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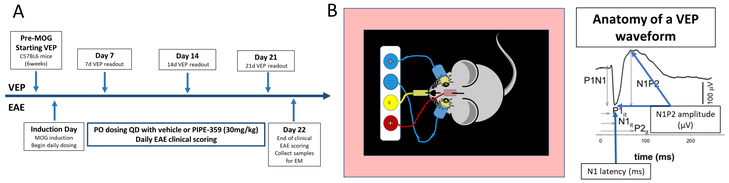
Geraldine C Edu*[1], Karin J Stebbins[1], Alexander R Broadhead[1], Michael M Poon[1], Ariana O Lorenzana[1], Thomas Schrader[1], Yifeng Xiong[1], Jill Baccei[1], Ari J Green[2], Jonah R Chan[2] and Daniel S Lorrain[1]. [1]Pipeline Therapeutics, San Diego, CA; [2]Neurol., UCSF, San Francisco, CA

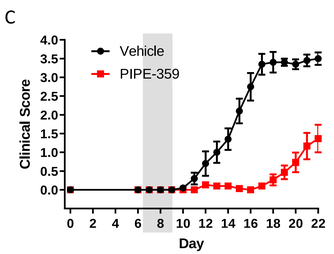
NO – PHOTO / RECORD

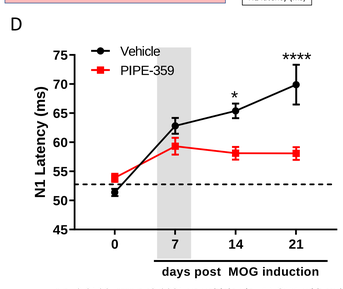
Introduction

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







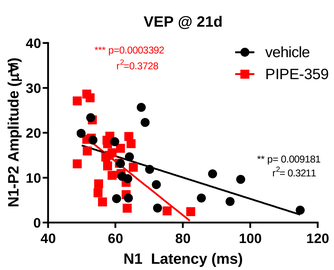


Fig 1A. Methods study timeline of PIPE-359 in the EAE model with flash VEP recordings performed in parallel. **1B.** EAE mice are dark adapted in a room with red lights for 1hr prior to recording. Mice are anesthetized with ketamine/xylazine (75/10mg/kg) and eyes are dilated with 1% Tropicamide for 2 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

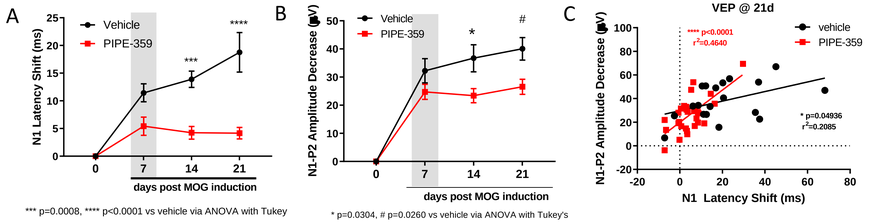


Fig 2A Differences in N1 latency shift can be detected at 7 days post MOG and becomes more significant overtime suggesting functional remyelination. **2B.** N1-P2 amplitude decreases are due to axonal damage at the retinal ganglion cells (You, Y. et.al 2011) and PIPE-359 prevents N1P2 amplitude degradation 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

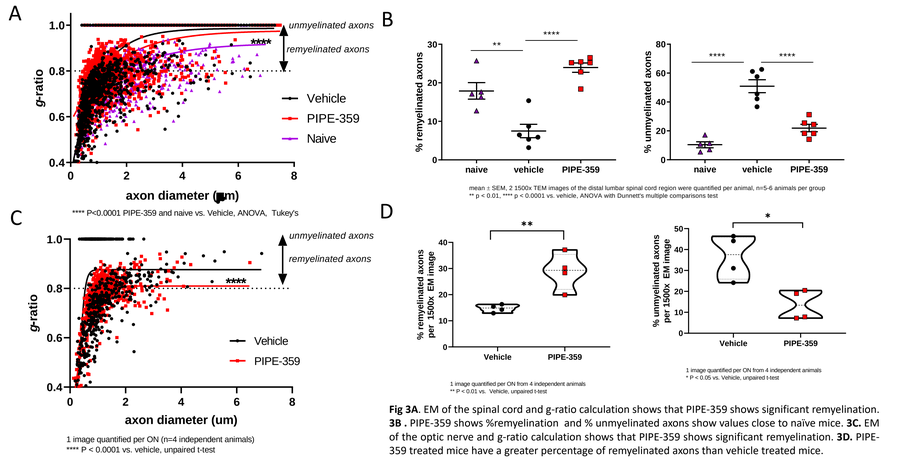


Fig 3A. EM of the spinal cord and g-ratio calculation shows that PIPE-359 shows significant remyelination. **3B** . PIPE-359 shows %remyelination and % unmyelinated axons show values close to naïve mice. **3C.** EM of the optic nerve and g-ratio calculation shows that PIPE-359 shows significant remyelination.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

VEP amplitude symmetry is preserved by M1 antagonists

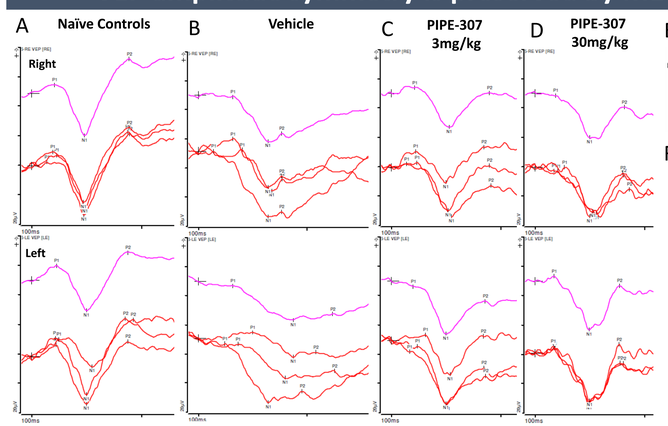


Fig 4A-D. Sample VEP waveform examples of each treatment group showing 3 VEP traces and the average trace. Top panel is the right eye, bottom panel is the left eye. **4E.** Standard VEP waveform components. Amplitude ratio description and calculation. **4F.** M1 antagonists do not show a degradation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

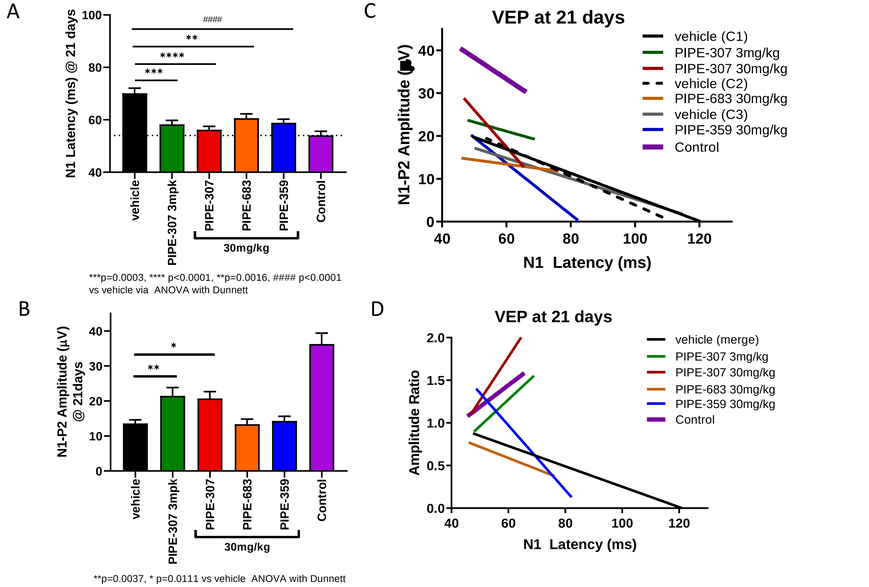


Fig 5A. M1 antagonists all showed significant N1 latency difference from vehicle treated EAE mice by 21 days post MOG induction. **B.** Not all M1 antagonists but PIPE-307 at both 3 and 30mg/kg showed a significant difference in N1P2 amplitude from vehicle at 21days. **C.** N1 latency vs N1P2 amplitude 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

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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.