effect on lysosome
- Test outcome in iPSCs
- Identify harmful drugs.

**Clinical application**
- Identification of clinical trial cohorts
- Clinical history of patients receiving the “bad drug”
- Analysis of carriers?

**Structural Analysis/In vivo**
- Identified BBB permeable drugs
- Test in animal model (Twi)
- No reported CNS toxicity
Isolated progenitor cells recapitulate in vivo defects

Proliferation

<table>
<thead>
<tr>
<th>proliferation rate (%) veh</th>
<th>veh</th>
<th>psy</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lysosomal pH

<table>
<thead>
<tr>
<th>lysosomal pH</th>
<th>WT</th>
<th>twi</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PND 17
A Specific Example….

**Drug Screen**
- Identify candidates
- Confirm cellular rescue
- Effect on lysosome
- Test outcome in iPSCs
- Identify harmful drugs.

**Oligodendrocyte Progenitors**
- Proliferation
- Differentiation (Lysosomal Function)

**Krabbe Disease**
- Lysosomal storage disease
- Known mutation in GALC
- Heterozygosity associated with mild phenotype

**Clinical application**
- Identification of clinical trial cohorts
- Clinical history of patients receiving the “bad drug”
- Analysis of carriers?

**Structural Analysis/In vivo**
- Identified BBB permeable drugs
- Test in animal model (Twi)
- No reported CNS toxicity
Unbiased screening

Focus on correcting important cellular behaviors, not on specific proteins or genes

Top hits rescue cellular defect associated with lysosomal dysfunction in vitro
A Specific Example:

Oligodendrocyte Progenitors
- Proliferation
- Differentiation
  (Lysosomal Function)

Drug Screen
- Identify candidates
- Confirm cellular rescue
- Effect on lysosome
- Test outcome in iPSCs
- Identify harmful drugs.

Krabbe Disease
- Lysosomal storage disease
- Known mutation in GALC
- Heterozygosity associated with mild phenotype

Clinical application
- Identification of clinical trial cohorts
- Clinical history of patients receiving the “bad drug”
- Analysis of carriers?

Structural Analysis/In vivo
- Identified BBB permeable drugs
- Test in animal model (Twi)
- No reported CNS toxicity
Top hits rescue cellular defect associated with lysosomal dysfunction in vivo

**Survival**
- Median survival (d)
- AAV
- BMT
- veh
- NKH

**Motor function**
- Time (ms)
- WT
- twi

**Myelination**
- Myelin intensity (% of WT)
- DAPI
- fluoromyelin
- veh
- NKH

(A) Stance time (B) Break time (C) Propel time
A Specific Example…

- **Oligodendrocyte Progenitors**
  - Proliferation
  - Differentiation (Lysosomal Function)

- **Krabbe Disease**
  - Lysosomal storage disease
  - Known mutation in GALC
  - Heterozygosity associated with mild phenotype

- **Drug Screen**
  - Identify candidates
  - Confirm cellular rescue
  - Effect on lysosome
  - Test outcome in iPSCs
  - Identify harmful drugs.

- **Clinical application**
  - Relevant “recovery” readouts
  - Physiology Imaging
  - Identification of clinical trial cohorts
  - Clinical history of patients receiving the “bad drug”
  - Analysis of carriers?

- **Structural Analysis/In vivo**
  - Identified BBB permeable drugs
  - Test in animal model (Twi)
  - No reported CNS toxicity

- **Physiology Imaging**
  - Identification of clinical trial cohorts

- **Glaucoma**

- **MS**
Proposed time line and human capital

Patient cohorts
- Access
- Genetics, Disease
  Specific Natural History
  Krabbe, Rett, Gaucher,
  Hurler, Batten, MLD, A-T,
  VWM

Clinical trial design
- Relevant “recovery”
  readouts
- Diagnostic/ Biomarkers

Define cellular endpoints

Establish cell system

Test candidate lysosomal drugs on target cells

Confirm candidate “hits” using in vivo models

Test in iPSCs with distinct genetic background

Human iPSCs

Mouse mutant

Jonathan, Alex, John

Mark

6-12 months

12 months

12 months

MDs
1 PhD

Statistician

2 PhD
2 TAs

2 PhD
3 TAs

Chris
Margot

Krabbe, Rett, Gaucher, Hurler, Batten, MLD, A-T, VWM

MDs
1 PhD

Statistician

2 PhD
2 TAs

2 PhD
3 TAs

Confirm candidate “hits” using in vivo models

Test in iPSCs with distinct genetic background

MDs
1 PhD
Challenges

• Specific cellular targets might not been known (aka ASD)
• Lysosomal defects might not be a general cellular pathology
• Patient material might not be readily available for iPSC cell derivation
• Genetic profile might not be sufficient to identify relevant pathways

Solutions

• An unbiased approach might identify novel targets (see A-T work)
• In addition to lysosomal defects we can screen cells for mitochondrial and ER defects
• Crisper/Cas technology might allow to introduce defects in normal cells overcoming the need for patient material
• Proteomic or/and lipidomic analysis can be added to the genetic profiling
Collaborative published work:


A Value-Added Rare Disease Center

1. Common denominator
   - Drug discovery
2. Broad Impact