Myelin Repair for Multiple Sclerosis

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Outline

- MS pathology
- Screening for compounds that drive endogenous myelination pathways
- Modeling the inhibitory effects of inflammation on remyelination
- Translation to the clinic: trial design, outcomes, and interpretation

MS is a complex disease with multiple contributing pathophysiological mechanisms

**Inflammation:**
- Cellular cytotoxicity
- C/Ab-mediated damage
- Cytokines/soluble factors
- Neurotrophic factors

**Demyelination:**
- Remyelination?
- Result of toxic insult
- Degenerative?

**Axonal damage/gliosis:**
- Result of toxic insult:
  - immune-mediated
  - excitotoxicity
  - Degenerative?
Remyelination occurs in ~20% of people with MS

- Restores axonal function & health
  - saltatory conduction of action potentials
  - trophic support to underlying axon
  - prevents neurodegeneration
  - protects against glial scarring
- Spontaneous remyelination fails later in disease: why?


Premyelinating Oligodendrocytes present in chronic MS lesions

Factors affecting remyelination

- Age
  - Number of OPCs
  - Macrophage recruitment (clearance of debris)
  - Epigenetic control of differentiation
- Ability to recruit OPCs
- Microenvironment unfavorable to oligodendrocyte differentiation (immune cell activation and glial scarring etc.)
- Axonal health (Neuroregulins? Probably other signals)

Oligodendrocyte Progenitor Cells (OPCs)

Nishiyama A. Neuroscientist 2007; 13; 62
Factors affecting remyelination

Gliogenesis Therapeutics

- Enhance *in vivo* generation of new oligodendrocytes from resident adult progenitors.
- Screen OPCs with a drug/small molecule library
- Progenitor cells derived from NG2-DsRed:PLP-eGFP mice
- Fate mapping with PDGFraCreER: Rosa26YFP reporter
Reporter Mice

- NG2 DsRed
  - Oligodendrocyte Progenitor Cell (OPC)
- PLP eGFP
  - Oligodendrocyte (OL)

OPCs OLs

Reporter OPCs are differentiated into OLs via T3

Green = PLP+ Oligodendrocyte
Thyroid Hormone Receptors

- Thyroid Hormone Receptor alpha (cardiac)
- Thyroid Hormone Receptor beta (liver)
- Tom Scanlan, Professor of Physiology and Pharmacology at OHSU, developed selective thyromimetic GC-1 (Sobetirome), which was shown to have log fold higher affinity for TR beta than TR alpha and therefore have lipid lowering properties without the cardiotoxicity.

GC-1 induces OL differentiation of reporter OPCs

eGFP Induction

Baxi et al; Glia 2014
GC-1 Induces OL Differentiation from Human OPCs

Validate hits from murine drug screen with human OPCs

Drug Screen in OPCs
How do we test compounds in vivo?

- EAE, may depend on the model
  - MOG peptide mice have extensive axonal damage
  - Full length rMOG1-125 in rats have more primary demyelination
- Cuprizone
  - Can compounds promote remyelination of corpus callosum or hippocampus?
  - Endogenous remyelination occurs too readily
- OPC lineage tracing mice during development
  - Can compounds promote differentiation of PDGFRα+ OPCs into OLs?

Cuprizone model of demyelination

Black gold staining of corpus callosum
Cre-lox fate mapping of OPCs with PDGFR<sub>ER</sub>-Cre;Z/EG or Rosa26YFP double Tg mice

Shin Kang, Bergles Lab

Thyroid hormone beta receptor agonist promotes oligodendrogenesis in the postnatal corpus callosum

Baxi et al, Glia 2014
Will manipulating developmental OPC differentiation pathways such as TH or anti-LINGO be effective in MS and do we have the right models to screen therapeutic agents?

• Need to recapitulate inflammatory environment
• In vitro cytokine modeling
• Popko CNS IFNg model
• T cell infiltration with primary demyelination in vivo

IFNγ modulates OPCs

• Inhibits cell cycle exit
• Suppresses OPC maturation and myelin gene expression
• Increases OPC apoptosis
• IFNγ induces STAT1 binding and IRF-1 in OPCs

V. Gallo and others
Effects of IFN-γ on demyelination and remyelination in cuprizone-treated transgenic mice in which astrocytes make IFNg upon withdrawal of Doxy

Lin W et al. Brain 2006;129:1306-1318

Inhibitory Effects of Cytokines on OPC Differentiation

Figure 2: The inhibitory effect of T_{eff} cells on OPC differentiation. Immunocytochemistry analysis was performed on primary rat OPCs cultured with T_{eff} supernatants for 4 days prior to fixation and staining with MBP (1:1000) and Olig2 (1:1000). Two doses of supernatants was used 1:10 and 1:1 diluted in OPC media. Neutralization antibody against IFNγ (αIFNγ) was applied to determine IFNγ specific activity. T-cell subtype supernatants were generated from CD4+ or CD8+ isolated cells that were polarized to TH1/17 and TC1/17, respectively.
Transcriptome profiling of OPC in response to immune mediators

Rat cells
Cultured with PDGF
Withdraw PDGF
48 / 96hrs
Transcriptome profiling

# of unique genes with significant expression change (FDR<0.05)

<table>
<thead>
<tr>
<th></th>
<th>Fold change &gt;=1.5</th>
<th>T3+IL4 vs. T3</th>
<th>T3+IFNg vs. T3</th>
<th>T3+IL10 vs. T3</th>
<th>T3+IL13 vs. T3</th>
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<tbody>
<tr>
<td>48 Hr</td>
<td>Up-regulated</td>
<td>6</td>
<td>796</td>
<td>11</td>
<td>4</td>
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<tr>
<td></td>
<td>Down-regulated</td>
<td>5</td>
<td>769</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>96 Hr</td>
<td>Up-regulated</td>
<td>27</td>
<td>691</td>
<td>103</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Down-regulated</td>
<td>15</td>
<td>611</td>
<td>29</td>
<td>23</td>
</tr>
</tbody>
</table>

In vivo modeling of the effects of inflammation on OPCs and remyelination

- To determine how various subsets of polarized Th cells differentially affect OPC differentiation and remyelination using the cuprizone model
  - Combination of inflammation and demyelination provides a model that more closely mimics MS and provides a window of demyelination in which to test potential remyelinating drugs
AT-Cuprizone Design

Th17 cells migrate most effectively to the brain, but become IL17+/IFNγ+ in CNS
Axons are not lost but show signs of stress

Baxi et al; J. Neurosci, 2015
Lineage Tracing in PDGF-Rα_CreER; Rosa26_YFP reporter mice following cuprizone mediated demyelination

Next Steps

- Pathway analysis from gene array to understand mechanism of IFNg inhibition
- Sort YFP cells from reporter mice with AT-Cup
- Screen for compounds that would suppress these pathways
- In vivo testing of compounds in AT-Cup
- Translate to the clinic
How are we going to test protective and reparative drugs in MS?

• Unlike RRMS, there is no rapid phase 2 trial paradigm or outcome to do short proof of concept studies
• Progression occurs slowly in most patients
• Can we measure lesion damage or repair?
  ▪ Track lesion evolution by MRI
  ▪ Examine sequelae of optic neuropathy by optical coherence tomography (OCT) of retina

What are the best outcome measure for remyelination agents?

• In studies of anti-inflammatory agents we could look at new/active MRI lesions and relapse rate
• MRI: MT, DTI, atrophy, myelin water (lack specificity for myelin)
• PET (low SNR and lacks specificity for lamellar myelin)
• Clinical: Slowed worsening or even improvement in quantitative behavioral outcomes; dynamometry, vibration, 9-hole peg test, timed gait (may lag tissue remyelination)
• OCT: RNFL, GCL (indirect measures of failed remyelination)
• VEP: Recovery of latencies
Imaging the Anterior Visual Pathway

Pathophysiology of Retinal Changes in MS

Optic nerve inflammation & demyelination → Retrograde axonal degeneration → Ganglion cell death

Could rapid remyelination protect against this process?

Microscopic cross-sectional view through the optic nerve including the retinal layers
http://hubel.med.harvard.edu/book/82.jpg

Saidha et al. CML – Multiple Sclerosis 2010; 2: 33-43
**Clemastine Remyelination Trial**

Ari Green and Jonah Chan

### Studies

- **150 day crossover trial**

### Visits

<table>
<thead>
<tr>
<th>Day</th>
<th>Sc</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-7</td>
<td>14</td>
<td>pre</td>
<td>30</td>
<td>90</td>
</tr>
</tbody>
</table>

### Compound

- **Primary Endpoints**
  - VEP latency
  - Safety
  - Tolerability

- **Secondary Endpoints**
  - LCVA
  - MAF (fatigue) SDMT
  - MRI Myelin water fraction/MTR/FA
  - RNFL thickness on therapy (OCT)
  - EDSS
  - 6 Minute walk test
  - SF-36
  - MSFC

### Subjects: 50 Total, 25 per arm

**Inclusion:** RRMS, RNFL thickness > 70, VEP latency > 118 ms in at least one eye

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**Anti-LINGO Development**

- Enhances OPC→Oligodendrocyte in vitro (Sha Mi Nature Medicine 2005)
- Enhances remyelination in whole MOG rat EAE and cuprizone models (Mi, Nature Medicine 2007 and Annals of Neurology)
- Phase 1 trial of anti-LINGO Mab completed
- Phase 2 trials:
  - Treat Acute Optic Neuritis (OCT and VEP)
    - 30% greater recovery of latency w/ anti-LINGO
  - RRMS patients (Improvement in EDSS, MRI Lesions)
- Caveats: Is LINGO expressed in adult brain? Will release of this brake be enough to overcome all the inhibitory cues?
RENEW Study Design

AON onset

- **Primary endpoint:** improvement in optic nerve conduction latency by full-field (FF) VEP
  - Latency at end of treatment (Week 24), study end (Week 32) for affected vs. unaffected fellow eye at baseline

**Participants**

- With first episode unilateral AON
- Screening (pre-treatment)
- N ~ 80
- 3–5 days IV steroids

**Treatment Groups**

- **Opicinumab 100 mg/kg IV every 4 weeks x 6 doses (n ~ 40)**
- **Placebo IV every 4 weeks x 6 doses (n ~ 40)**

**Visit Schedule**

- Day -28
- Day 1 Baseline
- 4 8 12 16 20 24 32

**End of**

- End of treatment visit
- End of study/follow-up visit

**RENEW Study Populations**

- **Intent-to-treat (ITT) population:** 82 patients
  - Randomized and received ≥ 1 dose
  - Opicinumab 100 mg/kg IV (n = 41)
  - Placebo (n = 41)

- **Per-protocol (PP) population:** 69 patients
  - Completed the study; missed ≤ 1 study dose; did not receive MS-modifying therapy
  - Opicinumab 100 mg/kg IV (n = 33)
  - Placebo (n = 36)

**Notes**

- MS = multiple sclerosis.
- For patients with AON who had not already been treated.

[Diagram showing participants with first episode unilateral AON and treatment groups with time points and end of visits.]

**Abbreviations**

- AON = acute optic neuritis
- VEP = visual evoked potential
- IV = intravenous
- MS = multiple sclerosis
Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ITT Placebo</th>
<th>ITT Opicinumab</th>
<th>PP Placebo</th>
<th>PP Opicinumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, %</td>
<td>76%</td>
<td>66%</td>
<td>75%</td>
<td>64%</td>
</tr>
<tr>
<td>Mean ± SD age, yr</td>
<td>32.4 ± 8.9</td>
<td>31.8 ± 7.2</td>
<td>32.2 ± 8.8</td>
<td>31.2 ± 7.1</td>
</tr>
<tr>
<td>FF-VEP conduction block affected eye, n (%)</td>
<td>5 (12%)</td>
<td>10 (24%)</td>
<td>5 (14%)</td>
<td>6 (18%)</td>
</tr>
<tr>
<td>Mean ± SD FF-VEP latency fellow eye, ms</td>
<td>101.7 ± 5.3</td>
<td>102.7 ± 6.4</td>
<td>101.0 ± 4.9</td>
<td>102.6 ± 6.1</td>
</tr>
<tr>
<td>Mean ± SD RGCL/IPL thickness affected eye, µm a</td>
<td>66.0 ± 6.9</td>
<td>63.8 ± 7.4</td>
<td>65.9 ± 7.2</td>
<td>63.6 ± 8.1</td>
</tr>
<tr>
<td>Mean ± SD LCLA score affected eye, 1.25% Sloan chart b</td>
<td>4.0 ± 8.3</td>
<td>5.1 ± 9.7</td>
<td>3.5 ± 6.9</td>
<td>5.7 ± 10.6</td>
</tr>
<tr>
<td>Mean ± SD LCLA score affected eye, 2.5% Sloan chart b</td>
<td>7.4 ± 12.8</td>
<td>12.4 ± 13.9</td>
<td>6.8 ± 12.5</td>
<td>12.5 ± 14.4</td>
</tr>
</tbody>
</table>

LCLA = low-contrast letter acuity; µm = microns; ms = milliseconds; RGCL/IPL = retinal ganglion cell layer/inner plexiform retinal layer; SD = standard deviation; yr = years. a n = 38 in the placebo group and n = 40 in the opicinumab group for the ITT population; n = 34 in the placebo group and n = 32 in the opicinumab group for the PP population. b n = 39 in the opicinumab group for the ITT population; n = 31 in the opicinumab group for the PP population.

Primary Endpoint: Recovery of FF-VEP Latency

<table>
<thead>
<tr>
<th>Population analyzed</th>
<th>Week 24 a</th>
<th>Week 32 b</th>
<th>Week 24 a</th>
<th>Week 32 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT</td>
<td>20.83 n = 41</td>
<td>17.34 n = 41</td>
<td>21.15 n = 41</td>
<td>15.08 n = 41</td>
</tr>
<tr>
<td>PP</td>
<td>22.24 n = 36</td>
<td>14.69 n = 33</td>
<td>22.35 n = 36</td>
<td>13.22 n = 33</td>
</tr>
</tbody>
</table>

Adjusted mean change in FF-VEP latency, ms

<table>
<thead>
<tr>
<th>Week 24 a</th>
<th>Week 32 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average difference</td>
<td>-3.48 ms</td>
</tr>
<tr>
<td>95% CI</td>
<td>-10.61 to 3.65</td>
</tr>
<tr>
<td>P-value</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Average difference | -7.55 ms | -9.13 ms |
95% CI | -15.12 to 0.01 | -16.11 to -2.14 |
P-value | 0.05 | 0.01 |

CI = confidence interval. a Analysis of covariance (ANCOVA). b Mixed-effect model repeated measure (MMRM).
Secondary Outcomes at Week 24

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>ITT population treatment difference at Week 24 vs. placebo</th>
<th>PP population treatment difference at Week 24 vs. placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean percentage change in RNFL thickness (SD-OCT; 95% CI)</td>
<td>-3.9 (-9.7 to 1.9) $P = 0.19$ (ANCOVA)</td>
<td>-4.8 (-11.3 to 1.7) $P = 0.15$</td>
</tr>
<tr>
<td>Mean change in RGCL/IPL thickness, µm (SD-OCT; 95% CI)</td>
<td>-1.2 (-4.5 to 2.2) $P = 0.50$</td>
<td>-1.8 (-5.5 to 2.0) $P = 0.35$</td>
</tr>
<tr>
<td>Change in LCLA, 1.25% Sloan chart (95% CI)</td>
<td>-1.6 (-6.9 to 3.6) $P = 0.54$</td>
<td>-1.2 (-6.6 to 4.3) $P = 0.66$</td>
</tr>
<tr>
<td>Change in LCLA, 2.5% Sloan chart (95% CI)</td>
<td>-0.8 (-6.5 to 4.9) $P = 0.77$</td>
<td>-0.8 (-6.7 to 5.2) $P = 0.80$</td>
</tr>
</tbody>
</table>

CI = confidence interval; RNFL = retinal nerve fiber layer; SD-OCT = spectral-domain optical coherence tomography.

Trial Summary and Conclusions

- Results from RENEW are first to suggest biological activity of opicinumab in humans
- Support optic nerve remyelination based on greater improvement of FF-VEP latency in affected eyes
- Relative degrees of FF-VEP latency improvement may be age-dependent
- Fellow eye MF-VEP amplitude results provide additional novel evidence of biological effects of opicinumab in the non-AON visual pathway
Efficacy and Safety of anti-LINGO in RRMS: phase 2

Primary endpoint
- The primary efficacy endpoint is the percentage of participants experiencing confirmed improvement of neurophysical and/or cognitive function over 72 weeks (~18 months) of treatment as measured by a composite endpoint comprising the Expanded Disability Status Scale (EDSS),* Timed 25-Foot Walk (T25FW),* 9-Hole Peg Test (9HPT),* and 3-second Paced Auditory Serial Addition Test (PASAT-3,* Figure 2).

Thank you.
Questions?