Discovery of PIPE-505, a small molecule therapeutic for the treatment of sensorineural hearing loss (SNHL) associated with cochlear synaptopathy

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**PIE-505** is a gamma secretase inhibitor in development for the treatment of SNHL associated with cochlear synaptopathy. A series of in vitro and in vivo studies in animal models of auditory loss have demonstrated two distinct mechanisms of action (MOA) leading to restoration of hearing function. Specifically, PIPE-505 1) facilitates SGN neurite growth via the Netrin/DCC pathway leading to regeneration of inner hair cell ribbon synapses and 2) increases Atoh1 expression via reduced Notch signaling leading to outer hair formation. These cellular regenerative effects together, restore auditory function.

**PIE-505** restores auditory nerve synapses and hair cells

**PIE-505** causes dose dependent type I spiral ganglon neurite outgrowth

**PIE-505** increases hair cells via Notch inhibition

Ex vivo explant

In vivo drug treatment

**PIE-505** restores synapses in vivo, mouse model

**PIE-505** improves auditory measures

**PIE-505** restores synapses in vivo, guinea pig model

In summary, **PIE-505** restores SGN connections to inner hair cells and regenerates outer hair cells. These effects are mediated via two distinct γ-secretase substrates, DCC and Notch, respectively. A first-in-human study is planned to evaluate **PIE-505** in patients with SNHL associated with cochlear synaptopathy. Measures of audibility as well as speech intelligibility will be assessed.
Introduction

Inhibition of the muscarinic acetylcholine receptors by non-selective muscarinic antagonists (e.g., clemastine, benztropine) accelerates the differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs). Subsequent work has implicated the M1 isoform as being a key driver of this phenomenon. In-house chemistry efforts have identified a number of potent, selective M1 antagonists. Using these, we have characterized the effects of inhibiting M1 in a diverse set of in vitro assays, including OPC differentiation, cortical myelination, and organotypic brain slice. Our data show that a selective, small molecule inhibitor of M1 is sufficient to drive OPCs towards differentiation and that the resulting oligodendrocytes express myelin basic protein. Moreover, these OLs are functional, i.e., capable of axonal wrapping and induction of nodes of Ranvier. Of note, an M3 selective antagonist (Sagara et al., 2006) was not active in a rat OL differentiation assay. In concert with our in vivo data (also presented at this meeting), a strong case can be made that the development of an M1 selective small molecule antagonist is a promising approach for treating demyelinating diseases such as multiple sclerosis.

Calcium mobilization

<table>
<thead>
<tr>
<th>Compound</th>
<th>M1 IC50 (nM)</th>
<th>M2/M1</th>
<th>M3/M1</th>
<th>M4/M1</th>
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<tbody>
<tr>
<td>Benztropine</td>
<td>3.13</td>
<td>11.9</td>
<td>17.0</td>
<td>7.7</td>
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<tr>
<td>PIPE-359</td>
<td>12.9</td>
<td>21.0</td>
<td>23.0</td>
<td>3.3</td>
</tr>
<tr>
<td>PIPE-307</td>
<td>3.26</td>
<td>8.3</td>
<td>11.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Compound 23</td>
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<td>7.8</td>
<td>11.5</td>
<td>5.5</td>
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<tr>
<td>Compound 29</td>
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<tr>
<td>Compound 683</td>
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<tr>
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<td>17.8</td>
<td>17.8</td>
<td>2.3</td>
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<tr>
<td>Compound 51</td>
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<td>15.0</td>
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<tr>
<td>Compound 25</td>
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<td>12.9</td>
<td>18.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Compound 14</td>
<td>15.5</td>
<td>12.4</td>
<td>21.7</td>
<td>1.6</td>
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</table>

Table 2 Pipeline compounds are potent and selective in a cellular setting. Compounds were evaluated in CHO-K1 cells expressing one of M1-4 receptors for inhibition of ACh-induced calcium release at EC50 concentrations.

Lyssolecithin mouse brain slice

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIPE-683</td>
<td>18.9</td>
</tr>
<tr>
<td>PIPE-307</td>
<td>20.3</td>
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<tr>
<td>Compound 107</td>
<td>78.5</td>
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<tr>
<td>Compound 77</td>
<td>175</td>
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Table 4 Differentiated OLs are myelination competent. Myelination was evaluated in a rat cortical myelination assay as described previously (Larissa-Willingham et al., 2016). Myelin segments were identified by MBP colocalization with TuJ1 (axonal marker) and averaged per OL.

Rat OPC differentiation

Figure 1 Pipeline compounds induce OL differentiation in rat OPCs at nM potencies. Compounds were evaluated by immunocytochemistry in rat OPCs (Mei et al., 2016). ACh levels in OPC conditioned media measured by calcium flux in M1-CHO. Pulse dosing using PIPE-683, a structural analog of PIPE-307, shows 6h exposure is sufficient to initiate OPC differentiation.

Figure 2 Pipeline compounds induced Mbp in cultured cortical mouse brain slice demyelinated with lyssolecithin. Slices were cultured at postnatal day 17, demyelinated and treated with compound. Mbp was measured by quantitative PCR. The highly M1 selective peptide MT7 was used as a positive control.

Rat cortical myelination

Figure 3 Differentiated OLs are myelination competent. Myelination was evaluated in a rat cortical myelination assay as described previously (Larissa-Willingham et al., 2016). Myelin segments were identified by MBP colocalization with TuJ1 (axonal marker) and averaged per OL.

Human brain slice

Figure 4 Pipeline M1 antagonists induced Mbp in a naïve human cortical brain slice assay. Slices were incubated in MT7 or compound for 9 days prior to RNA isolation and QPCR.

Conclusion

Selective inhibition of M1 results in the differentiation of OPCs into mature oligodendrocytes. Here, we described the identification of potent, selective small molecule M1 antagonists as evaluated by [3H]NMS binding and calcium mobilization assays and further showed that these molecules induce myelination-competent oligodendrocytes. These molecules also induced Mbp in mouse and human organotypic slice models. Together, this provides compelling evidence that inhibition of M1 with small molecule antagonists developed at Pipeline have a positive impact in treating demyelinating disorders such as multiple sclerosis. At this point, a clinical development candidate has been identified and IND-enabling studies have been initiated.

References


**PIPE-359, a novel, potent and selective M1 muscarinic receptor antagonist as a therapeutic approach for remyelination in multiple sclerosis**


**Introduction**

Novel small molecule approaches aimed at stimulating remyelination would greatly complement immunotherapies and provide significant neural protection in demyelinating conditions such as multiple sclerosis (MS). Recently, we described the muscarinic M1 receptor (M1R) as an important regulator of oligodendrocyte precursor cell (OPC) differentiation and a promising target for drug discovery. We developed PIPE-359, a novel, potent and selective M1R antagonist and highlight its potential for remyelination.

**PIPE-359 binds to M1 with high affinity and demonstrates selectivity over other muscarinic receptors**

<table>
<thead>
<tr>
<th>Potency (nM)</th>
<th>Membrane binding, Ki</th>
<th>Calcium flux, IC50</th>
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</thead>
<tbody>
<tr>
<td><strong>0.144</strong></td>
<td>1.69</td>
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</table>

**Fold-selectivity**

<table>
<thead>
<tr>
<th>M2/M1</th>
<th>M3/M1</th>
<th>M4/M1</th>
<th>M5/M1</th>
</tr>
</thead>
<tbody>
<tr>
<td>130</td>
<td>14</td>
<td>45</td>
<td>17</td>
</tr>
</tbody>
</table>

**PIPE-359 promotes OPC differentiation in vitro and increases remyelination ex vivo**

- PIPE-359 dose-dependently differentiates rat OPCs in oligodendrocytes in vitro
- PIPE-359 increases MBP and Caspr protein in mouse cortical slices

**PIPE-359 improves behavioral score, VEP N1 latency and g-ratios in a murine MOG-EAE model**

- **Orally administered PIPE-359 occupies M1 receptors and inhibits M1 function in mouse forebrain**

- **PIEPE-359 enhances remyelination in a murine cuprizone model**

- Normal chow
- Vehicle or M1 antagonist x 2 weeks

**Conclusion**

- These data highlight the therapeutic potential of a selective M1R antagonist to benefit conditions such as MS in which demyelination plays a role.
- A clinical development candidate has been identified and IND-enabling studies have been initiated.
The muscarinic M1 antagonist PIPE-359 demonstrates remyelination in vivo through visual evoked potential (VEP) and electron microscopy (EM) of mice with experimental autoimmune encephalitis (EAE).

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Introduction

Multiple sclerosis is characterized by immune mediated medullary injury and progressive axonal loss. Visual evoked potential (VEP) is clinically translatable model used in patients with multiple sclerosis due to its ability to measure myelin damage of the visual pathway through the latency of VEP, which reflects the velocity of signal conduction along the visual pathway; while the amplitude of VEP is believed to be closely correlated with axonal damage of the retinal ganglion cells (RGC). PIPE-359 is a novel, potent and selective M1 antagonist with good oral exposure and brain penetration which is efficacious in rodent models of demyelination such as cuprizone and experimental autoimmune encephalitis (EAE). Flash VEPs were recorded from EAE mice to determine if a selective M3 antagonist can demonstrate functional remyelination. Spinal cords and optic nerves were collected for electron microscopy (EM) imaging and g-ratios were calculated to confirm remyelination.

PIPE-359 is efficacious in VEP of EAE mice

**Fig. 1**. VEP amplitude and latency is measured in naïve, untreated EAE mice and mice treated with vehicle or PIPE-359. The latency of VEP is delayed by PIPE-359, which is consistent with reduced axon diameter.

**Fig. 2**. Sham axons are similar to naïve; PIPE-359 restores axon diameter to naïve levels.

**Fig. 3**. In EAE, EM shows unmyelinated axons and reduced myelin thickness compared to naïve. PIPE-359 restores axon diameter and myelin thickness.

### Conclusions

- **VEP** is a sensitive measure of remyelination due to its ability to detect impairment in the visual pathway before the onset of clinical disability in EAE mice.
- M1 antagonists demonstrate robust remyelination and axonal protection as seen by reduced N1 latency shifts and preserved VEP amplitude waveform symmetry.
- Multiple compounds screened through this in vivo discovery paradigm have demonstrated remyelination thus confirming a small molecule selective M1 antagonist is a promising approach to treat multiple sclerosis.
- A clinical development candidate has been identified and IND-enabling studies have been initiated.

### References