Remibrutinib Inhibits Neuroinflammation Driven by B Cells and Myeloid Cells in Preclinical Models of Multiple Sclerosis

P134

Barbara Nuesslein-Hildesheim[1], Enrico Ferrero[1], Catherine Huck[1], Paul Smith[2], Denis Eichlisberger[1], Bruno Cenni[1]

[1]Novartis Institutes for Biomedical Research, Basel, Switzerland; [2]Connect Biopharma, San Diego, CA

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* (ECTRIMS 2022, Poster #EP0896)
- 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[5]

Objective

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

Methods

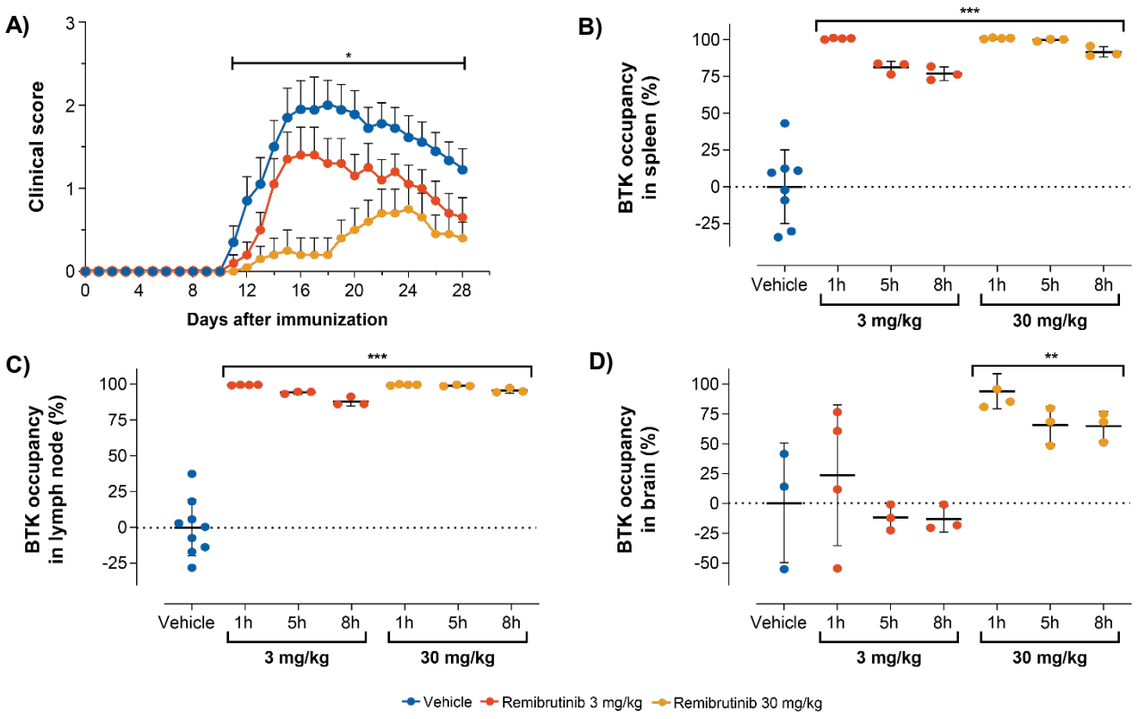
In both models C57BL/6 mice were immunized with either HuMOG or RatMOG to induce EAE. EAE clinical features were scored daily. BTK occupancy levels in spleen, lymph nodes and brain were determined with immunoassays for free BTK and total BTK protein[3]. Additional assessments were for *ex vivo* T-cell recall proliferative response, serum anti-MOG antibody response and serum neurofilament light chain (NfL) levels. Brain and spinal cord mRNA expression were analyzed 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 1A)**
- BTK occupancy measured at early timepoints of dosing at the end of the study was near-maximal in spleen and lymph nodes, and showed the expected decay due to BTK protein resynthesis **(Figure 1B and 1C)**
- Brain BTK occupancy was near-maximal for the 30 mg/kg dose, whereas it was marginal for 3 mg/kg dose **(Figure 1D)**

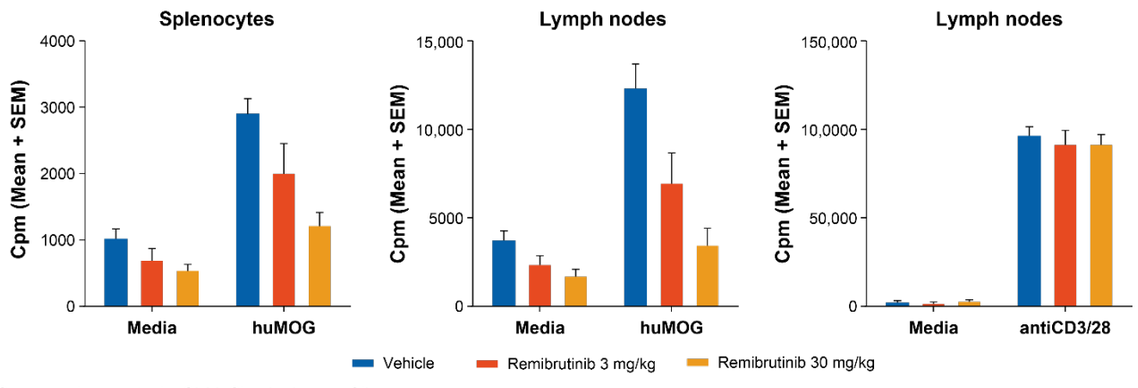
Figure 1. Efficacy and BTK occupancy in HuMOG EAE



ANOVA, Analysis of Variance; BTK, Bruton Tyrosine Kinase; MOG, 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 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



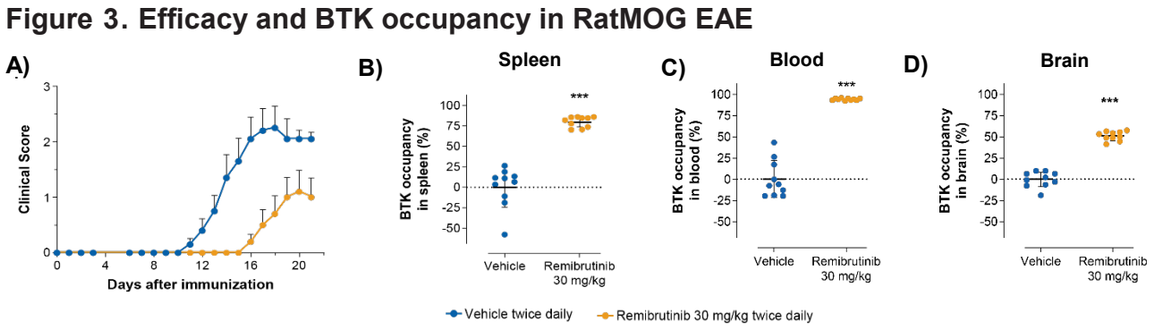
Cpm, counts per minute; SEM, Standard error of the mean

- *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 3A)**, suggesting that in absence of direct T-cell inhibition the efficacy in this RatMOG EAE model is mediated by myeloid cell and microglia inhibition
- In this study, BTK occupancy was measured 16 h after the last dose to assess minimal levels of BTK engagement and remibrutinib showed still notable levels of BTK occupancy in the spleen, blood and brain **(Figure 3B, 3C and 3D)**

Figure 3. Efficacy and BTK occupancy in RatMOG EAE



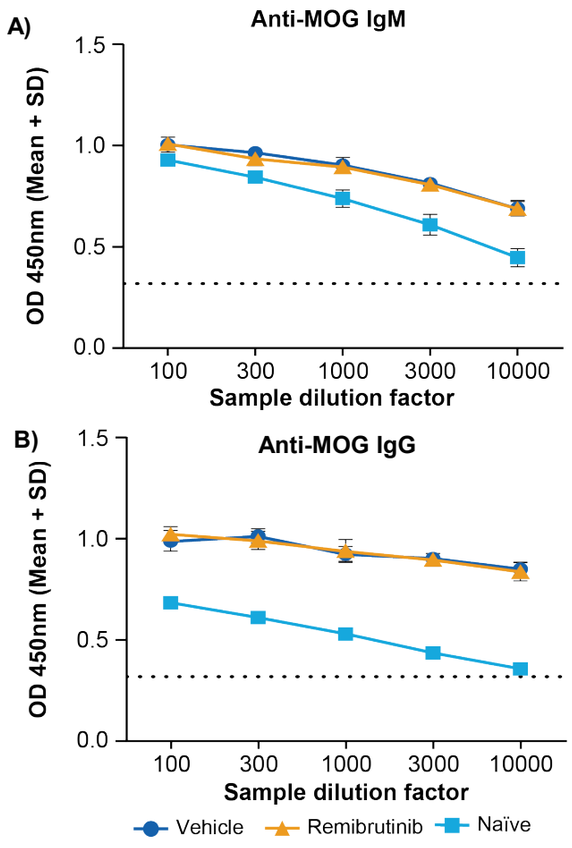
ANOVA, Analysis of Variance; BTK, Bruton Tyrosine Kinase; MOG, Myelin Oligodendrocyte Glycoprotein.
Statistical significance (ANOVA with Dunnett's test) is shown as *p < 0.05, **p < 0.01, ***p < 0.001.

- Remibrutinib treatment had no effect on the anti-MOG serum IgM **(Figure 4A)** and IgG levels **(Figure 4B)**
- Remibrutinib reduced serum NfL levels correlating with EAE clinical scores reduction **(Figure 5)**

Reduction of neuroinflammation gene signature in EAE microglia

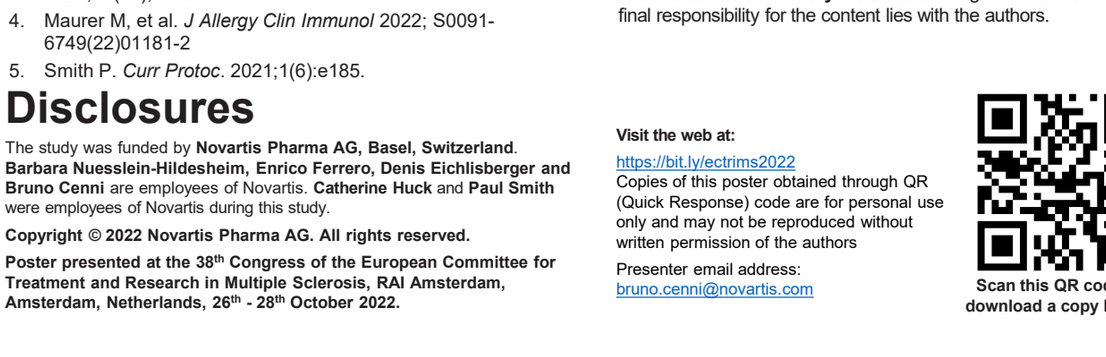
- Analysis of scRNA-seq data obtained from brains and spinal cords from a separate RatMOG EAE study revealed that remibrutinib significantly down-regulated multiple gene sets related to inflammation in microglia, suggesting it has an anti-inflammatory effect specifically in these cells **(Figure 6)**

Figure 4. RatMOG EAE antibody response



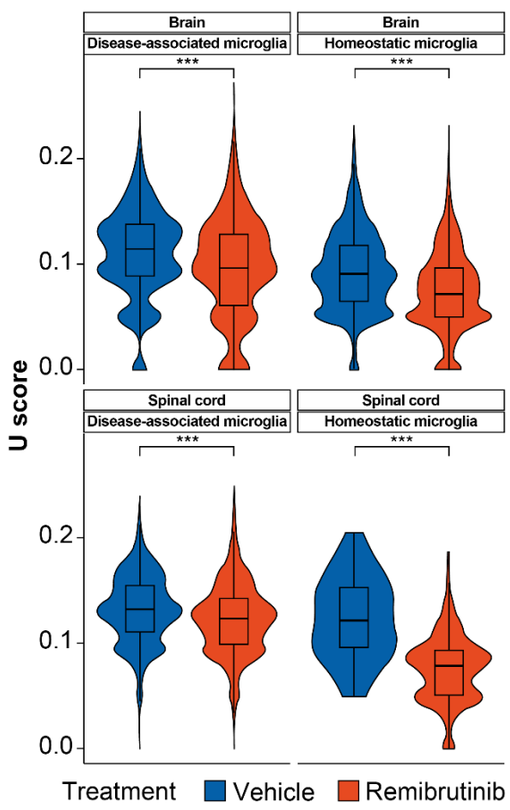
Ig, Immunoglobulin; MOG, myelin oligodendrocyte glycoprotein; OD, optical density, SD, standard deviation

Figure 5. NfL concentration in serum



NfL, neurofilament light chain

Figure 6. Remibrutinib reduces EAE microglia neuroinflammation gene expression



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
- In both models, remibrutinib efficacy was correlated with sustained and high BTK occupancy in peripheral tissues and brain, supporting the use of higher doses to maximize efficacy
- The efficacy of remibrutinib in these *in vivo* models, as well as its dual mode of action directed towards pathogenic B cells and microglia, support clinical development in chronic inflammatory diseases such as MS

References

1. Steinmaurer A, et al. *Curr Pharm Des*. 2021;27:1-8.
2. Kaul M, et al. *Clin Transl Sci*. 2021;14(5):1756-1768.
3. Angst D, et al. *Journal of Medicinal Chemistry*. 2020;63(10),5102-5118.
4. Maurer M, et al. *J Allergy Clin Immunol* 2022; S0091-6749(22)01181-2
5. Smith P. *Curr Protoc*. 2021;1(6):e185.

Acknowledgments

The authors acknowledge the following Novartis employees: **Vaishnavi Khandal** and **Sreelatha Komatireddy** for medical writing assistance and coordinating author reviews, and **Madhavi Kanithi** and **Mantosh Roy for** creative design assistance. The final responsibility for the content lies with the authors.

Disclosures

The study was funded by **Novartis Pharma AG, Basel, Switzerland**. **Barbara Nuesslein-Hildesheim, Enrico Ferrero, Denis Eichlisberger and Bruno Cenni** are employees of Novartis. **Catherine Huck** and **Paul Smith** were employees of Novartis during this study.

Poster presented at the 38th Congress of the European Committee for Treatment and Research in Multiple Sclerosis, RAI Amsterdam, Amsterdam, Netherlands, 26th - 28th October 2022.

Visit the web at:

https://bit.ly/ectrims2022

Presenter email address:
bruno.cenni@novartis.com

Scan this QR code to download a copy Poster