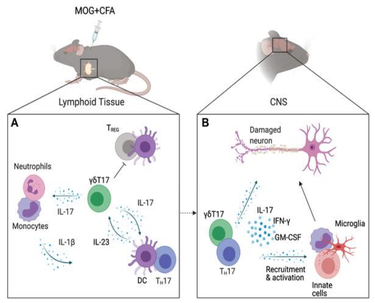
EAE Model of Multiple Sclerosis: Prophylactic Versus Treatment Fingolimod

Aggelika Gana[1], Erwina Stojek[1], Dervla Moore-Frederick[1], Róisín Winters[1], Alicja Zochowska-Klepanska[2], Ewa Sokolowska[2], Ashley Bernardo[3] Jack Prenderville[1]

Transpharmation Ltd. Ireland[1], Transpharmation Ltd. Poland, Transpharmation Ltd. Canada

INTRODUCTION

Experimental autoimmune encephalomyelitis (EAE) is a robust model of autoimmune inflammatory disease like multiple sclerosis (MS). The EAE model has CNS-related histopathology, motor deficits and inflammatory responses. Fingolimod (Fin), an S1P modulator, is the first oral drug approved for treating MS and is used as a positive control in EAE mice. We aimed to characterize the prophylactic (Pro) and treatment (Tx) effects of Fin in EAE mice by investigating disease progression and assessing neuroinflammatory and axonal damage biomarkers, and histological changes.

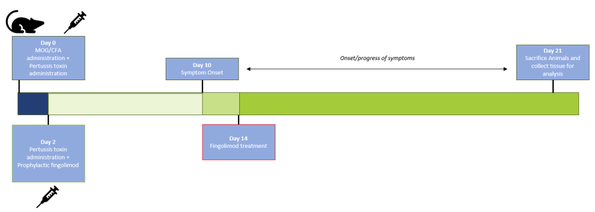


Agerholm and Bekiaris, European Journal of Immunology 2021

METHODS

Female C57BL/6J mice (n=34) were immunized with myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant followed by pertussis toxin (PT). On Day 2, another PT injection was administered and the Pro group (n=10) received daily administration of Fin. The Tx group started receiving daily Fin when 50% showed clinical symptoms (Day 14). Mice were checked daily for clinical symptoms and body weight (Day 0 -21). Levels of neurofilament light chain (NfL) in plasma and neuroinflammatory biomarkers were analysed by the MesoScale Discovery panel. Inflammatory cell infiltration in the spinal cord was evaluated by H&E staining.

0	no signs
0.5	little weight loss and paralysis of half of the tail
1	little weight loss and complete paralysis of the tail
1.5	both lots of weight loss and some loss of power in tail
2	complete paralysis of the tail and slight difficulty in gait
2.5	complete paralysis of the tail and mild weakness of hind legs
3	complete paralysis of the tail and severe weakness of hind legs
3.5	complete paralysis of the tail and paralysis of one hind leg
4	complete paralysis of the tail, paralysis of both hind legs (only pulled by front legs)

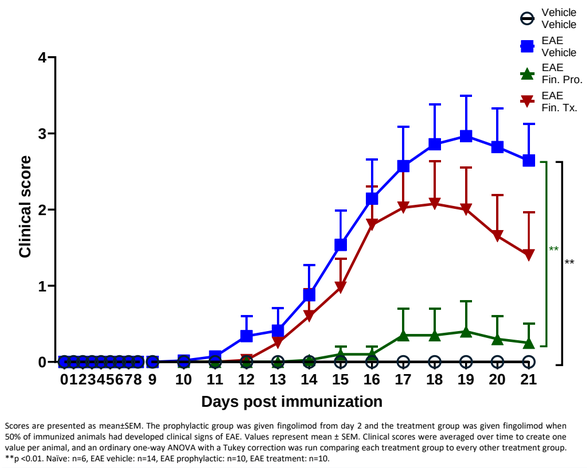


RESULTS

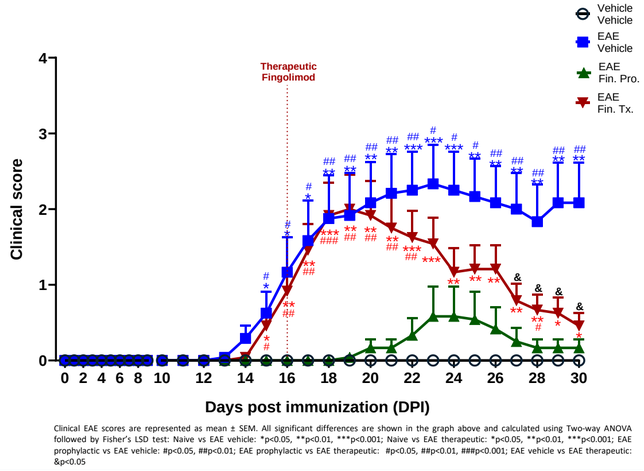
IN-LIFE

Results showed a significant clinical difference between groups (F(3,36)=6.797; p=0.001). Day 21: Naïve: 0±0, EAE Veh: 2.7±0.5, EAE Pro: 0.25±0.25, EAE Tx:1.4±0.6). The Prophylactic group showed lower daily clinical scoring evaluating the extent of paralysis. 9 out of 10 Prophylactic mice did not develop any clinical symptoms while paralysis progressed in the Treatment group.

In-life data was repeatable, in addition prolonged treatment with fingolimod for 14 days daily resulted in a significant reduction in the development of clinical symptoms ($p<0.05$) in the Treatment group compared to the EAE vehicle group from Day 27.



Scores are presented as mean±SEM. The prophylactic group was given fingolimod from day 2 and the treatment group was given fingolimod when 50% of immunized animals had developed clinical signs of EAE. Values represent mean ± SEM. Clinical scores were averaged over time to create one value per animal, and an ordinary one-way ANOVA with a Tukey correction was run comparing each treatment group to every other treatment group. **p <0.01. Naïve: n=6, EAE vehicle: n=14, EAE prophylactic: n=10, EAE treatment: n=10.

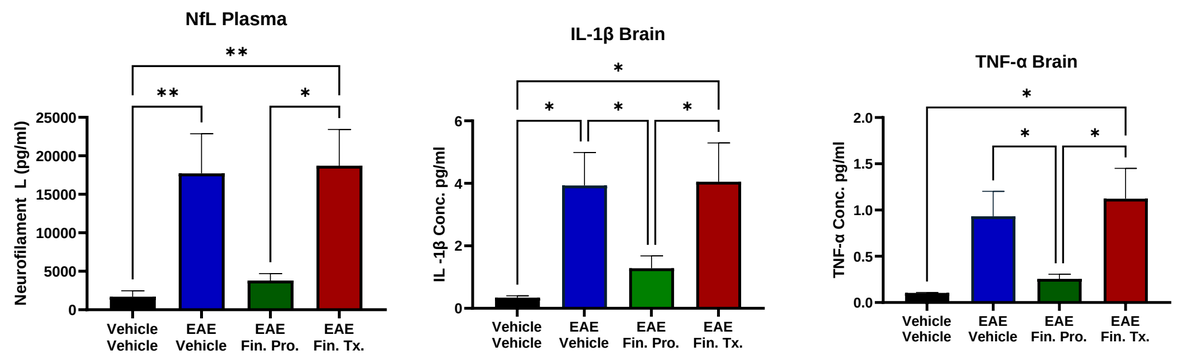


Clinical EAE scores are represented as mean ± SEM. All significant differences are shown in the graph above and calculated using Two-way ANOVA followed by Fisher's LSD test: Naive vs EAE vehicle: *p<0.05, **p<0.01, ***p<0.001; Naïve vs EAE therapeutic: *p<0.05, **p<0.01, ***p<0.001; EAE prophylactic vs EAE vehicle: #p<0.05, ##p<0.01; EAE prophylactic vs EAE therapeutic: #p<0.05, ##p<0.01, ###p<0.001; EAE vehicle vs EAE therapeutic: &p<0.05

BIOMARKERS

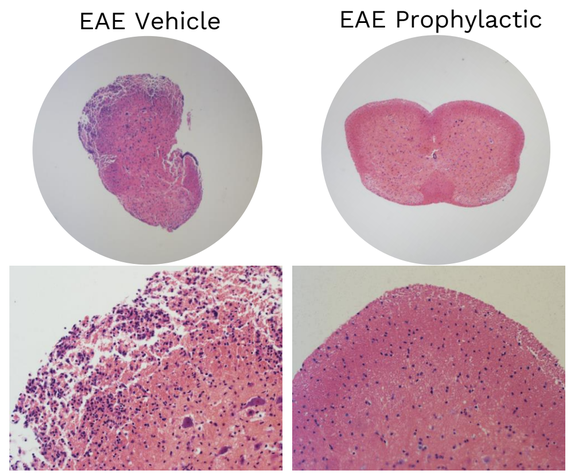
Biomarker results corroborate the clinical scores. NfL plasma levels were increased in EAE Veh animals (F(3,33)=4.124;p=0.01); Naïve: 1702±764, EAE Veh: 17721±5136, EAE Pro: 3798±907, EAE Tx: 18718±4718.

In the brain, increased IL-1β (F(3, 35) = 3.282; p=0.03) and TNFα (F(3, 34) = 3.320; p=0.03) were found in EAE Veh compared to Naïve and EAE Pro. (IL-1β: Naïve: 0.34±0.06, EAE Veh: 3.9±1, EAE Pro: 1.3±0.4, EAE Tx: 4.1±1.2; TNFα: Naïve: 0.1±0.005, EAE Veh: 0.9±0.3, EAE Pro: 0.3±0.05, EAE Tx: 1.1±0.3).



HISTOLOGY

H&E staining indicated increased penetration of inflammatory cells in the spinal cord in the prophylactic group compared to the vehicle. Representative images of H&E staining of spinal cord sections 20x. EAE vehicle: Cumulative Clinical Score: 28.5, Symptom Onset: Day 14; EAE prophylactic: Cumulative Clinical Score: 0, Symptom Onset: n/a



CONCLUSIONS

- This study reports that the EAE mouse model can effectively mimic MS symptoms
- Temporally-dependent start of a daily dose of Fingolimod can prevent disease onset, development of axonal damage, neuroinflammation and inflammatory cell infiltration in the spinal cord.
- The EAE model could be a useful approach to studying treatments that may modify these processes and compare their efficacy with Fingolimod.

REFERENCES

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