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

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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.

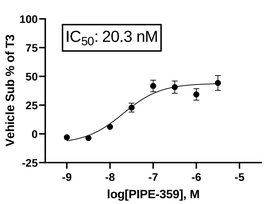
PIPE-359 binds to M1 with high affinity and demonstrates selectivity over other muscarinic receptors

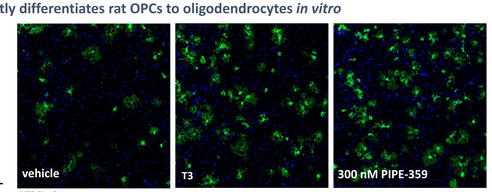
	Potency (nM)
Membrane binding, Ki	0.144
Calcium flux, IC_{50}	1.69

	Fold-selectivity			
	M2/M1	M3/M1	M4/M1	M5/M1
Membrane binding, Ki	130	14	45	17
Calcium flux, IC_{50}	102	43	26	315

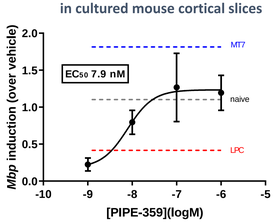
PIPE-359 promotes OPC differentiation *in vitro* and increases remyelination *ex vivo*

PIPE-359 dose-dependently differentiates rat OPCs to oligodendrocytes *in vitro*

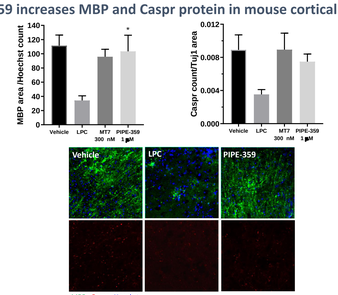




PIPE-359 dose-dependently induces *Mbp* in cultured mouse cortical slices

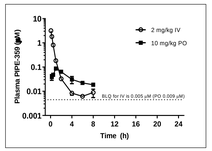


PIPE-359 increases MBP and Caspr protein in mouse cortical slices



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

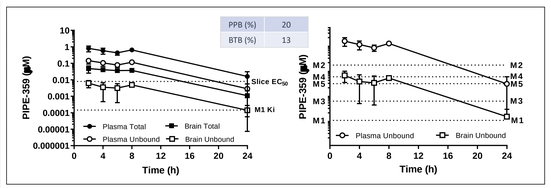
PIPE-359 Exposure in rat



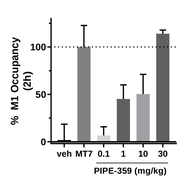
$[Brain]_{2h}$ (µM)	0.026
$[Plasma]_{2h}$ (µM)	0.080
$[CSF]_{2h}$ (µM)	<0.004
K_p, $K_{p,uu}$	0.32, 0.21

$AUC_{P.O.}$ (hr*µg/mL)	0.14
$t_{1/2,\ P.O.}$ (hr)	8.6
C_{max} (µM)	0.09 @ 1h
F (%)	4.5
$AUC_{I.V.}$ (hr*µg/mL)	0.5
Cl (mL/min/kg), ER	61, 0.87
V_d (L/kg)	2.2
$t_{1/2,\ I.V.}$ (hr)	0.9
µsome Cl_{int} (µL/min/mg)	23
PPB (%)	20
BTB (%)	13

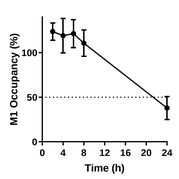
30 mg/kg PIPE-359 provides full M1 coverage for 8h in mouse brain



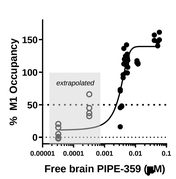
M1 occupancy ED_{50} ~ 2 mg/kg



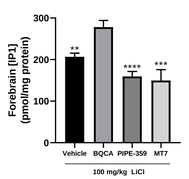
30 mg/kg PIPE-359 provides ≥ 50% M1 occupancy for ~20h



M1 occupancy EC_{50} ~ 3 nM



30 mg/kg PIPE-359 fully inhibits M1-mediated IP1 accumulation



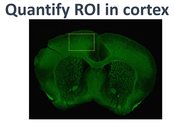
** p<0.01, *** p<0.001, **** p<0.0001 vs. BQCA, ANOVA with Dunnett's multiple comparison test

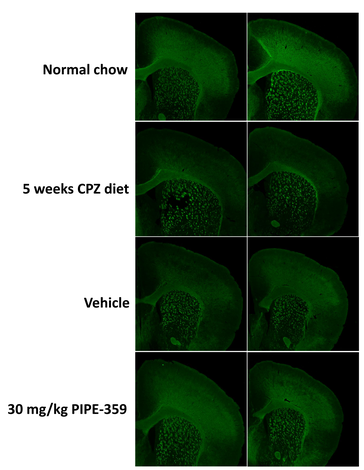
PIPE-359 enhances remyelination in a murine cuprizone model

0.2% cuprizone diet x 5 weeks → demyelination → remyelination → Free floating brain sections → Confocal microscopy

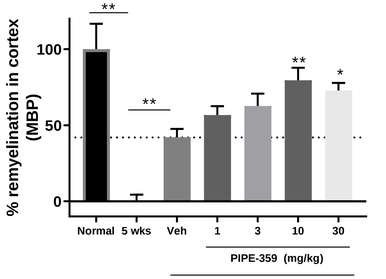
Normal chow
Vehicle or M1 antagonist
x 2 weeks

Quantify ROI in cortex





PIPE-359 dose-dependently enhances remyelination in a non-immune-mediated cuprizone model



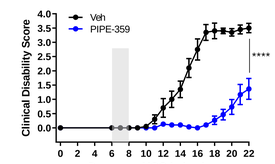
* p<0.05, ** p<0.01, **** p<0.00 vs. vehicle, ANOVA with Dunnett's multiple comparisons test
** p<0.05 Normal chow vs. 5 weeks CPZ diet, Student's t-test

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

Visual Evoked Potential (baseline, d7, d14, d21)

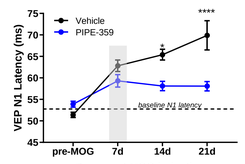
Induce MOG (d0) → Vehicle or PIPE-359 QD daily behavioral score (d0-d21) → TEM on spinal cord and optic nerves → Image analysis (g-ratios)

30 mg/kg improves behavioral score



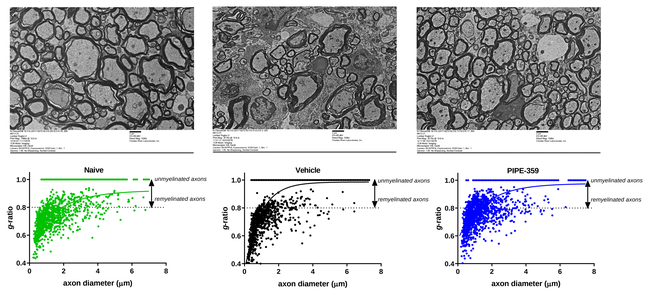
**** p<0.0001, mixed-effects model with Sidak's multiple comparisons test

30 mg/kg improves VEP N1 latency

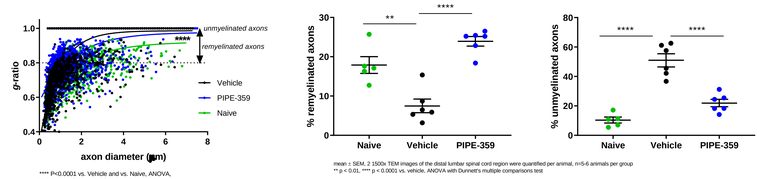


* P <0.05, **** P < 0.0001 vs. vehicle, 2way ANOVA, Tukey's multiple comparison test

30 mg/kg PIPE-359 remyelinates axons in the spinal cord



PIPE-359 increases the percentage of remyelinated axons and decreases the percentage of unmyelinated axons



mean ± SEM, 2 1500x TEM images of the distal lumbar spinal cord region were quantified per animal, n=5-6 animals per group
** p < 0.01, **** p < 0.0001 vs. vehicle, ANOVA with Dunnett's multiple comparisons test

**** P<0.0001 vs. Vehicle and vs. Naive, ANOVA, Tukey's multiple comparisons test

PIPE-359 also promotes remyelination in optic nerves from this study (see Edu et al. poster)

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.