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

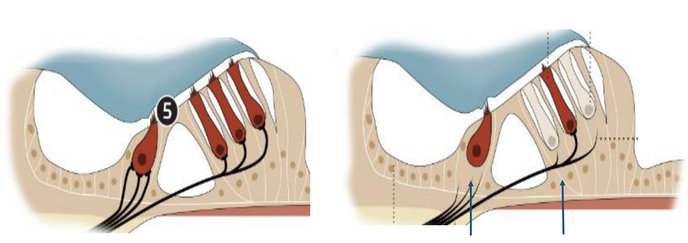
***Daniel S Lorrain**, Michael Poon, Karin J Stebbins, Geraldine Cabrera, Alex Broadhead, Jon Seiders, and Jeff Roppe
Pipeline Therapeutics, San Diego, CA

304.01

NO – PHOTO / RECORD

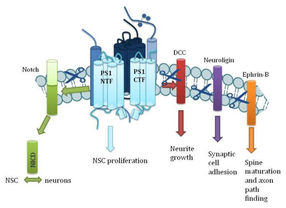
Introduction

PIPE-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.

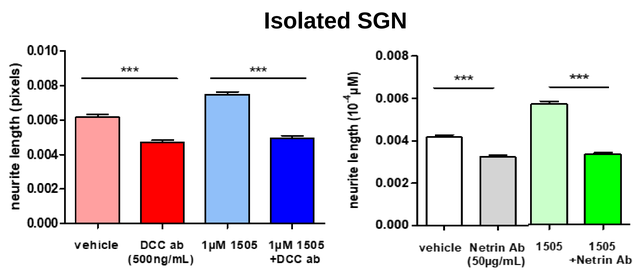


PIPE-505 restores auditory nerve synapses and hair cells

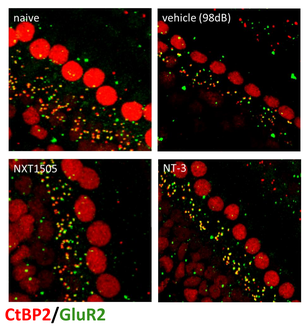
Neurite outgrowth mediated by Netrin/DCC pathway

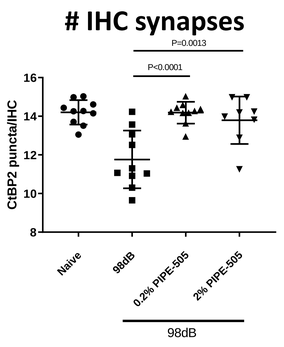


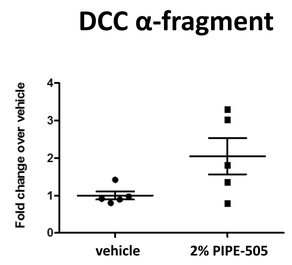
Brain Research Bulletin 2016



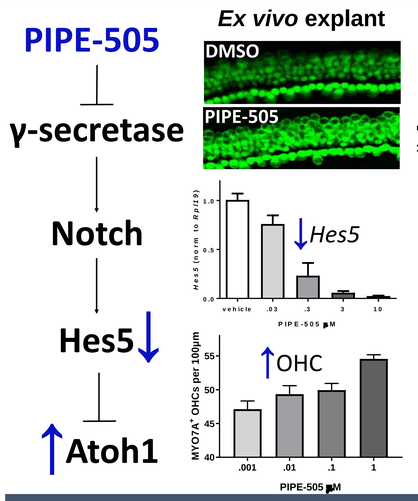
PIPE-505 restores synapses *in vivo*, mouse model

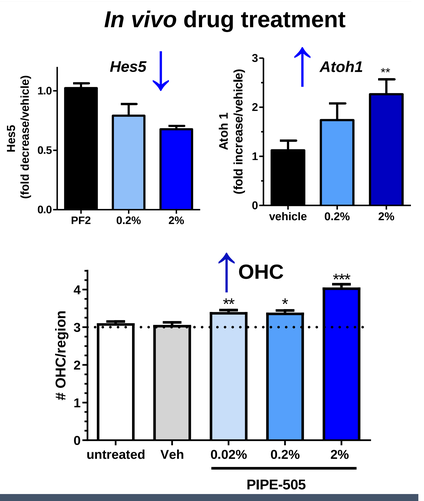






PIPE-505 increases hair cells via Notch inhibition

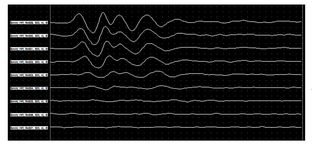




PIPE-505 improves auditory measures

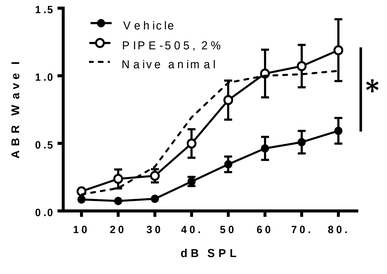
ABR Wave 1

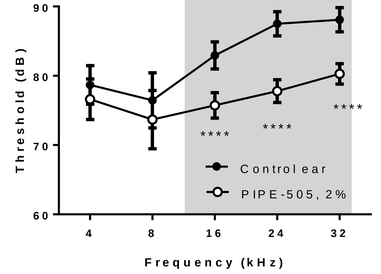
PIPE-505 restores ABR wave 1 amplitude, a measure of AN function



ABR Threshold

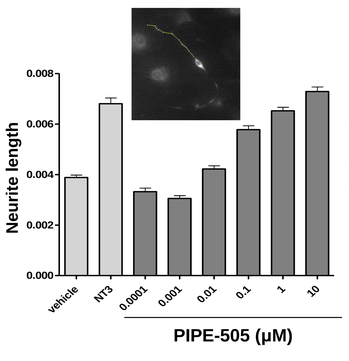
PIPE-505 improves ABR threshold, a measure of OHC function

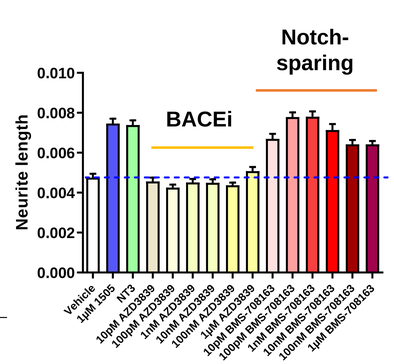




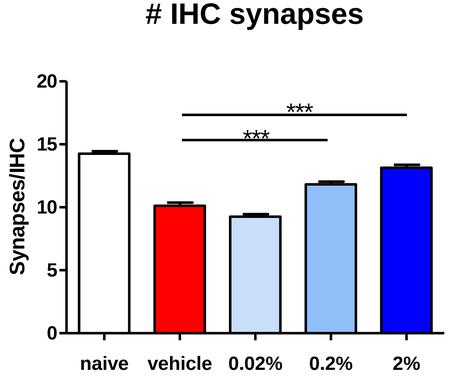
In summary, PIPE-505 restores SGN connections to inner hair cells and regenerates outer hair cells. These effects are mediated via two distinct γ-secretase substrates, DCC and Notch, respectively. A first-in-human study is planned to evaluate PIPE-505 in patients with SNHL associated with cochlear synaptopathy.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

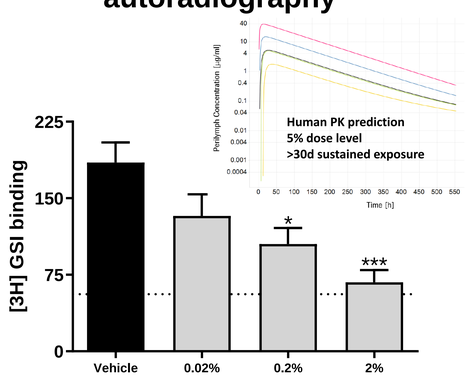
PIPE-505 causes dose dependent type I spiral ganglion neurite outgrowth





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





Small molecule inhibition of the muscarinic M1 acetylcholine receptor by potent, selective antagonists facilitate OPC differentiation

206.19

*Michael M Poon[1], Kym I Lorrain[1], Ariana O Lorenzana[1], Karin J Stebbins[1], Alexander Broadhead[1], Thomas O Schrader[2], Yifeng Xiong[2], Jill Baccei[2], Ari J Green[4], Jonah R Chan[4], Daniel S Lorrain[3]; [1]Biol., [2]Chem., [3]Pipeline Therapeutics, San Diego, CA; [4]Neurol., UCSF, San Francisco, CA

NO – PHOTO / RECORD

Introduction

Inhibition of the muscarinic acetylcholinergic 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.

[^{3}H]NMS membrane binding

Compound	M1 Avg Ki (nM)	Fold selectivity against M1			
		M2/M1	M3/M1	M4/M1	M5/M1
Benztropine	1.14	16	2.67	8.21	2.7
PIPE-359	0.144	130	14.4	45.1	17.4
PIPE-307	0.349	73	18.5	38	259
Compound 57	1.13	22	7.11	29.9	5.37
Compound 25	1.41	160	8.81	189	736
Compound 77	1.48	8.8	41.1	13.5	54.5
Compound 51	2.34	390	113	148	538
Compound 29	2.55	90	17.4	1.95	6.07
Compound 14	3.6	>7692	59.5	174	583
PIPE-683	4.04	87	13.3	121	167
Compound 107	7.55	120	38.4	93.1	*n.d.*

Table 1 Pipeline compounds are potent and selective for human M1 in an mAChR recombinant membrane binding assay.

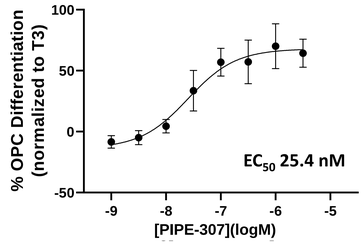
*Small molecule M3 selective antagonist from Banyu (Sagara et al 2006).
Ki (nM) for M1: 4670, M2: 6730, M3: 25.5, M4: 3600 in 3HNMS membrane binding

Calcium mobilization

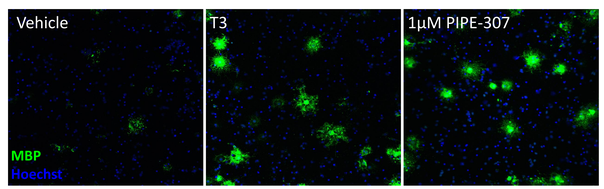
Compound	M1 IC50 (nM)	Fold selectivity against M1		
		M2/M1	M3/M1	M4/M1
Benztropine	3.19	16.9	11.2	4.78
Compound 57	0.716	343	763	430
PIPE-359	1.69	102	43	26
Compound 77	2.1	98.8	1270	212
PIPE-307	2.35	555	64.2	54.2
Compound 29	6.69	57.4	347	91.9
PIPE-683	7.45	698	175	292
Compound 107	8.91	178	117	313
Compound 51	13.5	417	1590	24.5
Compound 25	19.6	128	199	241
Compound 14	51.5	124	217	58.4

Table 2 Pipeline compounds are potent and selective in a cellular setting. Compounds were evaluated in CHO-K1 cells overexpressing one of M1-4 receptors for inhibition of ACh-induced calcium release at EC_{80} concentrations.

Rat OPC differentiation



Compound	EC50 (nM)
PIPE-683	18.9
PIPE-359	20.3
PIPE-307	25.4
Compound 107	78.5
Compound 77	175
M3 selective*	> 3.00E+03



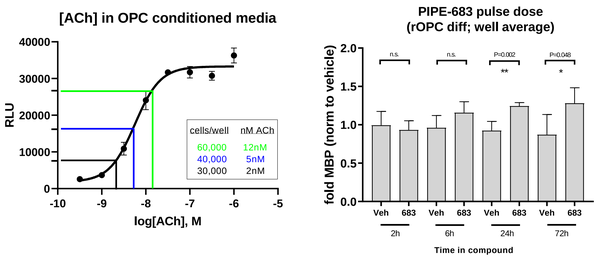
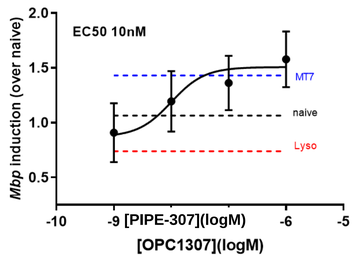


Figure 1 Pipeline compounds induce OL differentiation in rat OPCs at nM potencies. Compounds were evaluated by immunocytochemistry in rat OPCs (Mei et al 2016). ACh levels in OPC conditioned media measured by calcium flux in hM1-CHO. Pulse dosing using PIPE-683, a structural analog of PIPE-307, shows 6h exposure is sufficient to initiate OPC differentiation.

Lysolecithin mouse brain slice



Compound	EC50 (nM)
PIPE-359	7.9
PIPE-307	10
Compound 107	30
PIPE-683	36.2
Compound 77	44.3

Figure 2 Pipeline compounds induced *Mbp* in cultured cortical mouse brain slice demyelinated with lysolecithin. 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

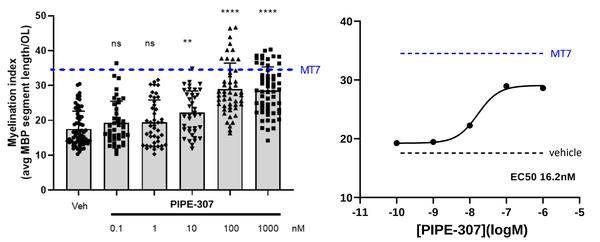
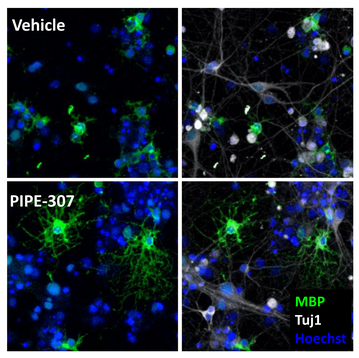


Figure 3 Differentiated OLs are myelination competent. Myelination was evaluated in a rat cortical myelination assay as described previously (Lariosa-Willingham et al 2016). Myelin segments were identified by MBP colocalization with Tuj1 (axonal marker) and averaged per OL.



Human brain slice

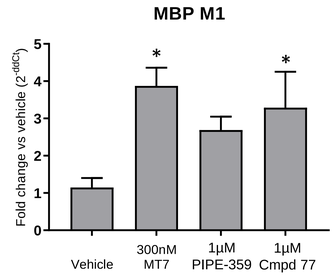


Figure 4 Pipeline M1 antagonists induced *Mbp* in a naïve human cortical brain slice assay. Slices were incubated in MT7 or compound for 9 days prior to RNA isolation and QPCR.

Dunnett's multiple comparisons test	Significant?	Summary	Adjusted P Value
Vehicle vs. MT7	Yes	*	0.0136
Vehicle vs. PIPE-359	No	ns	0.1802
Vehicle vs. Compound 77	Yes	*	0.0444

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 [^{3}H]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

Sagara, Y. et al. Identification of a novel 4-aminomethylpiperidine class of M3 muscarinic receptor antagonists and structural insight into their M3 selectivity. *J Med Chem*, 2006;*49*(19), 5653–5663.

Lariosa-Willingham, K.D., et al. Development of a central nervous system axonal myelination assay for high throughput screening. *BMC Neuro*, 2016;*17*(6).

Mei, F. et al. Accelerated remyelination during inflammatory demyelination prevents axonal loss and improves functional recovery. *eLife*, 2016; 5: e18246.

206.18

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

*** Karin J Stebbins**[1], Michael M Poon[1], Alexander Broadhead[1], Geraldine Edu[1], Ariana Lorenzana[1], Kym I Lorrain[1], Thomas Schrader[1], Yifeng Xiong[1], Jill Baccei[1], Christopher Baccei[1], Ari J Green[2], Jonah R Chan[2] and Daniel S Lorrain[1] ., [1] Pipeline Therapeutics, San Diego, CA; [2]UCSF, Department of Neurology, San Francisco, CA

NO – PHOTO / RECORD

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.

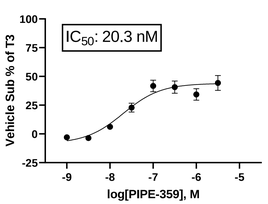
PIPE-359 binds to M1 with high affinity and demonstrates selectivity over other muscarinic receptors

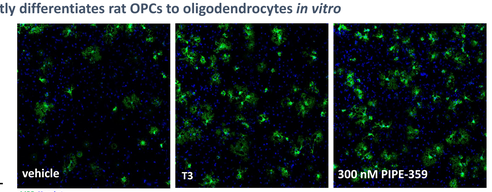
	Potency (nM)
Membrane binding, Ki	0.144
Calcium flux, IC_{50}	1.69

	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

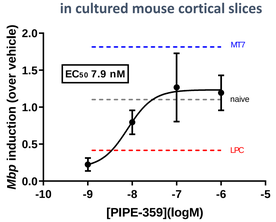
PIPE-359 promotes OPC differentiation *in vitro* and increases remyelination *ex vivo*

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

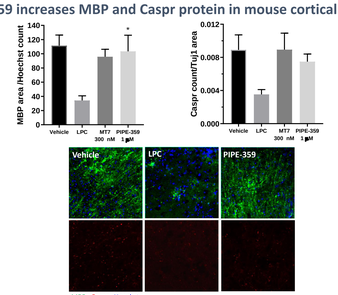




PIPE-359 dose-dependently induces *Mbp* in cultured mouse cortical slices

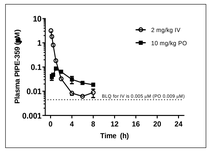


PIPE-359 increases MBP and Caspr protein in mouse cortical slices



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

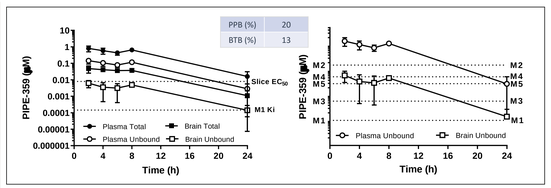
PIPE-359 Exposure in rat



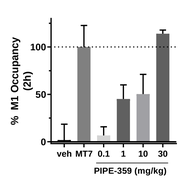
$[Brain]_{2h}$ (µM)	0.026
$[Plasma]_{2h}$ (µM)	0.080
$[CSF]_{2h}$ (µM)	<0.004
K_p, $K_{p,uu}$	0.32, 0.21

$AUC_{P.O.}$ (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

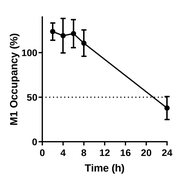
30 mg/kg PIPE-359 provides full M1 coverage for 8h in mouse brain



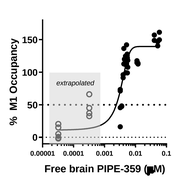
M1 occupancy ED_{50} ~ 2 mg/kg



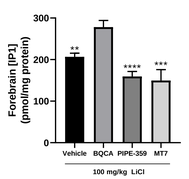
30 mg/kg PIPE-359 provides ≥ 50% M1 occupancy for ~20h



M1 occupancy EC_{50} ~ 3 nM



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



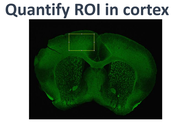
** p<0.01, *** p<0.001, **** p<0.0001 vs. BQCA, ANOVA with Dunnett's multiple comparison test

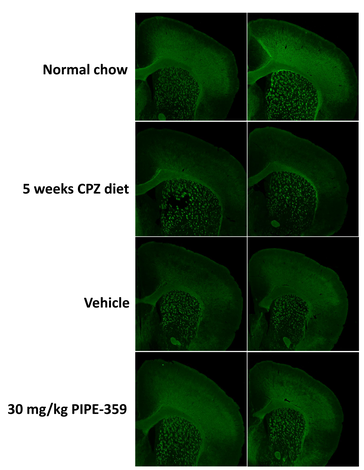
PIPE-359 enhances remyelination in a murine cuprizone model

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

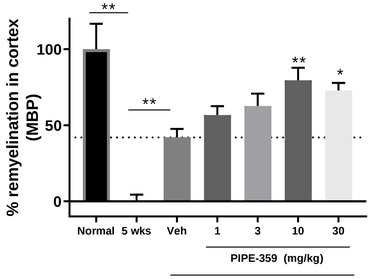
Normal chow
Vehicle or M1 antagonist
x 2 weeks

Quantify ROI in cortex





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



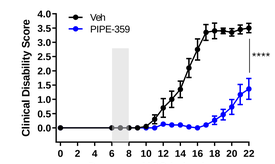
* p<0.05, ** p<0.01, **** p<0.00 vs. vehicle, ANOVA with Dunnett's multiple comparisons test
** p<0.05 Normal chow vs. 5 weeks CPZ diet, Student's t-test

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

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

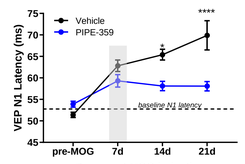
Induce MOG (d0) → Vehicle or PIPE-359 QD daily behavioral score (d0-d21) → TEM on spinal cord and optic nerves → Image analysis (g-ratios)

30 mg/kg improves behavioral score



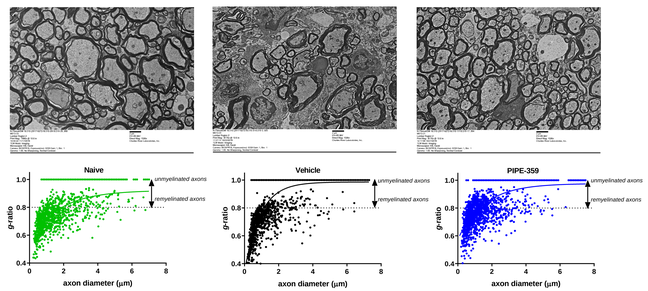
**** p<0.0001, mixed-effects model with Sidak's multiple comparisons test

30 mg/kg improves VEP N1 latency

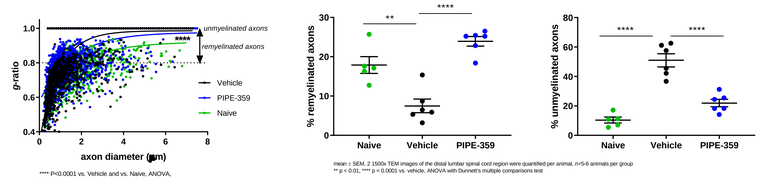


* P <0.05, **** P < 0.0001 vs. vehicle, 2way ANOVA, Tukey's multiple comparison test

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.

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)

206.19

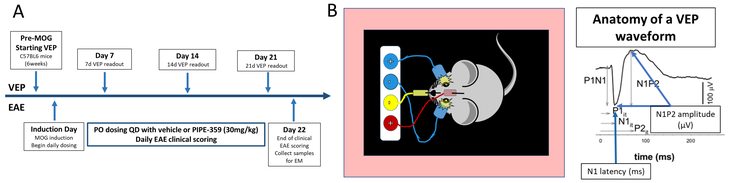
Geraldine C Edu*[1], Karin J Stebbins[1], Alexander R Broadhead[1], Michael M Poon[1], Ariana O Lorenzana[1], Thomas Schrader[1], Yifeng Xiong[1], Jill Baccei[1], Ari J Green[2], Jonah R Chan[2] and Daniel S Lorrain[1]. [1]Pipeline Therapeutics, San Diego, CA; [2]Neurol., UCSF, San Francisco, CA

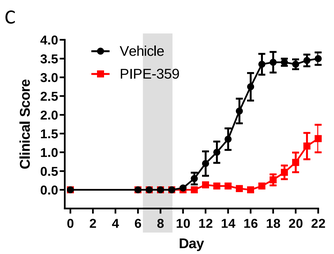
NO – PHOTO / RECORD

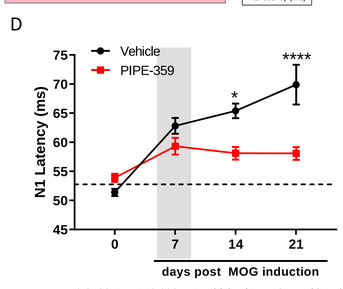
Introduction

Multiple sclerosis is characterized by immune mediated myelin injury and progressive axonal loss. Visual evoked potential (VEP) is a 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[1] - 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)[3]. 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 M1 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







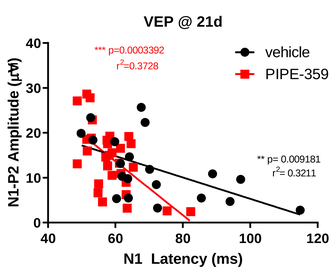


Fig 1A. Methods study timeline of PIPE-359 in the EAE model with flash VEP recordings performed in parallel. **1B.** EAE mice are dark adapted in a room with red lights for 1hr prior to recording. Mice are anesthetized with ketamine/xylazine (75/10mg/kg) and eyes are dilated with 1% Tropicamide for 2 minutes. Genteal eyedrops were applied for lubrication and mice are placed on the Diagnosys Celeris Model D430 (Lowell, MA) 37°F heated platform. Electrodes are carefully placed and flash VEP execution is performed 10 minutes post anesthesia wherein each exam consists of at least 3 runs, with pulse intensity 3 cd.s/m2, frequency 1 Hz, on time 4 ms, pulse color: white-6500K, 100 sweeps per result. Flash VEPs are recorded from each eye independently and simultaneously. N1 latency is determined by the average N1 from 3 VEP traces. **1C.** Clinical signs of EAE are first detected at day 11 and by the end of the study PIPE-359 significantly alleviates EAE clinical disability. **1D.** In same animals in parallel, flash VEP detects a difference in N1 latency with PIPE-359 as early as 7days post MOG induction, over time the effect becomes more significant out to 21days. **1E.** Each individual eye scatter plot show that N1-P2 amplitude and N1 latency measures are significantly correlated measures at 21 days post MOG induction and PIPE-359 (Y = -0.03580*X + 2.971) has a significantly more positive slope than vehicle (Y = -0.01338*X + 1.656).

PIPE-359 reduces N1 latency shifts and N1P2 amplitude degradation

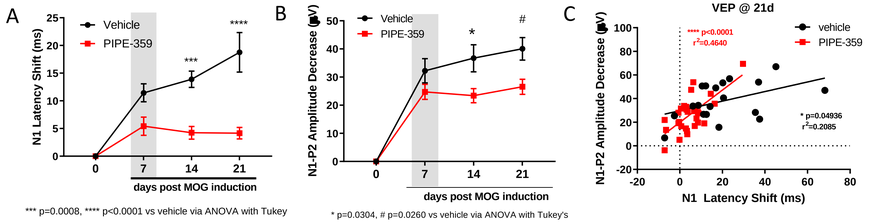


Fig 2A Differences in N1 latency shift can be detected at 7 days post MOG and becomes more significant overtime suggesting functional remyelination. **2B.** N1-P2 amplitude decreases are due to axonal damage at the retinal ganglion cells (You, Y. et.al 2011) and PIPE-359 prevents N1P2 amplitude degradation over time. **2C.** At 21days in a scatter plot of each eye N1 latency shifts and N1-P2 amplitude decreases are significantly correlated measures. PIPE-359 (Y = 1.365*X + 19.88) had a significantly more positive slope than vehicle (Y = 0.4056*X + 29.71).

EM of spinal cord and optic nerve show remyelination by PIPE-359

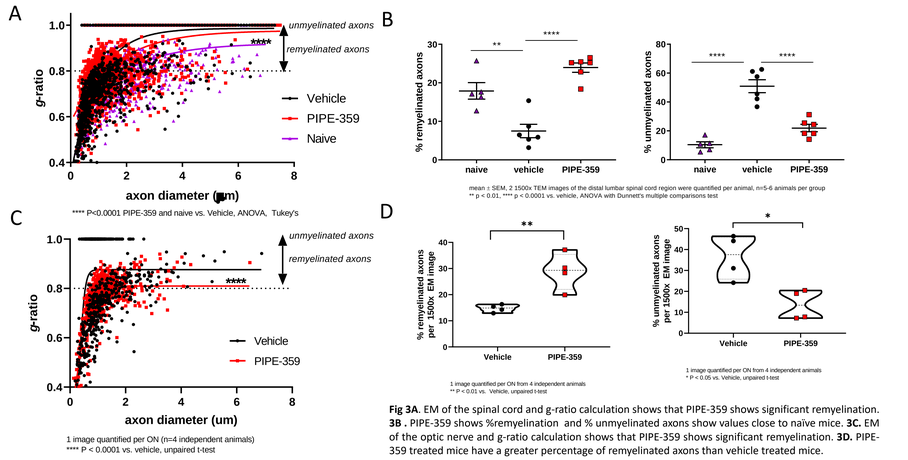


Fig 3A. EM of the spinal cord and g-ratio calculation shows that PIPE-359 shows significant remyelination. **3B** . PIPE-359 shows %remyelination and % unmyelinated axons show values close to naïve mice. **3C.** EM of the optic nerve and g-ratio calculation shows that PIPE-359 shows significant remyelination. **3D**. PIPE-359 treated mice have a greater percentage of remyelinated axons than vehicle treated mice.

VEP amplitude symmetry is preserved by M1 antagonists

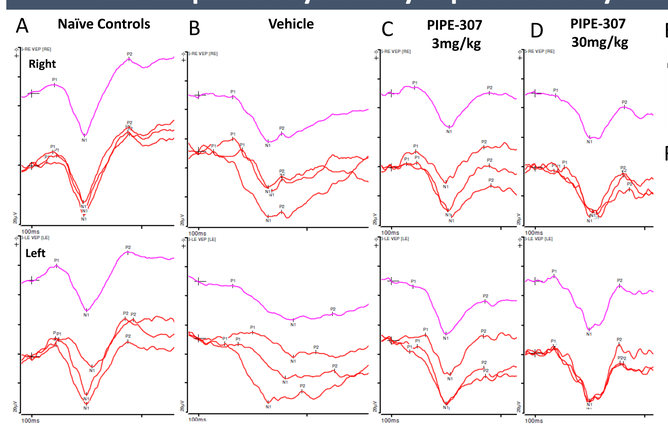


Fig 4A-D. Sample VEP waveform examples of each treatment group showing 3 VEP traces and the average trace. Top panel is the right eye, bottom panel is the left eye. **4E.** Standard VEP waveform components. Amplitude ratio description and calculation. **4F.** M1 antagonists do not show a degradation of N1P2 amplitude values and thus preserve of the symmetry of P1N1 and N1P2 amplitude waveforms

**p=0.0026, ## p=0.0032. *p=0.0356 vs vehicle via ANOVA with Dunnett

Profiling M1 antagonists through VEP

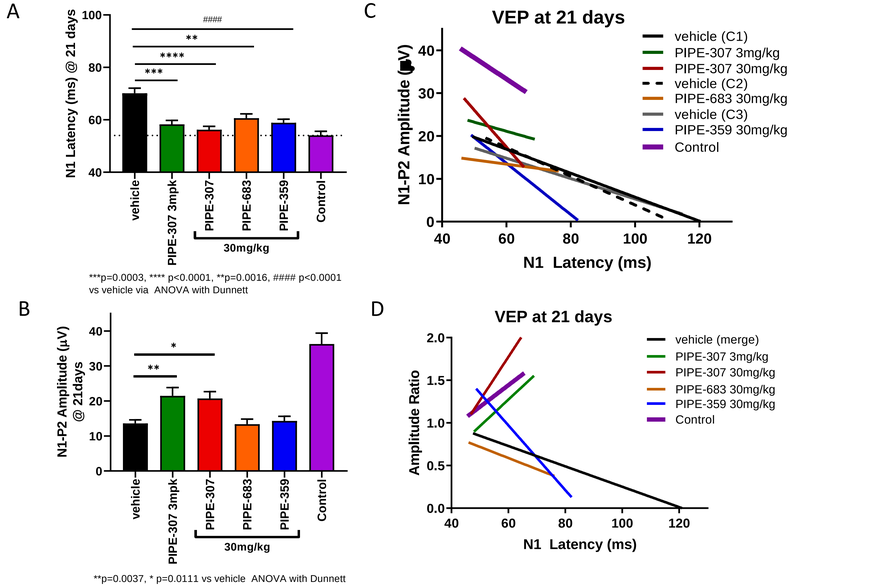


Fig 5A. M1 antagonists all showed significant N1 latency difference from vehicle treated EAE mice by 21 days post MOG induction. **B.** Not all M1 antagonists but PIPE-307 at both 3 and 30mg/kg showed a significant difference in N1P2 amplitude from vehicle at 21days. **C.** N1 latency vs N1P2 amplitude linear regression (xy scatter data points not shown) at 21 days of each M1 antagonist where the desired profile is low N1 latency and high N1-P2 amplitude. **D.** N1 latency vs amplitude ratio linear regression (xy scatter data points not shown) at 21 days the desired profile is a positive slope where demyelination is seen with a negative slope (Y = *-0.01194*X + 1.446). M1 antagonist PIPE-307 at both 3mg/kg (Y = *0.03111*X - 0.5940) and 30mg/kg (Y = *0.05091*X - 1.282) achieves a positive slope very close to control mice (Y = *0.02540*X - 0.08203) .

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

1. Green AJ, Gelfand JM, Cree BA, Bevan C, Boscardin WJ, Mei F, Inman J, Arnow S, Devereux M, Abounasr A, Nobuta H, Zhu A, Friessen M, Gerona R, von Büdingen HC, Henry RG, Hauser SL, Chan JR. Clemastine fumarate as a remyelinating therapy for multiple sclerosis (ReBUILD): a randomised, controlled, double-blind, crossover trial. Lancet. 2017 Dec 2;390(10111):2481-2489.
2. You Y, Klistorner A, Thie J, Graham SL. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. Invest Ophthalmol Vis Sci. 2011;52(9):6911–6918.
3. You Y, Klistorner A, Thie J, Gupta VK, Graham SL. Axonal loss in a rat model of optic neuritis is closely correlated with visual evoked potential amplitudes using electroencephalogram-based scaling. Invest Ophthalmol Vis Sci. 2012;53:3662.