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P1480

Remibrutinib Ameliorates CNS Autoimmune Disease - Insights From EAE

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SUMMARY

1 Remibrutinib (LOU064) is a potent, highly selective covalent Bruton's tyrosine kinase inhibitor (BTKi) with a promising profile for multiple sclerosis (MS) and other autoimmune or autoallergic indications. The efficacy and mechanism of action of remibrutinib was assessed in two experimental autoimmune encephalomyelitis (EAE) mouse models for MS

2 Remibrutinib inhibited EAE by a two-pronged mechanism based on the inhibition of pathogenic B-cell autoreactivity, as well as direct anti-inflammatory effects in microglia. Remibrutinib showed efficacy in both models in absence of B-cell depletion, broad T-cell inhibition or reduction of total immunoglobulin (Ig) levels. These findings support the view that remibrutinib may represent a novel treatment option for patients with MS

INTRODUCTION

- BTK regulates the functions of B and myeloid cells, implicated in the pathogenesis of MS[1]. Remibrutinib is a covalent, oral BTKi exhibiting high selectivity and potency, with the potential to minimise off-target toxicity and is currently being investigated in phase 3 trials for the treatment of MS (NCT05147220, NCT05156281)[2]

OBJECTIVE

- To assess the in vivo efficacy of remibrutinib and to better understand its impact on inflammation and tissue destruction in the central nervous system (CNS) in an MS animal model

METHODS

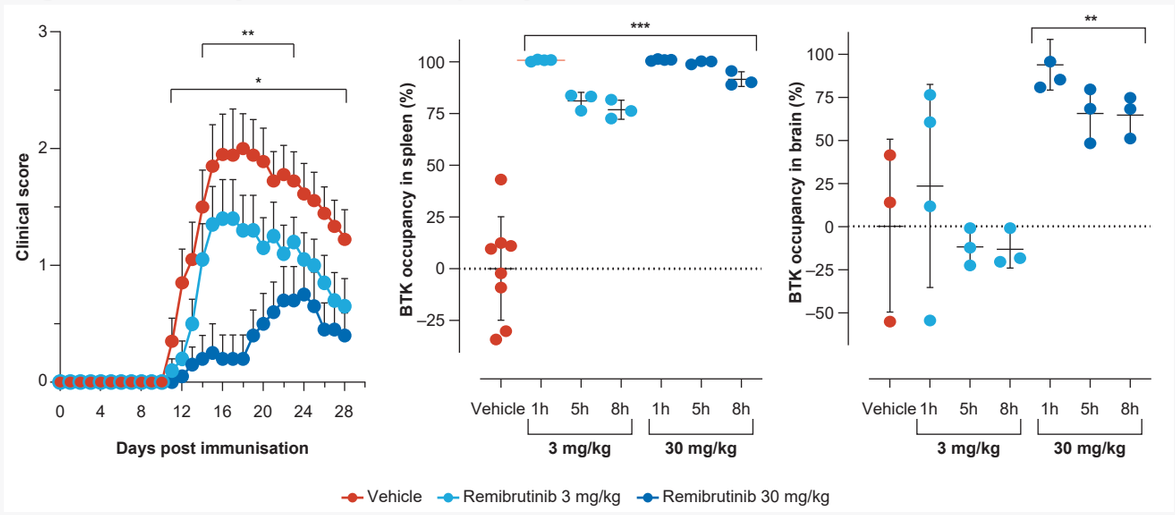
- EAE was induced by immunisation with either human myelin oligodendrocyte glycoprotein (HuMOG), causing a B cell–dependent disease, or rat MOG, causing B cell–independent pathology
- Target engagement was assessed in tissue, and clinical disease activity was determined
- Inflammation, demyelination and the degree of reactive gliosis were studied using immunohistochemistry

RESULTS

REMIBRUTINIB INHIBITS B CELL–DEPENDENT HUMOG EAE

- Remibrutinib dose dependently reduced HuMOG EAE symptoms. Peak spleen BTK occupancy was near-maximal for the 30 mg/kg group and lower for the 3 mg/kg group after the last dose. Interestingly, brain BTK occupancy showed dose-dependent differences with 3 mg/kg reaching only minimal BTK occupancy and 30 mg/kg reaching relatively sustained BTK occupancy[3] (**Figure 1**)

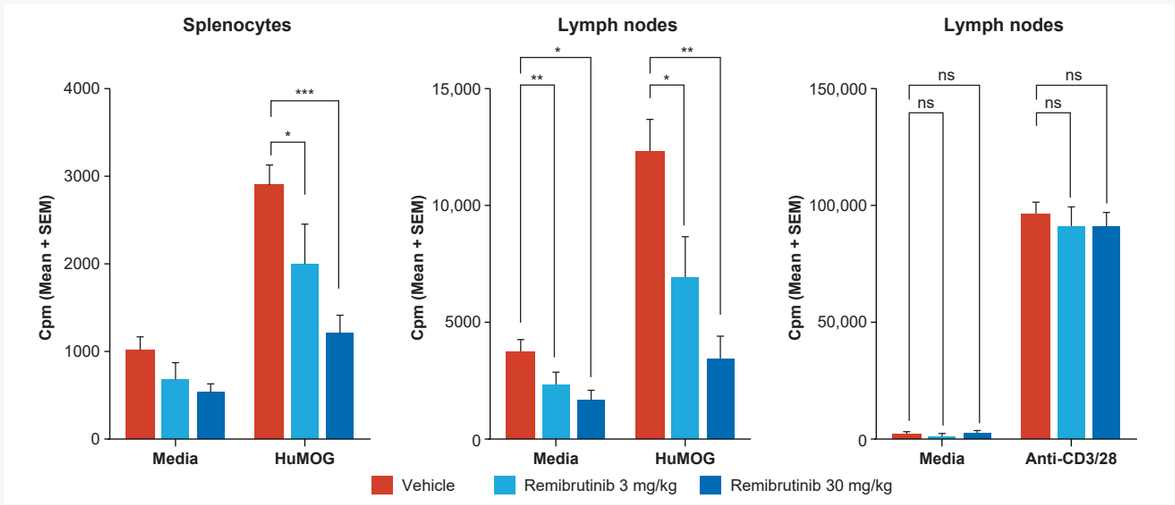
Figure 1. Efficacy and BTK Occupancy in HuMOG EAE



ANOVA, analysis of variance; BTK, Bruton's tyrosine kinase; EAE, experimental autoimmune encephalomyelitis; h, hour; HuMOG, human myelin oligodendrocyte glycoprotein.
Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05, **p<0.01, ***p<0.001.

- Remibrutinib inhibited ex vivo HuMOG-specific splenocyte T-cell recall proliferative response, but not polyclonal T-cell proliferation to anti-CD3/CD28, indicating the absence of direct T-cell immune suppression[3] (**Figure 2**)

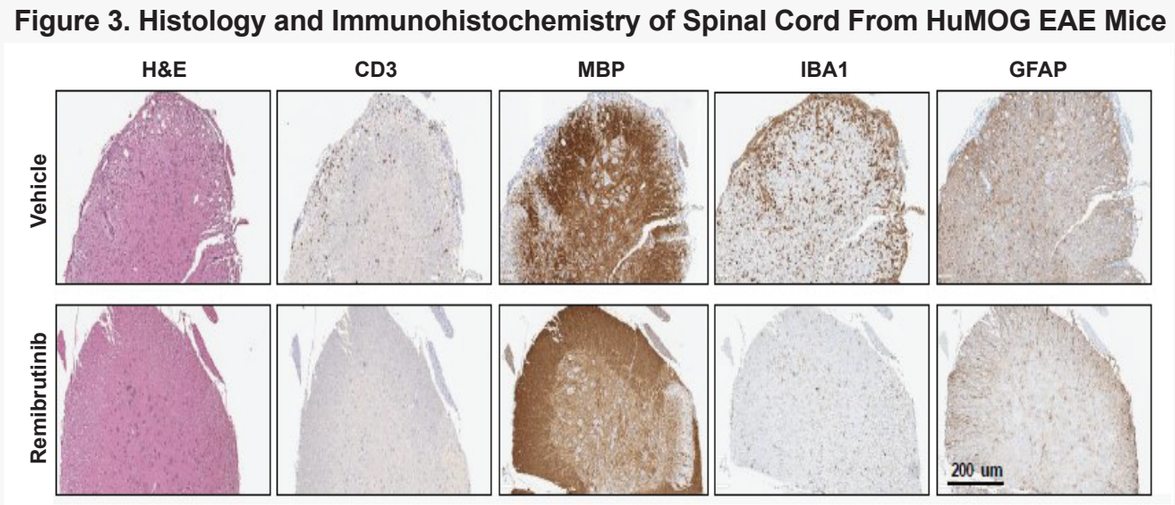
Figure 2. Ex Vivo Splenocyte HuMOG EAE Recall Proliferative Response



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.
Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05, **p<0.01, ***p<0.001.

- Ex vivo analysis of splenocytes, lymph node cells, and blood revealed no changes in B-cell populations or IgG antibody levels, but a clear reduction of CD4+ T-helper 17 (Th17) cells (data not shown)

Figure 3. Histology and Immunohistochemistry of Spinal Cord From HuMOG EAE Mice

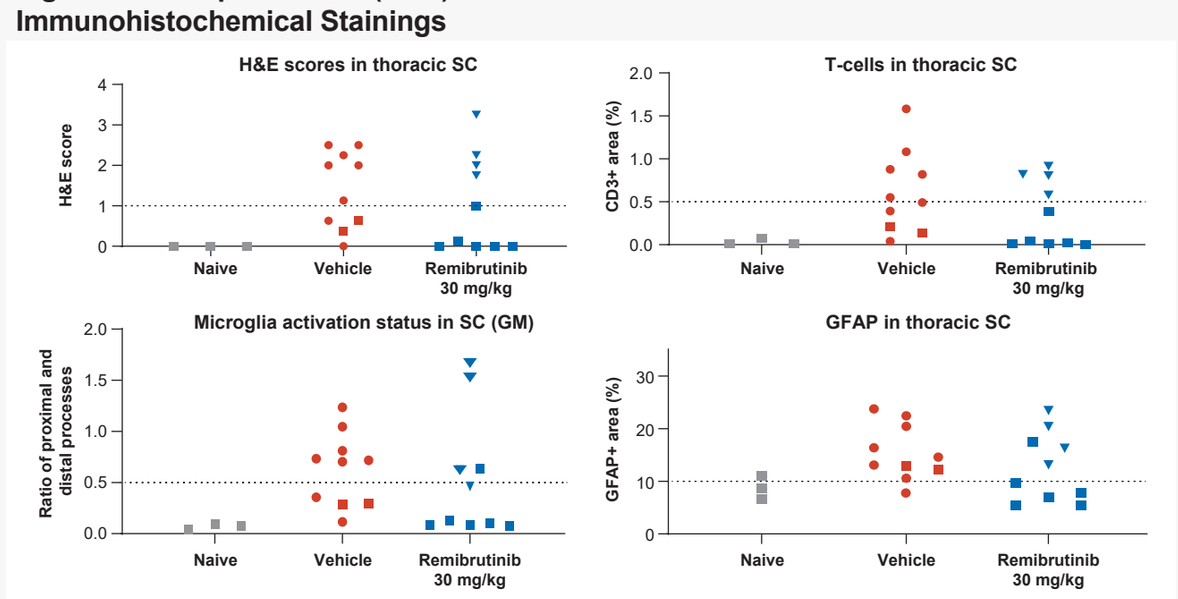


Spinal cords from HuMOG-induced EAE mice were collected on Day 19 post-immunisation at the peak of the disease. H&E sections were scored on the scale 0-4. Immunohistochemically stained sections were analysed using image analysis platforms HALO® and ASTORIA.

CD3, cluster of differentiation 3 – marker of T cells; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein – marker of astrocytes; H&E, hematoxylin and eosin; HuMOG, human myelin oligodendrocyte glycoprotein; IBA1, ionised calcium-binding adaptor molecule 1 – marker of microglia; MBP, myelin basic protein.

- Histological analysis of the spinal cord at Day 19 post immunisation (**Figure 3–4**) revealed protective effect of remibrutinib at 30 mg/kg b.i.d. by reducing the density and activation status of microglia and astrocytes. Infiltrating T cells were slightly reduced, while demyelination was not changed upon treatment (data not shown)

Figure 4. Semiquantitative (H&E) and Quantitative Results of the Immunohistochemical Stainings

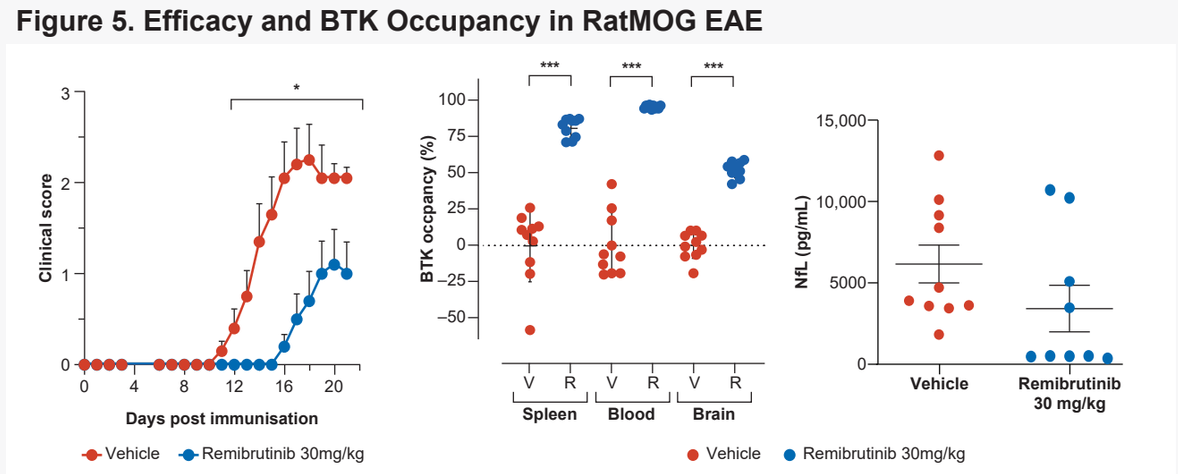


Squares indicate naive mice with clinical score 0.
CD, cluster of differentiation; GFAP, glial fibrillary acidic protein; GM, gray matter; H&E, hematoxylin and eosin; SC, spinal cord.

REMIBRUTINIB INHIBITS RATMOG EAE

- Remibrutinib orally dosed at 30 mg/kg b.i.d. reduced EAE clinical symptoms (**Figure 5**), suggesting that in the absence of direct T-cell inhibition, the efficacy in this RatMOG EAE model is mediated by myeloid cell and microglia inhibition[3]
- BTK occupancy was measured 16 h after the last dose, showing notable levels of BTK occupancy in the spleen, blood and brain (**Figure 5**)
- Remibrutinib led to a trend in reduction of plasma neurofilament light chain (NfL), a biomarker for neuroinflammation

Figure 5. Efficacy and BTK Occupancy in RatMOG EAE

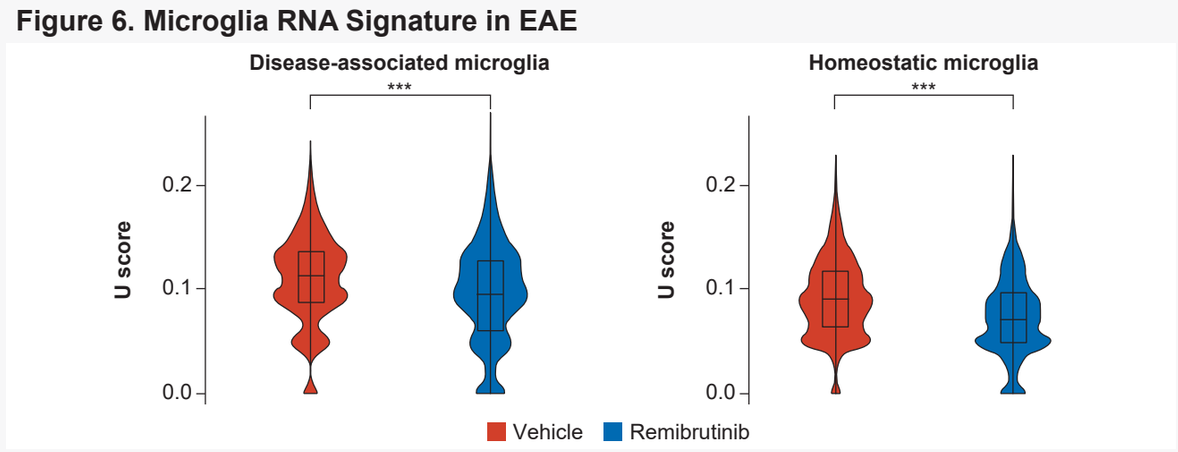


ANOVA, analysis of variance; BTK, Bruton's tyrosine kinase; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; NfL, neurofilament light chain; R, remibrutinib; V, vehicle.
Statistical significance (ANOVA with Dunnett's test) is shown as *p<0.05, ***p<0.001.

REDUCTION OF NEUROINFLAMMATION GENE SIGNATURE IN EAE MICROGLIA

- Analysis of scRNA-seq data obtained from brain (**Figure 6**) and spinal cord (data not shown) revealed that remibrutinib significantly downregulated multiple gene sets related to inflammation in microglia, suggesting it has an anti-inflammatory effect specifically in these cells[3]

Figure 6. Microglia RNA Signature in EAE



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

CONCLUSIONS

- Remibrutinib demonstrated clinical efficacy in a BTK-dependent EAE model, which was associated with CNS tissue protection
- Our findings in an experimental MS model support the view that BTK might represent a promising target for treating MS
- The clinical outcomes of the ongoing phase 3 trials studying remibrutinib in MS are warranted to allow translation from model systems to patients

References: 1. Steinmaurer A, et al. *Curr Pharm Des.* 2022;28(6):437-444; **2.** Wiendl H, et al. Poster presented at: American Academy of Neurology; April 2-7, 2022. P7-003; **3.** Nuesslein-Hildesheim B, et al. *J Neuroinflammation*. 2023;20(1):194.

Abbreviations: ANOVA, analysis of variance; **b.i.d.,** twice daily; **BTKi,** Bruton's tyrosine kinase inhibitor; **CD,** cluster of differentiation; **cpm,** counts per minute; **CNS,** central nervous system; **EAE,** experimental autoimmune encephalomyelitis; **GFAP,** glial fibrillary acidic protein; **GM,** gray matter; **h,** hour; **H&E,** hematoxylin and eosin; **HuMOG,** human MOG; **IBA,** ionised calcium-binding adaptor molecule; **IgG,** immunoglobulin G; **MBP,** myelin basic protein; **MOG,** myelin oligodendrocyte glycoprotein; **MS,** multiple sclerosis; **NfL,** neurofilament light chain; **ns,** not significant; **R,** remibrutinib; **RNA,** ribonucleic acid; **SC,** spinal cord; **scRNA,** single-cell RNA; **SEM,** standard error of the mean; **V,** vehicle.

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