The role of human and mouse BTK in myeloid cells.

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Disclosures:

Roland Grenningloh was an **employee of EMD Serono** at the time of the study. Ursula Boschert, is an **employee of Ares Trading SA, Eysins, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany**.

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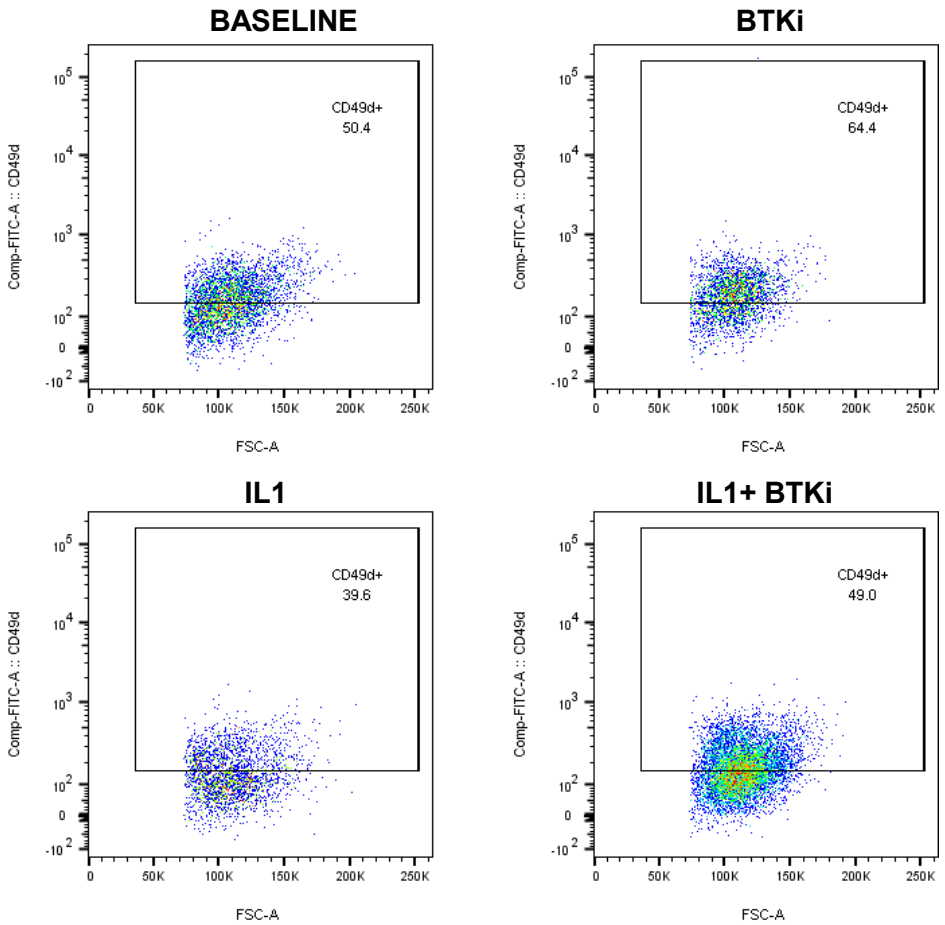
BACKGROUND:

Bruton's tyrosine kinase (BTK) is a member of the TEC family of non-receptor tyrosine kinases expressed in cells of hematopoietic origin, including B lymphocytes and myeloid cells, but not in T or NK cells. Selective BTK inhibitors (BTKi) have shown efficacy in Phase 2 trials in multiple sclerosis (MS)

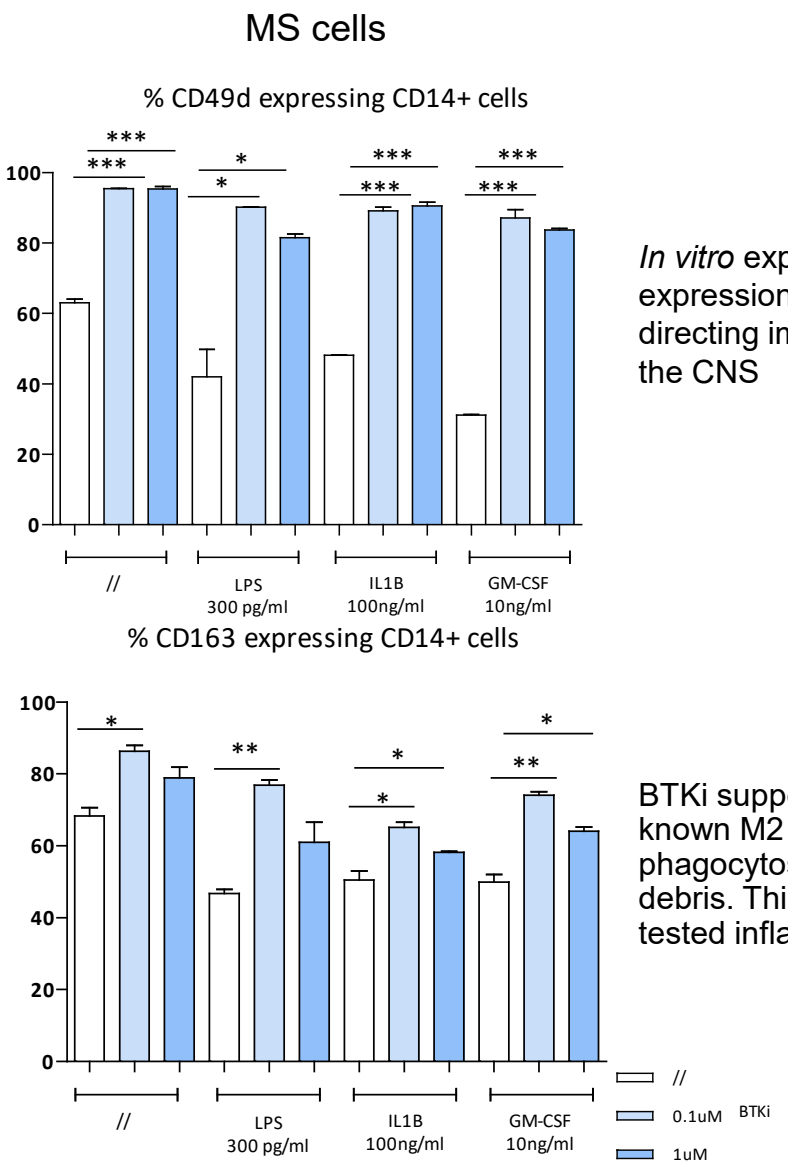
OBJECTIVE:

Here we aimed to investigate the role of BTK in human and mouse myeloid cells by *in vitro* and *in vivo* studies

1) BTKi supports high expression of CD49d and CD163 on MS and control monocytes



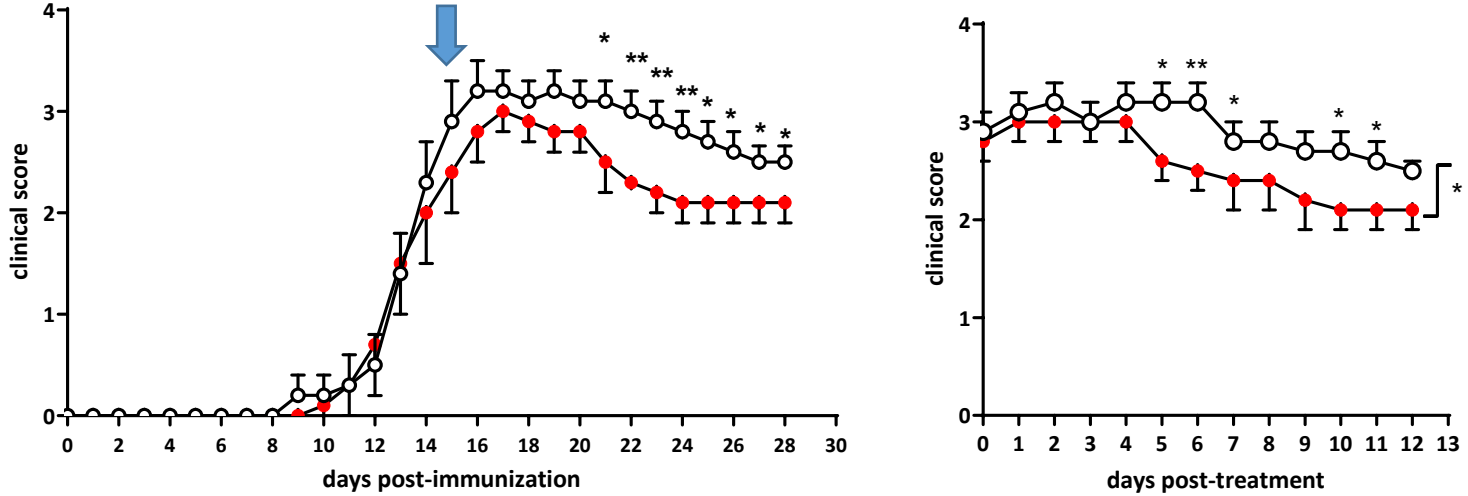
Peripheral blood mononuclear cell isolation from healthy subjects and treatment-naive patients with RR-MS, 2h pre-incubation with BTKi (1μM MSC2406639B-2), addition of stimuli (300 pg/ml LPS, 100 ng/ml IL1beta, 10 ng/ml GM-CSF) for 18h, staining for surface markers on monocytes, flow cytometry acquisition and analysis



In vitro exposure to BTKi enhances the expression of VLA4/CD49d, an integrin directing immune cell migration towards the CNS

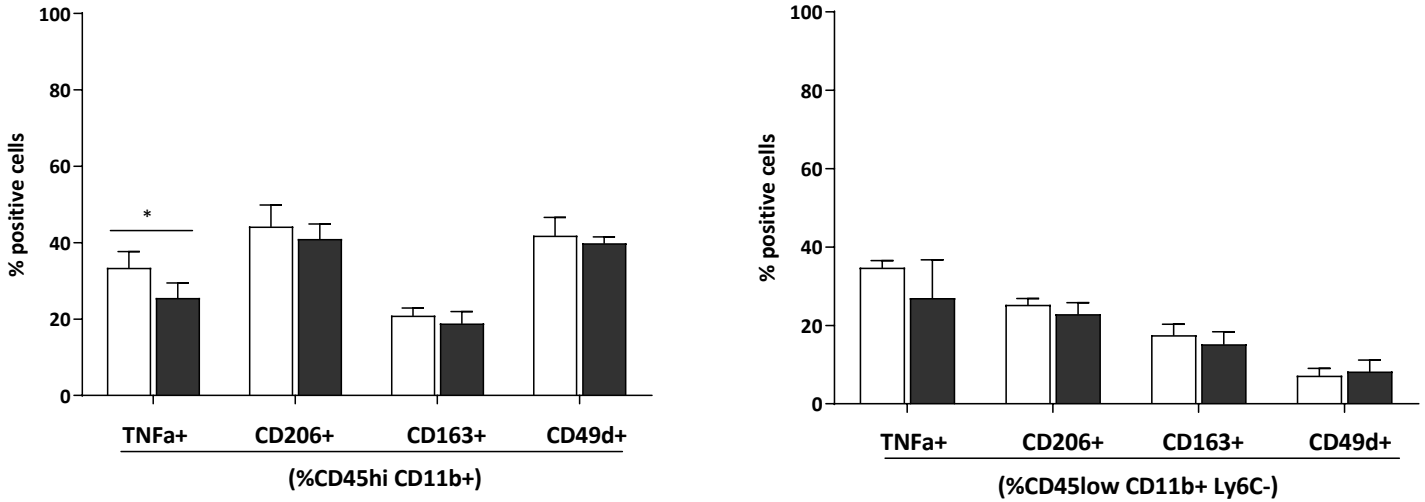
BTKi supports expression CD163, a well known M2 marker potentially involved in phagocytosis and removal of myelin debris. This effect is maintained under all tested inflammatory settings

2) Evobrutinib (10 mg/kg, oral) ameliorates EAE and reduces the frequency of CNS-infiltrating TNFα-producing myeloid cells



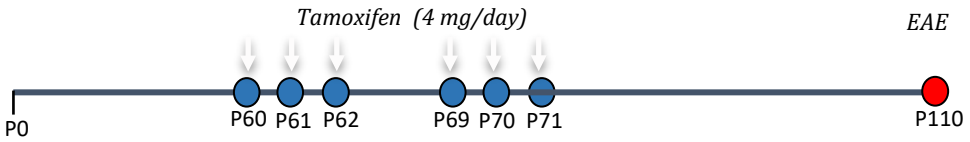
MOG_{35-55} peptide-experimental autoimmune encephalomyelitis (EAE) induction in C57BL6 mice, oral administration of 10 mg/kg/day evobrutinib starting the third day after EAE onset (arrow), clinical observation, sacrifice at day 28 for flow cytometry analysis of myeloid cells in the CNS

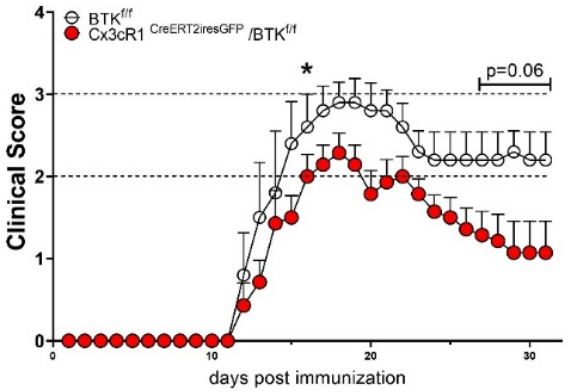
Clinical efficacy of evobrutinib is associated with a lower load of CNS-infiltrating inflammatory myeloid cells

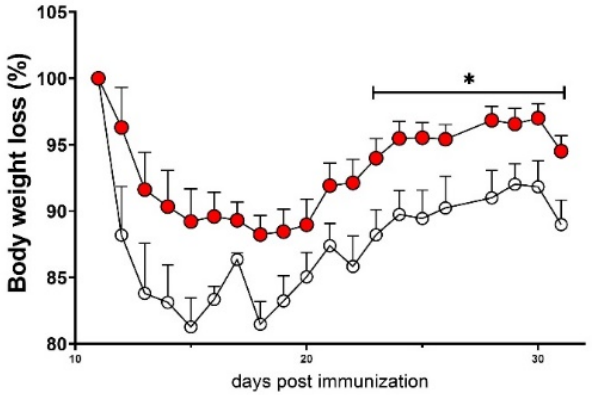


Evobrutinib reduces the fraction of TNFα-producing CD45 high CD11b+ CNS-infiltrating macrophages, while showing no effect on microglia (CD45 low CD11b+Ly6C- cells)

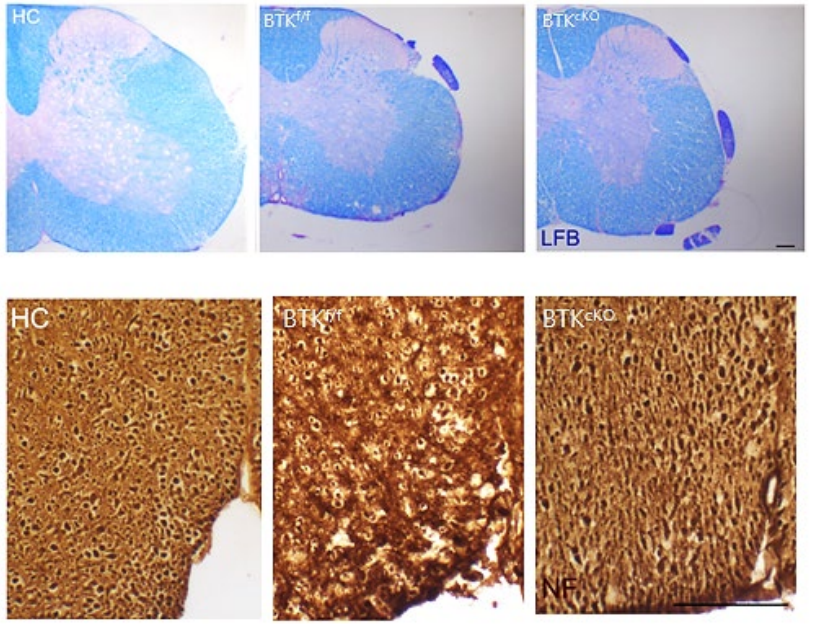
3) BTK deletion in Cx3CR1+ cells ameliorates EAE

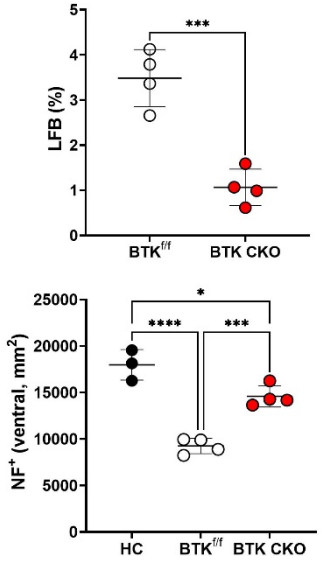






Conditional BTK inactivation was obtained crossing mice carrying a floxable BTK allele with mice carrying the Tamoxifen-inducible CreERT2 recombinase under Cx3CR1 promoter. Double transgenic and control mice received repeated tamoxifen (TAM) injections (4 mg/day, gavage) as shown in the schematic representation of the experiment. EAE was induced 38 days after the last TAM injection to allow the reconstitution of the circulating Cx3CR1+ cell population with BTK-expressing cells





After tamoxifen administration, circulating and tissue-resident Cx3CR1+ cells display distinct temporal requirements for their reconstitution. The pulse-chase tamoxifen protocol used in this experiment allows for reconstitution of wild-type circulating myeloid cells while maintaining BTK inactivation in Cx3CR1^{CreERT2}/BTK$^{f/f}$ tissue-resident, long living myeloid cells, including microglia. Such manipulation mitigates EAE progression. Percentages of demyelination measured by Luxol Fast Blue (LFB) staining were significantly reduced as well as we observed increased integrity of ventral spinal cord axonal pathways, labelled for Neurofilament (NF)

4) Conclusions

Overall, these experiments demonstrate that human and mouse myeloid cells may represent a cellular target for BTK inhibitors and that long-lived myeloid cells expressing BTK contribute to neuroinflammation. In particular, this study indicates that:

- BTKi supports expression CD163, a well known M2 marker potentially involved in phagocytosis and removal of myelin debris
- Evobrutinib injected EAE mice showed milder disease course
- Conditional BTK inactivation in Cx3cR1+ cells ameliorated EAE reducing demyelination and axonal damage