Protective and Remyelinating Potential of Siponimod in a Xenopus Model of Demyelination and a Mouse Model of Experimental Autoimmune Encephalomyelitis

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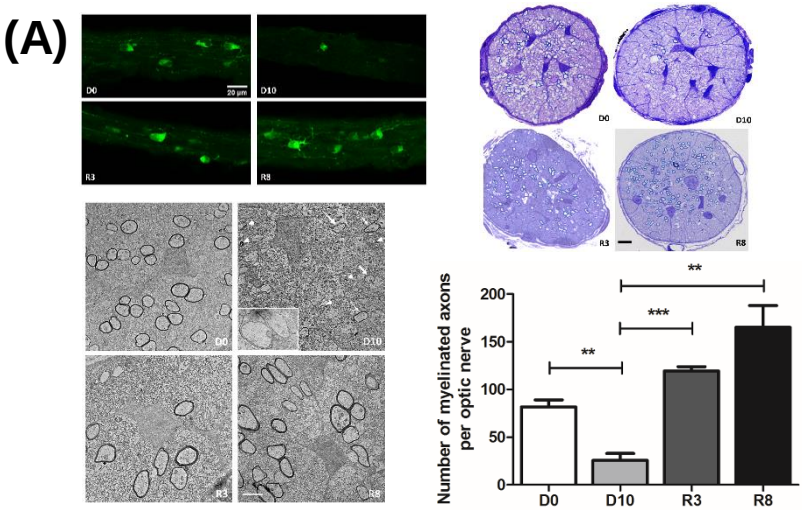
Introduction and Objectives

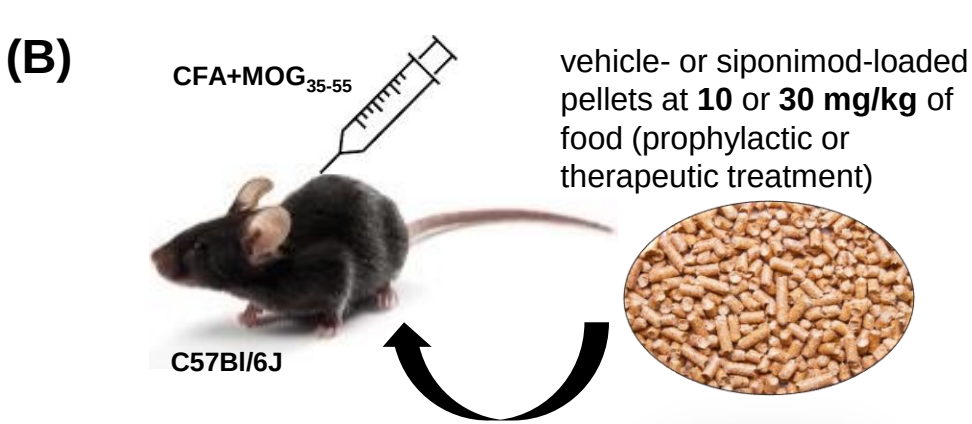
- In Multiple Sclerosis (MS), neurodegeneration is the main reason for chronic disability.[1]
- Siponimod is a potent, oral, selective sphingosine 1-phosphate receptor -1 and -5 modulator
- NDA approval for the treatment of “relapsing forms of MS to include CIS, RRMS and active SPMS” and of “active SPMS” in the US and for “active SPMS” in the EU.[2]

Aim: To assess remyelination and neuroprotective potential of siponimod in a model for Xenopus remyelination and a mouse experimental optic neuritis (EAEON) model using histological analysis and longitudinal visual system readouts.

1.Reich DS et al. N Engl J Med 2018; 378: 169–180
2.Kappos L et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): A double-blind, randomised, phase 3 study. Lancet 2018; 391: 1263–1273

Methods





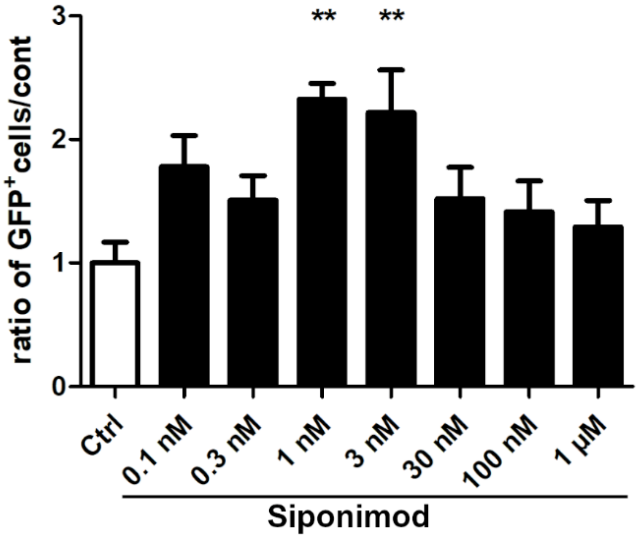
(A) Conditional demyelination transgenic Xenopus laevis model (MBP-GFP-NTR), oligodendrocyte apoptosis can be induced by metronidazole (MTZ) treatment.[3] Siponimod in swimming water (0.1nM-1µM).

(B) Experimental optic neuritis (EAEON) induced in female C57BL/6J mice immunized with myelin oligodendrocyte glycoprotein 35-55 (MOG_{35-55}).

(C) In mice, thickness of retinal layers and visual function assessed by optical coherence tomography (left) and optokinetic response (right).

3. Mannioui A and Zalc B. Conditional Demyelination and Remyelination in a Transgenic Xenopus laevis. Methods Mol Biol 2019; 1936: 239–248.

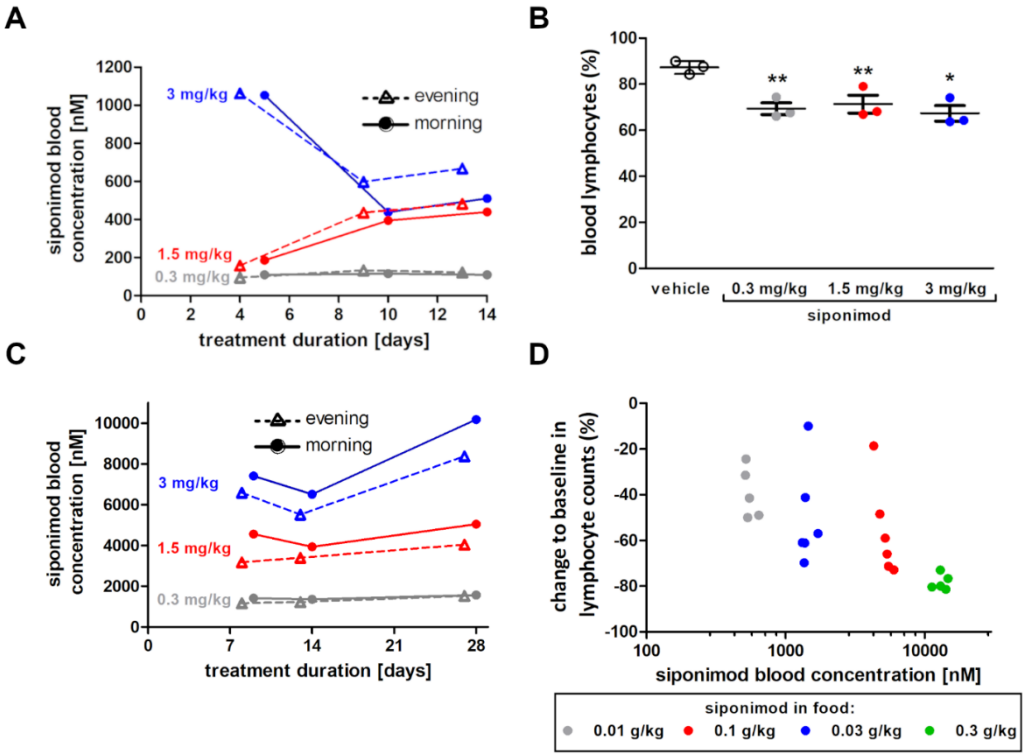
Dose response of siponimod remyelination potency



- Treatment of demyelinated tadpoles with siponimod (1nM in swimming water) improved remyelination 2.3±0.2 fold compared to control.
- The dose-response of siponimod efficiency to accelerate remyelination showed a bell-shaped curve.
- LC/MS/MS measurement: Maximum remyelination effect at concentrations between 70-80 nM in tissue.

Mean ± SEM, n = 5–8 tadpoles per group, **p<0.01 calculated using 1-way ANOVA followed by Dunn's post-hoc test to compare siponimod concentrations to control (Ctrl) condition

Administration route of Siponimod and pharmacokinetics



Supply via drinking water:

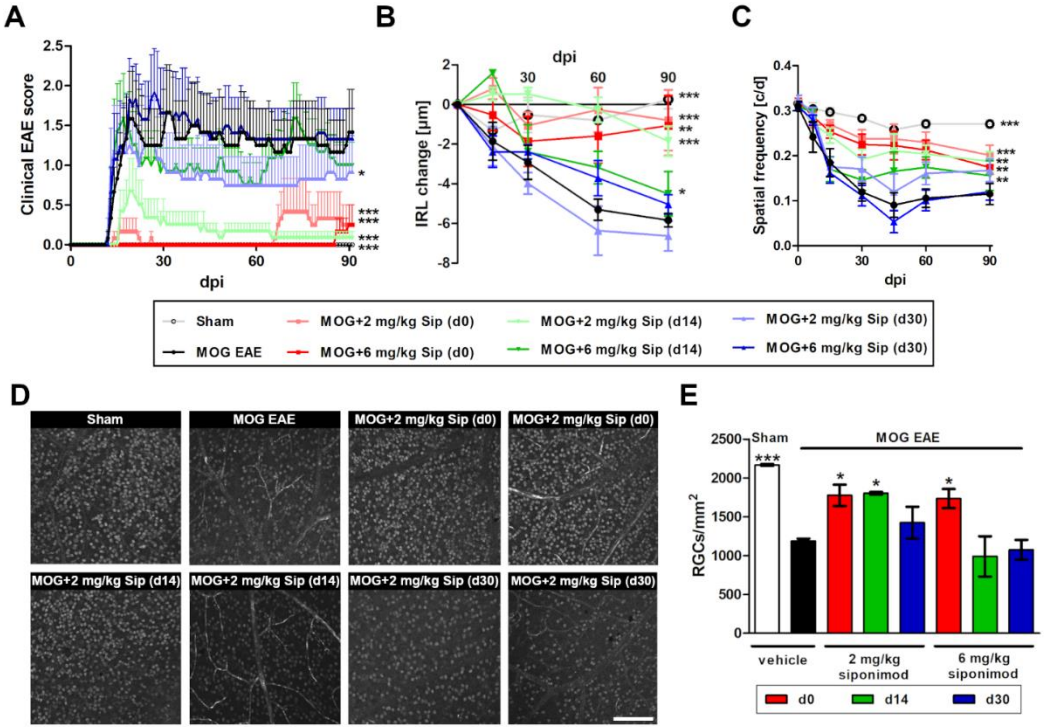
- poor dose-proportionality of siponimod blood levels (A).
- Reduction in blood lymphocytes counts of ~20% (B).

Supply via food pellets:

- Dose-dependent blood levels of siponimod (C).
- dose-related reduction in lymphocyte counts (D).
- good stability over time and low intra- and inter-individual variability

Pooled mean ± SEM, *p<0.05; **p<0.01; ***p<0.001, area under the curve compared by ANOVA with Dunnett's post hoc test for time courses and with *p<0.05, **p<0. 01, by ANOVA with Dunnett's post hoc test for scatter plots compared to control untreated mice.

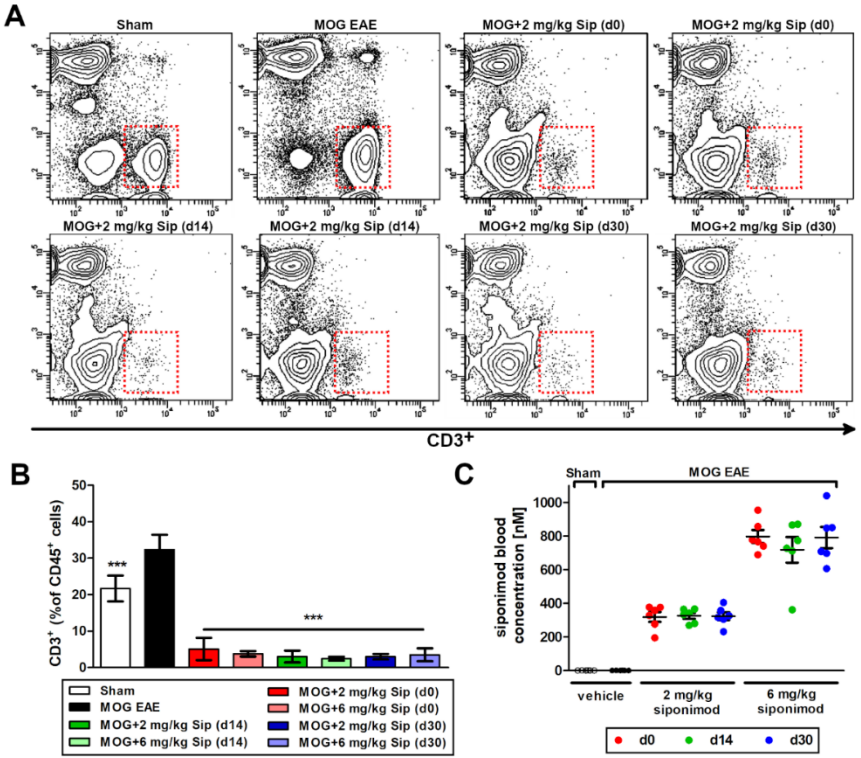
Siponimod attenuates MOG_{35-55}-induced EAE in C57BL/6J mice in a dose and time dependent manner.



- Prophylactic and therapeutic siponimod treatment had beneficial effect on clinical EAE scores (A) degeneration of the inner retinal layers (B) and visual function (C).
- Effects were reflected by the investigation of retinal ganglion cell (RGC) survival (D).
- Prominent RGC loss of untreated EAE animals was diminished by siponimod prophylactic diet and therapeutic treatment with 2 mg/kg (E).

Pooled mean ± SEM (n = 6 animals per group) with *p<0.05; **p<0.01; ***p<0.001, area under the curve compared by GEE or ANOVA with Dunnett's post hoc test for time courses compared to untreated MOG EAE. **p<0.01, ***p<0.001, by ANOVA with Dunnett's post hoc test compared to MOG EAE untreated mice for the bar graph

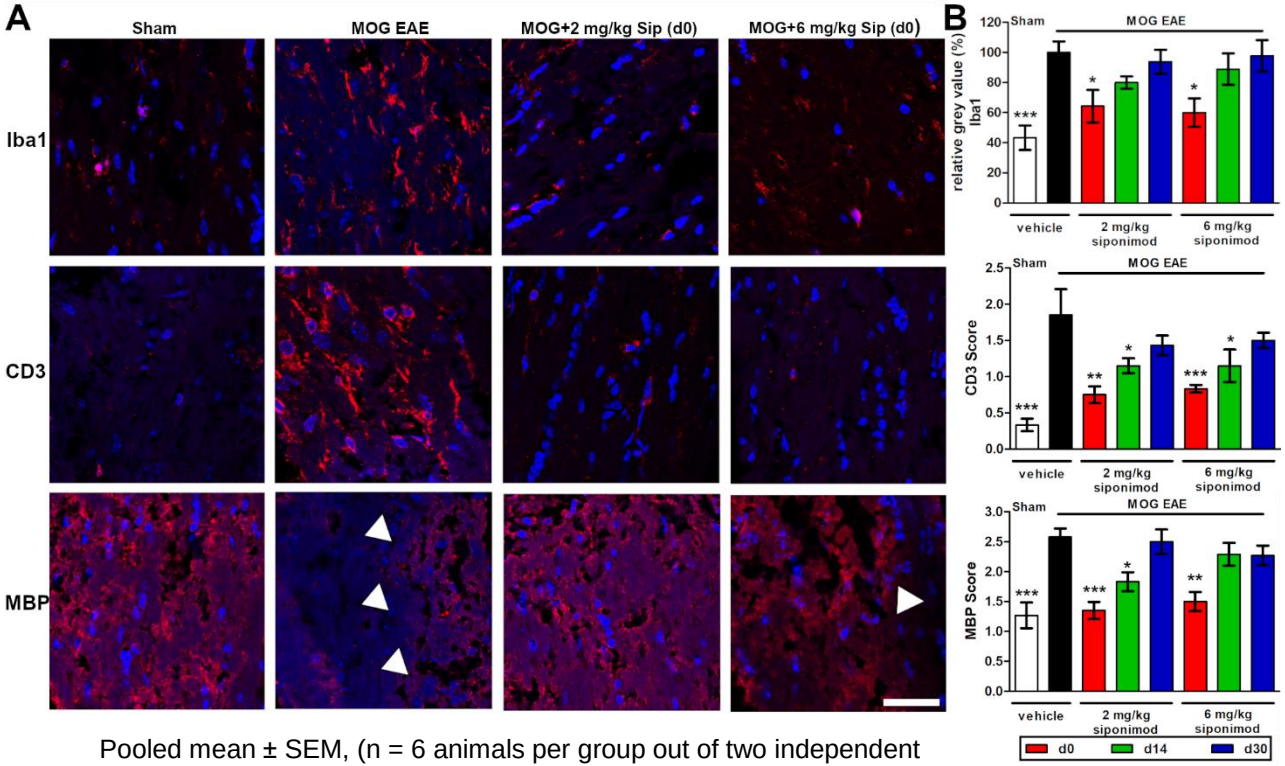
Siponimod treatment reduces the circulating CD3+ lymphocytes and shows dose dependent blood concentrations.



- CD3+ T-cell population (pregated for CD45+) was increased in MOG EAE mice 90 days after immunization (A).
- Siponimod therapy reduced the circulating CD3+ cells by approximately 90% at 2 and 6 mg/kg BW (A+B).
- Dose dependent concentrations between 300-400 nM and 700-900 nM were detected in mice treated at 2 or 6 mg/kg BW, respectively (C).

Pooled mean ± SEM (n = 6 animals per group out of two independent experiments) with ***p<0.001, by ANOVA with Dunnett's post hoc test compared to MOG EAE untreated mice.

Prophylactic siponimod therapy reduces immune cell infiltration and prevents demyelination



Pooled mean ± SEM, (n = 6 animals per group out of two independent experiments) with *p<0.05, **p<0.01, ***p<0.001 by ANOVA with Dunnett's post hoc test compared to MOG untreated mice

- Histological analyses of Iba1+ microglia/ macrophages, CD3+ T-cells and myelin status (MBP) of the optic nerve in longitudinal sections (A).
- Reduction of microglia/macrophage activity as well as T-cell infiltration in optic nerves of mice after prophylactic (d0) siponimod treatment (B).
- Prophylactic and therapeutic (d14) treatment showed beneficial effect (B).

Thank you for your interest!

Disclosures

MD received speaker honoraria from Novartis and Merck. HPH has received fees for serving on steering and data monitoring committees from Bayer Healthcare, Biogen, Celgene BMS, CSL Behring, GeNeuro, MedImmune, Merck, Novartis, Octapharma, Roche, Sanofi Genzyme, TG Therapeutic sand Viela Bio; fees for serving on advisory boards from Biogen, Sanofi Genzyme, Merck, Novartis, Octapharma, and Roche; and lecture fees from Biogen, Celgene BMS, Merck, Novartis, Roche, Sanofi Genzyme. PA received compensation for serving on Scientific Advisory Boards for Ipsen, Novartis, Biogen; he received speaker honoraria and travel support from Novartis, Teva, Biogen, Merz Pharmaceuticals, Ipsen, Allergan, Bayer Healthcare, Esai, UCB and Glaxo Smith Kline; he received research support from Novartis, Biogen, Teva, Merz Pharmaceuticals, Ipsen, and Roche. BZ received research support from Novartis and Merck and travel grant from Merck. CL has participated to advisory boards for Vertex, Roche, Biogen, Novartis, Genzyme, Merck, and speaking honoraria from Ipsen and Biogen. BS has received compensation for boards and/or lectures from Biogen, Sanofi-Genzyme, Novartis and Teva, and research grants from Merck, Roche, and Sanofi-Genzyme. PR, CB, CM, CA, ST and MB are employees of the Novartis Institutes for BioMedical Research. The other authors report no disclosures.