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Discovery of a Novel M1 Selective Antagonist, PIPE-307, for the Treatment of Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. Current treatments focus on suppressing the immune system to limit inflammation and the further loss of the myelin sheath. The next advance in the treatment of MS has focused on molecules that regulate remyelination. The M1 muscarinic acetylcholine receptor (M1R) has been shown to be a key regulator in the maturation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs), the cells that make myelin. This discovery was based on non-selective anti-muscarinic compounds such as Clemastine and Benzetropine and subsequently validated through cell type specific M1R knockout studies. Building from this initial discovery, Pipeline Therapeutics initiated a medicinal chemistry effort to discover a novel M1R selective antagonist. These efforts resulted in PIPE-307, a novel, potent, and selective, first-in-class small molecule antagonist of the M1 receptor. Significantly, PIPE-307 produces robust effects in OPCs driving them towards differentiation and expression of myelin basic protein. Furthermore, PIPE-307 elicited positive results in a diverse set of *in vitro* assays, including OPC differentiation, cortical myelination, and organotypic brain slice. *In vivo* visual evoked potential and MOG-EAE studies have confirmed that PIPE-307 induces functional remyelination as evidenced by positive results in these models. Taken altogether PIPE-307 represents a promising approach for treating demyelinating diseases such as multiple sclerosis.

PIPE-307 Selectively Binds Human M1

Figure 1a

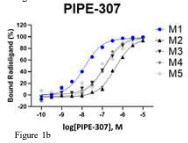


Table 1

	Fold Selectivity against M1			
M1 K_i (nM)	M2/M1	M3/M1	M4/M1	M5/M1
4.6 +/- 1.09 (n=4)	67x	17x	11x	17x

Figure 1b

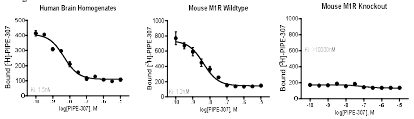


Figure 1a: PIPE-307 is a potent and selective M1 inhibitor in an mAChR recombinant membrane binding assay. Compound response curve of PIPE-307 in [3]H-NMS binding using membranes of CHO cell lines stably expressing the human gene for the M1, M2, M3, M4 and M5 muscarinic receptor subtypes. **Table 1:** Inhibition constant (K_i) (nM [3]H-NMS and fold selectivity of PIPE-307. PIPE-307 is potent and selective for human M1 in an mAChR recombinant membrane binding assay. **Figure 1b: [3H]-PIPE-307 shows potent and selective binding in both human and mouse brain tissue.** PIPE-307 was radiolabeled and tested in human and mouse brain homogenates. Data show similar binding in both human and mouse brain at 1.5 and 1.2nM K_i respectively. No binding was observed in M1R knockout mouse brain homogenates.

PIPE-307 Selectively Inhibits M1 Function

Figure 2a

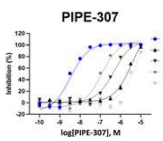


Table 2a

	Fold Selectivity against M1			
M1 IC_{50} (nM)	M2/M1	M3/M1	M4/M1	M5/M1
3.8 +/- 1.34 (n=4)	420x	55x	29x	950x

Figure 2: PIPE-307 is a potent and selective M1 inhibitor in an mAChR stable recombinant calcium mobilization assay. PIPE-307 was evaluated in recombinant CHO-K1 host cell lines stably expressing the human gene for the M1, M2, M3, M4, and M5 muscarinic receptor subtypes for inhibition of ACh-induced calcium release at EC_{80} concentrations. **Table 2:** The half maximal inhibitory concentration (IC_{50}) and fold selectivity of PIPE-307. PIPE-307 is potent and selective for human M1 in an mAChR recombinant calcium mobilization assay.

PIPE-307 Induces Rat OPC Differentiation

Figure 3a: Fluorescently tagged M1R selective peptide antagonist MT7 probe is expressed in oligodendrocyte precursor cells. MT7, a selective M1R antagonist was fluorescently labeled and added to OL precursor cells (OPCs). Approximately 30% of OPC's express M1R, as evidenced by the MT7 probe. This effect is reversed when cells are pre-incubated in MT7.

Figure 3b: PIPE-307 induces oligodendrocyte differentiation while Acetylcholine inhibits it in rat oligodendrocyte precursor cells (OPCs). OPCs isolated from rat postnatal day 8 cortex were treated with either PIPE-307 or Acetylcholine for 3 days following growth factor removal. Cells were PFA fixed and stained with MBP (mature oligodendrocytes) and Hoechst (DNA). Data are represented by vehicle subtracted percent of T3 control. Representative images show a decrease in basal differentiation with 1µM acetylcholine compared to vehicle control. Conversely, PIPE-307 induces oligodendrocyte differentiation to the level of the T3 control.

Figure 3c: PIPE-307 overcomes macrophage-mediated suppression of oligodendrocyte differentiation. Infiltrating immune cells provide rich source of acetylcholine in MS brain subsequently inhibiting OPC maturation. OPC's co-cultured with macrophages, immune cells rich in choline acetyltransferase (ChAT), show a significant reduction in their ability to differentiate to mature oligodendrocytes. This effect is reversed by the addition of PIPE-307.

Figure 3a

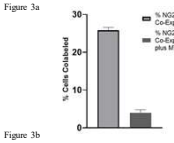
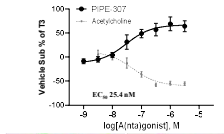


Figure 3b



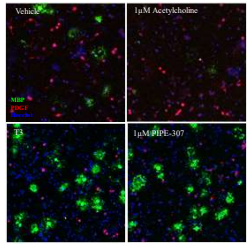
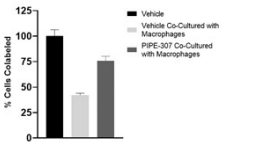


Figure 3c



PIPE-307 Induces Differentiated Functionally Competent Oligodendrocytes

Figure 4

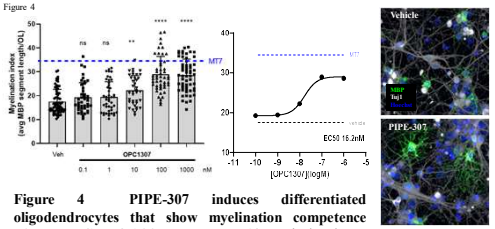
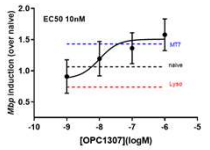


Figure 4 PIPE-307 induces differentiated oligodendrocytes that show myelination competence with an EC_{50} of 16.2nM. Mouse E18 cortical cultures treated with PIPE-307 for 9 days *in vitro*. Wells processed for immunocytochemistry against MBP and Tuj1. Myelin segments were identified by MBP colocalization with Tuj1 (axonal marker) and averaged per oligodendrocyte.

PIPE-307 Induces *Mbp* After *Ex Vivo* LPC Insult

Figure 5 PIPE-307 induced *Mbp* in cultured cortical mouse brain slice demyelinated with lysolecithin with an EC_{50} of 10nM. Mouse cortical slices were cultured at postnatal day 17, demyelinated with lysolecithin and treated with PIPE-307 for 3 days . *Mbp* was measured by quantitative PCR. The highly M1 selective peptide MT7 was used as a positive control.

Figure 5



PIPE-307 Increases CC-1+ Cells in Human Slice

Figure 6

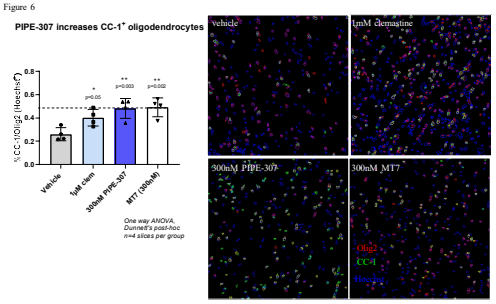


Figure 6 Human organotypic slices treated with PIPE-307 have increased *Mbp* RNA expression and CC-1+ oligodendrocytes.
Fresh human cortex from a 66 yo female donor (gray and white matter) was used to generate organotypic slices. Slices were treated for 9 days with PIPE-307, clemastine, or MT7 and processed for CC-1/Olig2 immunohistochemistry. Right, thresholded images showing co-localization of Olig2, CC-1 and Hoechst; left, quantification.

PIPE-307 Improves Clinical Score in EAE Model

Figure 6

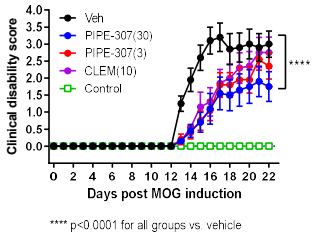


Figure 6 PIPE-307 at 3 and 30 mg/kg improves clinical disability score in a murine MOG-EAE model
C57BL/6N mice 10-13 weeks of age were induced on day 0 with MOG Kit (Hooke Labs, Hooke Kit MOG_{35-55}/CFA Emulsion PTX). Mice were orally dosed QD with Vehicle or PIPE-307 daily beginning on day 0. Daily clinical assessments were performed through day 22.

PIPE-307 Improves VEP Latency *In Vivo*

Figure 7

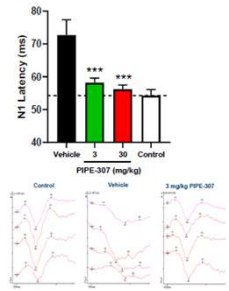


Figure 7 PIPE-307 at 3 and 30 mg/kg improves VEP N1 latency in a murine MOG-EAE model
C57BL/6N mice 10-13 weeks of age were induced on day 0 with MOG Kit (Hooke Labs, Hooke Kit MOG_{35-55}/CFA Emulsion PTX). Mice were orally dosed QD with Vehicle or PIPE-307 daily beginning on day 0. Visual evoked potential (VEP) is a clinically translatable model often used in patients with multiple sclerosis due to its ability to measure myelin degeneration of the optic nerve by determining the N1 latency in the VEP waveform. VEPs were recorded using a Celeris (Diagnosys Inc.) on days 21 and 22.

Conclusion

- Multiple sclerosis is a demyelinating disease that results in neurodegeneration through the death of myelin
- M1R has been shown to be a key regulator of oligodendrocytes, the cells that regulate myelin
- Pipeline Therapeutics has discovered PIPE-307, a novel first-in-class M1R selective antagonist
- PIPE-307 is shown to have robust effects at driving oligodendrocyte precursor cells to differentiate into mature myelin producing oligodendrocytes
- PIPE-307 has been shown to produce robust effects in several *in vivo* models of MS including VEP and MOG EAE