PIPE-791 inhibits LPA1 to promote myelination and limit neuroinflammation

Michael M Poon[1], Kym I Lorrain[1,3], Geraldine Edu[1,3], Karin J Stebbins[1,3], Alexander Broadhead[1,3], Ariana J Lorenzana[1,3], Jeffrey R Roppe[2,3], Austin C Chen[2,3], Daniel S Lorrain[3]; [1]Biol., [2]Chem., Pipeline Therapeutics[3], San Diego, CA

Abstract

Multiple sclerosis (MS) is an inflammatory demyelinating disease that results in the disruption of neuronal transmission and ultimately neurodegeneration. In MS, levels of the inflammatory lipid, lysophosphatidic acid (LPA) are elevated in part to due increased autotaxin (ATX) activity[1,2,3]. In addition to neuroinflammation, our data show that LPA also inhibits oligodendrocyte precursor cell (OPC) differentiation. Although LPA1–null mice show improved behavior in the MOG-EAE model, the lack of a brain penetrant LPA1 antagonist has hampered the ability to test this mechanism[4]. PIPE-791 is the first orally bioavailable, brain penetrant LPA1 antagonist. We have performed extensive characterization of PIPE-791 in numerous *in vitro* and *in vivo* contexts. Here, we present data showing that PIPE-791 induces OPC differentiation, impacts neuroinflammation, and restores function in the mouse MOG-EAE model of MS. In total, these data suggest that targeting LPA1 inhibition shows promise as a multifaceted mechanism of treatment for multiple sclerosis.

LPAR1 expression in OPCs and OLs

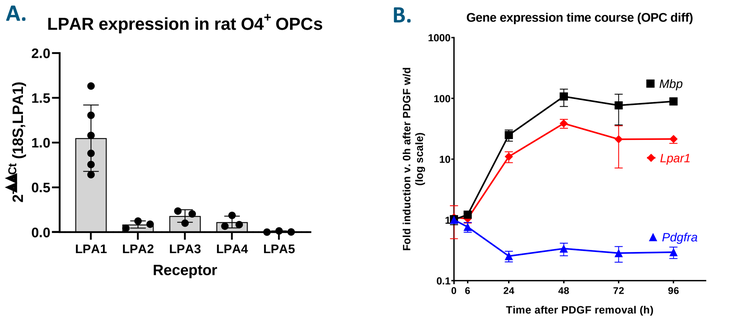

Figure 1. LPA1 is expressed in OPCs and oligodendrocytes. A. Quantitative PCR using primers against LPAR1-5 isoforms show LPAR1 is expressed in O4⁺ rat OPCs. B. LPAR1 expression increases as OPCs differentiate into oligodendrocytes. OPC differentiation indicated by an increase in *Mbp* (myelin basic protein) and a concomitant decrease in *Pdgfra* expression (mean ± SD).

PIPE-791 increases rat OPC differentiation (dissociated)

Assay	Value
$hLPA_1$ (RBA) K_i (nM)	0.75
$hLPA_1$ (Ca^{2+} flux) IC_{50} (nM)	9.9
Mouse Brain Slice IC_{50} (nM)	12
Rat OPC Differentiation IC_{50} (nM)	93

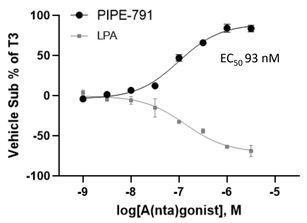

Figure 2 PIPE-791 induces OPC differentiation. Left, Table of PIPE-791 potencies across various assays. Right, OPC differentiation induced by PDGF removal is inhibited by further addition of LPA (gray line). Addition of PIPE-791 induces OPC differentiation. Values are expressed as a percentage of T3 (positive control) induction (mean ± SD).

PIPE-791 increases human CC1⁺ oligodendrocytes (slice)

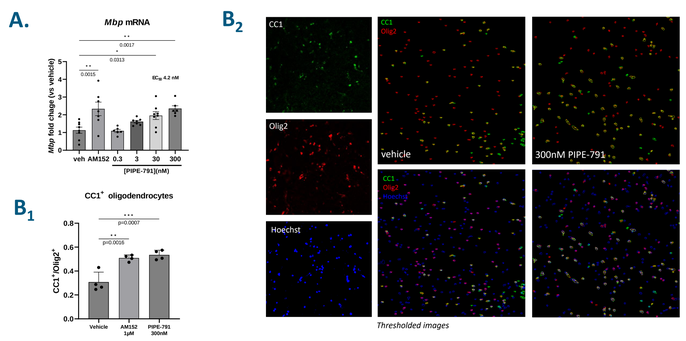

Figure 3 PIPE-791 increases the number of CC1⁺ oligodendrocytes in human brain slice cultures. A. Brain tissue from a 53yo female donor was used to generate cortical slice cultures. Addition of PIPE-791 dose dependently increases *Mbp* expression by qPCR , EC50 4.2 nM (mean ± SEM, ANOVA w/ Dunnett's), B_1. Addition of PIPE-791 increases the number CC1⁺ oligodendrocytes (mean ± SD, ANOVA w/ Tukey's). B_2 Representative images. Left column are raw images of oligodendrocyte marker CC1 (green), oligodendroglial marker Olig2 (red) and nuclear marker (Hoechst). Panel to the right are representative thresholded images of the same. Only cells expressing all three markers were counted as oligodendrocytes. AM152 is a potent, selective and structurally distinct LPA1 antagonist.

References

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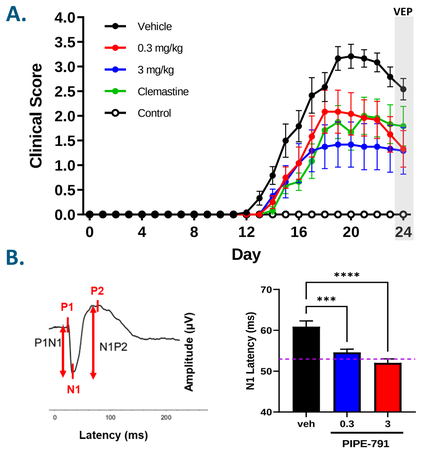
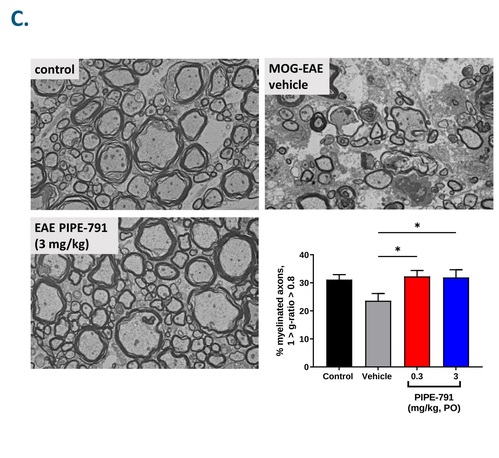
PIPE-791 promotes myelination in MOG-EAE model and limits neuroinflammation *in vivo*

(A - C.) PIPE-791 is efficacious in the mouse MOG-EAE model of MS





D. Inflammatory factors are induced over course of MOG-EAE

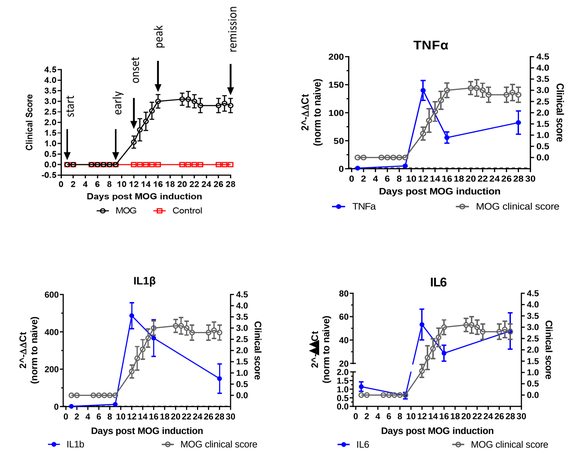


Figure 4. PIPE-791 is efficacious in the mouse MOG-EAE model of MS. A. Clinical scores. PIPE-791 or clemastine (10 mpk, positive control) was dosed after MOG induction for 25 days, q.d., PO. B. Visual evoked potentials were recorded for PIPE-791 and vehicle treated groups at day 24. Demyelination results in an increase in latency which is restored by PIPE-791 (mean ± SD , ANOVA with Tukey's, *** p=0.003, ****p<0.0001). Purple dash: latency of non-MOG mice. C. Representative EM micrographs showing more myelinated axons in PIPE-791 treated MOG-EAE spinal cords compared to vehicle. Controls are non-MOG-EAE mice dosed with vehicle. Graph of myelinated axons with g-ratio greater than 0.8 and less than 1. Axons in this range may be comprised of remyelinating axons (Mei et al., 2016 [5]) (mean ± SEM, * p < 0.05, ANOVA with Dunnett's, n ≥ 7). D. In a separate study, spinal cords were harvested at different timepoints after MOG-EAE induction and analyzed by qPCR against panel of proinflammatory factors. Expression graphed as overlay with corresponding clinical score (mean ± SEM, n=5)

Immune cells may be a local source of inflammatory LPA in MS

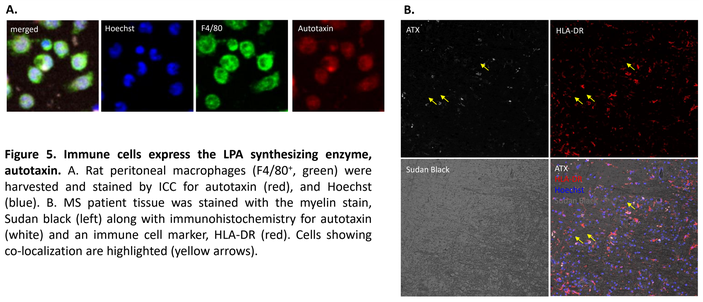
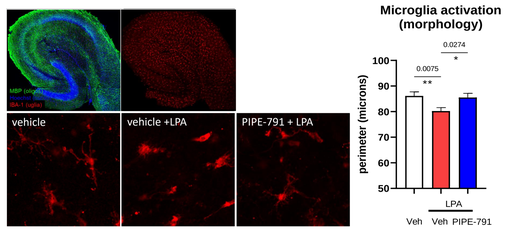


Figure 5. Immune cells express the LPA synthesizing enzyme, autotaxin. A. Rat peritoneal macrophages (F4/80⁺, green) were harvested and stained by ICC for autotaxin (red), and Hoechst (blue). B. MS patient tissue was stained with the myelin stain, Sudan black (left) along with immunohistochemistry for autotaxin (white) and an immune cell marker, HLA-DR (red). Cells showing co-localization are highlighted (yellow arrows).

PIPE-791 inhibits LPA induced microglia activation

Figure 6. Microglial activation by LPA is suppressed by PIPE-791. Acute mouse hippocampal slices were generated and treated for 2 h with vehicle or PIPE-791. LPA or vehicle was then added for 3 h, then slices fixed and processed for IHC against IBA1. Microglia activation was determined by measuring cell perimeter (mean ± SEM, ANOVA w/ Dunnett's)



PIPE-791 does not inhibit microglial phagocytosis

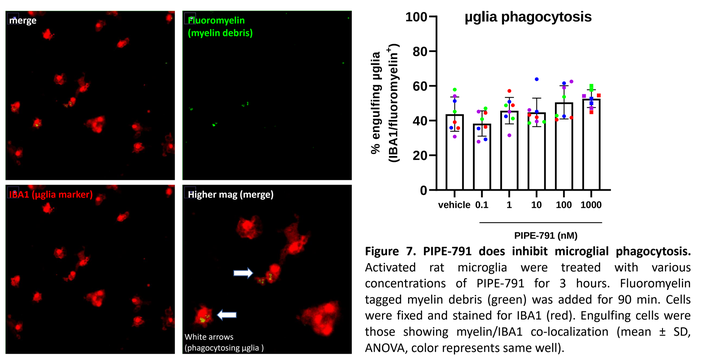
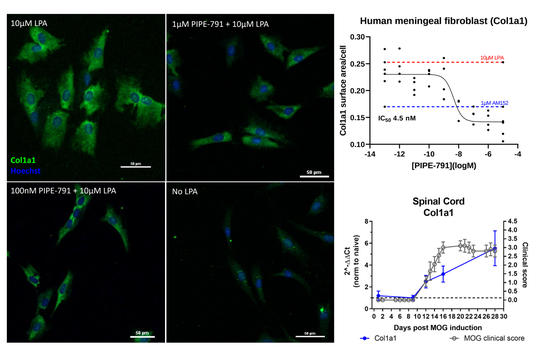


Figure 7. PIPE-791 does inhibit microglial phagocytosis. Activated rat microglia were treated with various concentrations of PIPE-791 for 3 hours. Fluoromyelin tagged myelin debris (green) was added for 90 min. Cells were fixed and stained for IBA1 (red). Engulfing cells were those showing myelin/IBA1 co-localization (mean ± SD, ANOVA, color represents same well).

PIPE-791 inhibits human brain meningeal fibroblast activation

Figure 8. PIPE-791 inhibits LPA induced meningeal fibroblast activation. Human leptomeningeal fibroblasts were starved in DMEM overnight then treated with vehicle or various concentrations of PIPE-791 for 6 hours. 10μM LPA was then added for 4h to activate fibroblasts. Cells were fixed and stained for Col1a1. Activation was measured as surface area/cell (IC_{50} 4.5 nM). Bottom graph, Col1a1 mRNA expression over course of mouse MOG-EAE (same study as Figure 4).



E. PIPE-791 (brain-penetrant) reduces neuroinflammation after acute LPS AM152 (peripherally restricted LPA1 antagonist) is less effective

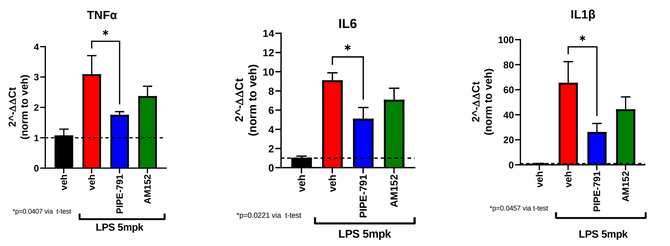


Figure 4 cont'd E. Acute lipopolysaccharide (LPS) was used to induce neuroinflammation. Mice were dosed with two LPA1 antagonists, PIPE-791 (brain penetrant) and AM152 (peripherally restricted). Two hours later, brains were collected and proinflammatory cytokines analyzed by qPCR. PIPE-791 reduces cytokine levels more effectively than a non-brain penetrant antagonist (AM152). Brain concentrations were also measured supporting improved brain penetration with PIPE-791.

Compound	Dose	Time	Brain (μM) Mean	$C_{u,brain}$ (μM) Mean
PIPE-791	3 mg/kg PO	4 hr	0.107	0.004
AM152	30 mg/kg PO	3 hr	< 0.041	< 0.0002

PIPE-791 inhibits retinal microglia activation *in vivo*

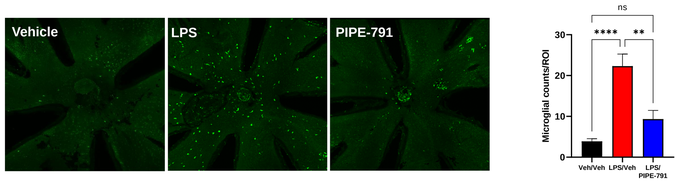


Figure 9. PIPE-791 inhibits retinal microglia activation. Mice were dosed PO with 3 mg/kg PIPE-791 for 1 h, then injected IP with 5 mg/kg LPS. 3 days later, retina were collected and immunostained for IBA1 (green). (mean ± SEM, ANOVA w/ Tukey's, n ≥3 animals per group.

PIPE-791 does not inhibit phagocytosis *in vivo*

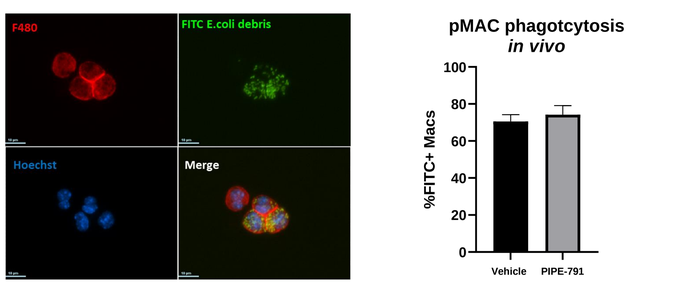


Figure 10. PIPE-791 does not impact phagocytosis *in vivo*. Mice were dosed with PIPE-791 (3 mg/kg, PO, 24 h) or vehicle 2h prior to FITC labeled *E. coli* debris (0.5 mg/mL). After 2 h, peritoneum was lavaged with cold PBS and cells stained with macrophage marker, F4/80 (red). Phagocytosis was measured as percentage of FITC⁺, F4/80⁺, Hoechst⁺ cells over total macrophages (mean ± SEM, n=5).

Conclusions

- **PIPE-791, a CNS penetrant, orally bioavailable LPA1 antagonist, reduces neuroinflammation and promotes myelination and is a promising treatment for MS**
- **Inhibition of LPA1 with PIPE-791 results in OPC differentiation into oligodendrocytes in rodent and human *in vitro* contexts**
- **PIPE-791 is efficacious in the MOG-EAE model of MS as assessed by clinical score and VEP latency, a functional measure of optic nerve myelination.**
- **PIPE-791 reduces the induction of neuroinflammatory cytokines, fibrosis, and microglia activation and is more efficacious *in vivo* than a peripheral LPA1 antagonist.**