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Poster 43

Remibrutinib Inhibits Neuroinflammation Driven by B Cells and Myeloid Cells in EAE

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CONCLUSIONS

- Remibrutinib exhibited dose-dependent efficacy in the HuMOG EAE model underscoring its direct effects on pathogenic autoreactive B-cell antigen presenting function. In addition, the efficacy of remibrutinib in the B-cell–independent RatMOG EAE model indicates significant contribution from the inhibition of pathogenic myeloid cells like microglia
- The effect of remibrutinib in these models is not mediated by B-cell depletion, T-cell inhibition, or reduction of antibody levels
- The efficacy of remibrutinib in these in vivo models, as well as its inhibitory effect on both pathogenic B cells and microglia, support clinical development in chronic inflammatory diseases such as MS

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INTRODUCTION

- Bruton's tyrosine kinase (BTK) is a key signalling node in B-cell receptor and Fc receptor signalling[1]
- Inhibition of BTK offers an attractive mechanism to modulate immune regulatory networks and related neuroinflammation via inhibition of B cells and myeloid cells[1]
- BTK inhibitors are a novel class of oral therapies to prevent inflammation and disease progression in multiple sclerosis (MS), without depleting B cells[1]
- Remibrutinib is a potent, highly selective covalent BTK inhibitor with a promising preclinical and clinical profile for MS treatment[2-4]
- Remibrutinib has shown to exhibit improved target selectivity and potency in vitro[5]
- This study describes the efficacy and mechanism of action of remibrutinib in the B-cell–dependent recombinant human myelin oligodendrocyte glycoprotein (HuMOG)-induced experimental autoimmune encephalomyelitis (EAE) mouse model and in the B-cell–independent RatMOG-induced EAE model[6]

OBJECTIVE

- To assess the mechanism of action and efficacy of remibrutinib in EAE mouse models for MS

METHODS

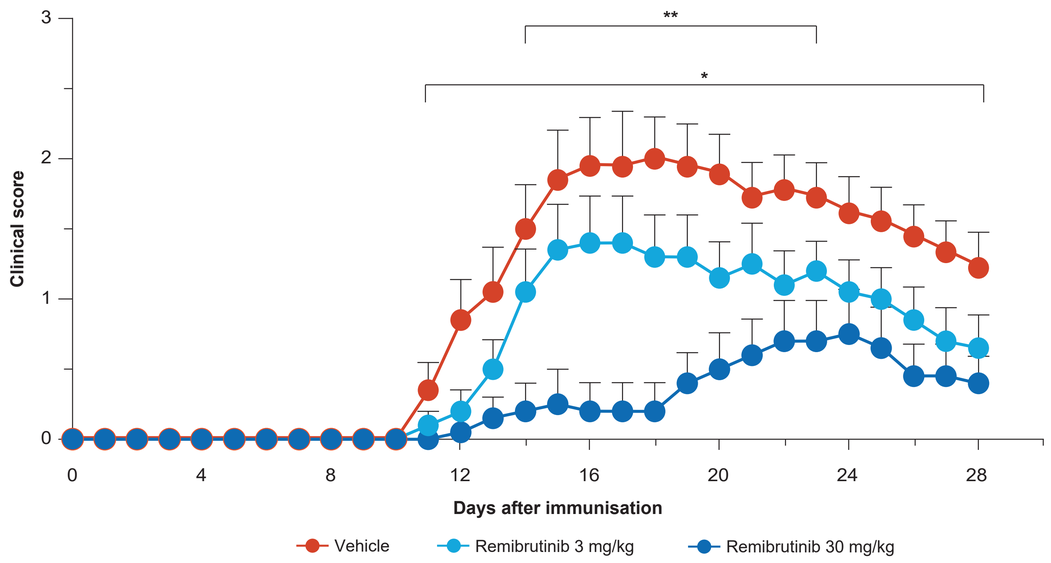
- In both models C57BL/6 mice were immunised with either HuMOG or RatMOG to induce EAE. EAE clinical features were scored daily. BTK occupancy levels in spleen, lymph nodes and brain were determined with immunoassays for free BTK and total BTK protein[3]
- Additional assessments were for ex vivo T-cell recall proliferative response and serum anti-MOG antibody response. Brain and spinal cord mRNA expression were analysed in RatMOG EAE by single-cell RNA-sequencing (scRNASeq)

RESULTS

Remibrutinib Inhibits B-Cell–Dependent HuMOG EAE

- Oral dosing of 3 or 30 mg/kg b.i.d. remibrutinib dose dependently reduced clinical scores in HuMOG EAE mice with a statistical significant inhibition for the 30 mg/kg dose (**Figure 1**)
- BTK occupancy was near maximal in the spleen and brain with 30 mg/kg (**Please refer to Poster 42**)

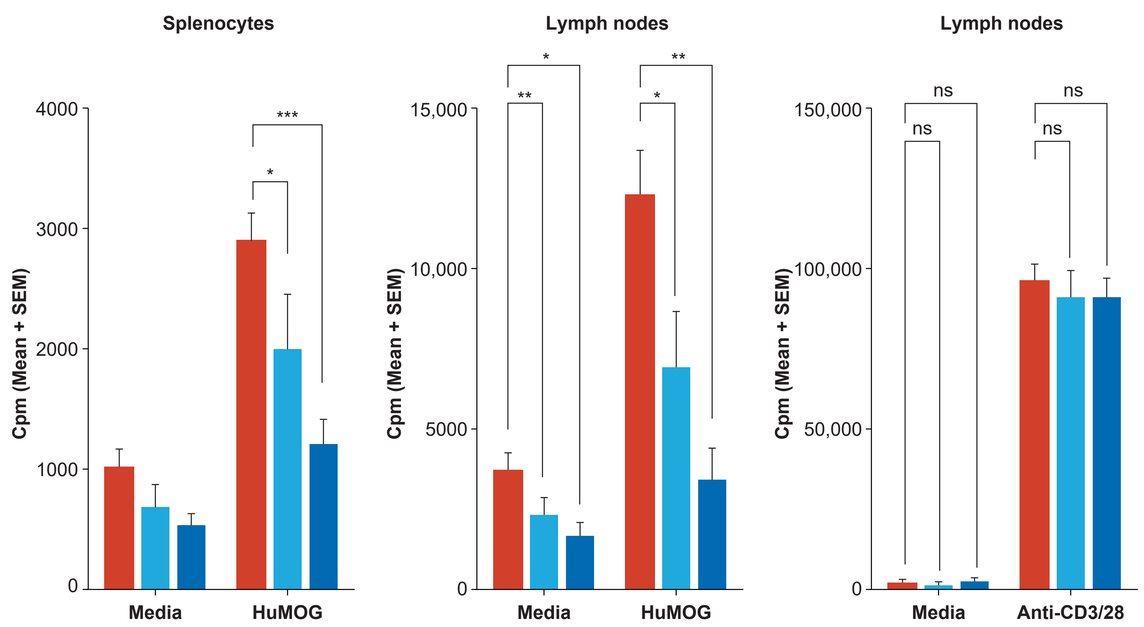
Figure 1. Efficacy in HuMOG EAE



Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05, **p<0.01.
ANOVA, analysis of variance; EAE, experimental autoimmune encephalomyelitis; HuMOG, human myelin oligodendrocyte glycoprotein.

- Remibrutinib inhibited ex vivo HuMOG-specific splenocyte T-cell recall proliferative response, but not the polyclonal T-cell proliferation to anti-CD3/CD28, indicating the absence of direct T-cell immune suppression (**Figure 2**)

Figure 2. Ex vivo splenocyte HuMOG EAE recall proliferative response



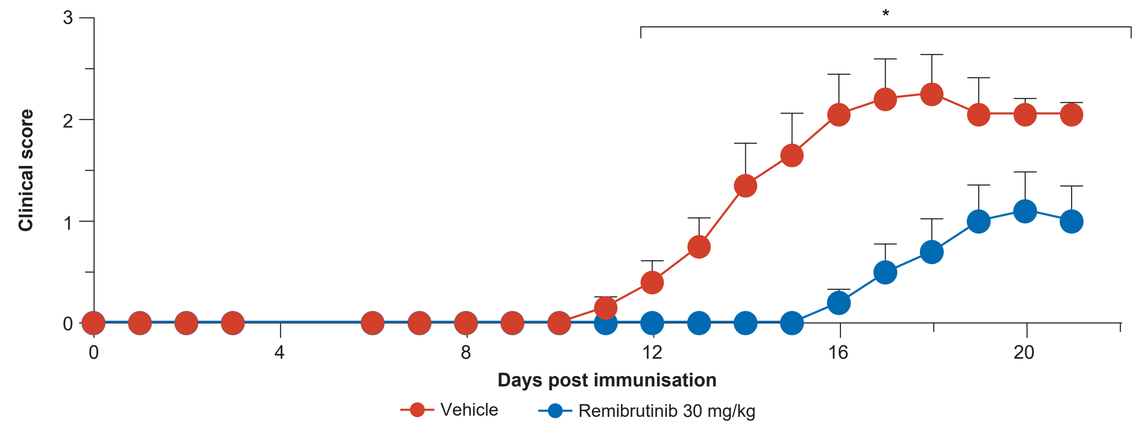
Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05, **p<0.01, ***p<0.001.
ANOVA, analysis of variance; CD, cluster of differentiation; Cpm, counts per minute; EAE, experimental autoimmune encephalomyelitis; HuMOG, human myelin oligodendrocyte glycoprotein; ns, not significant; SEM, standard error of the mean.

- Ex vivo analysis of isolated splenocytes, lymph node cells and blood revealed no significant changes in total B-cell populations, but a clear reduction of CD4+ T-helper 17 (Th17) cells (data not shown). Similarly, remibrutinib did not reduce total immunoglobulin (Ig) G antibody levels (data not shown)

Remibrutinib Inhibits RatMOG EAE

- Remibrutinib orally dosed at 30 mg/kg b.i.d. reduced EAE clinical symptoms (**Figure 3**), suggesting that in absence of direct T-cell inhibition the efficacy in this RatMOG EAE model is mediated by myeloid cell and microglia inhibition
- BTK occupancy measured at 16 hours after the last dose showed notable levels in the spleen, blood and brain (**Please refer to Poster 42**)

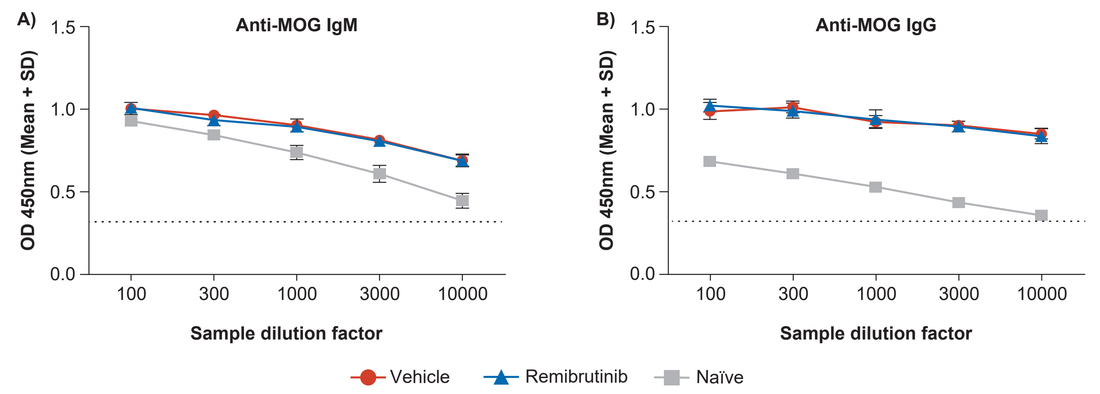
Figure 3. Efficacy in RatMOG EAE



Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05.
ANOVA, analysis of variance; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein.

- Remibrutinib treatment had no effect on the anti-MOG serum IgM (**Figure 4A**) and IgG levels (**Figure 4B**)

Figure 4. RatMOG EAE antibody response

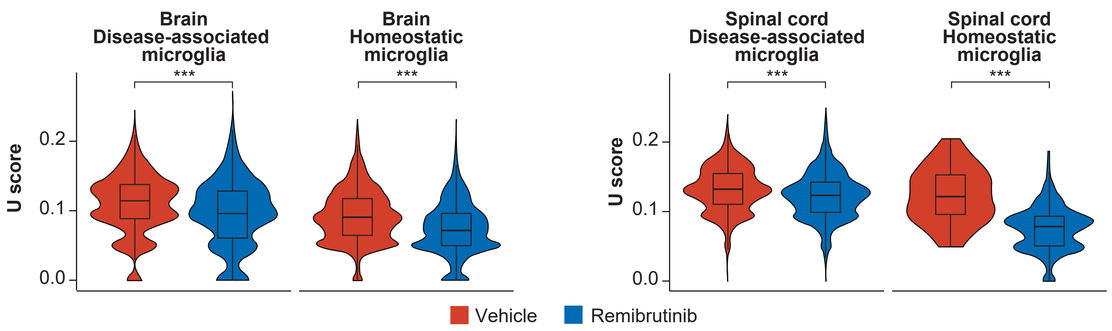


EAE, experimental autoimmune encephalomyelitis; Ig, Immunoglobulin; MOG, myelin oligodendrocyte glycoprotein; OD, optical density; SD, standard deviation.

Reduction of Neuroinflammation Gene Signature in EAE Microglia

- Analysis of scRNA-seq data obtained from brains and spinal cords from a separate RatMOG EAE study revealed that remibrutinib significantly downregulated multiple gene sets related to inflammation in microglia, suggesting it has an anti-inflammatory effect specifically in these cells (**Figure 5**)

Figure 5. Remibrutinib reduces EAE microglia neuroinflammation gene expression



***p<0.001, one-tailed Mann-Whitney U test.
EAE, experimental autoimmune encephalomyelitis.

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Disclosures

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