The role of human and mouse BTK in myeloid cells

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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).

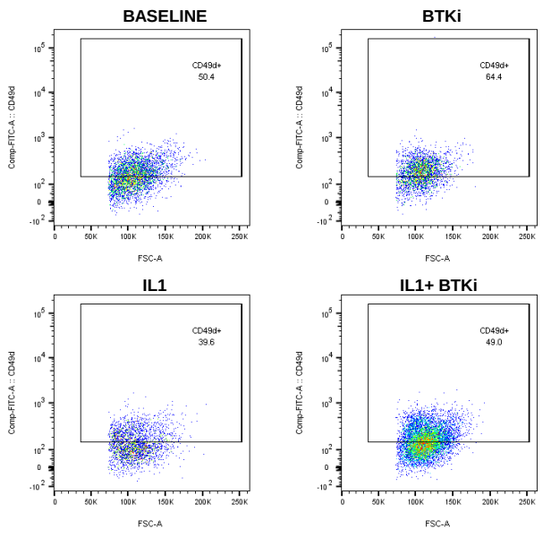
OBJECTIVE

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

The experimental system

Peripheral blood mononuclear cell isolation from healthy and/or treatment-naive MS subjects with the relapsing-remitting form of disease, 2h pre-incubation with BTKi (1μM MSC2406639B-2), addition of stimuli (300 pg/ml LPS, 100 ng/ml IL1, 10 ng/ml GM-CSF) for 18h, staining for surface markers on monocytes, flow cytometry acquisition and analysis.

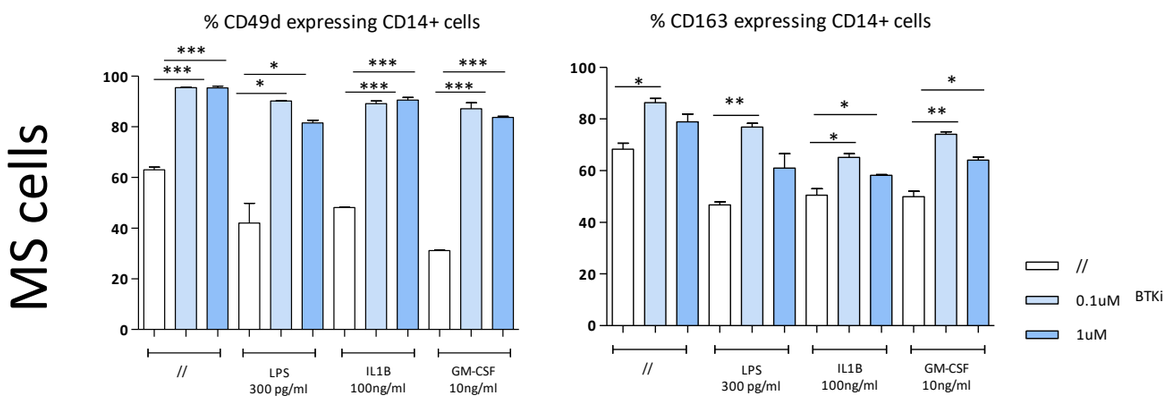


Representative stainings for CD49d/VLA4 on CD14-positive monocytes:

While IL1 downregulated CD49d, pre-exposure to BTKi counterbalanced this action, so that human monocytes maintained high levels of this marker even under inflammatory stress.

The Markers	The Functions	Effect of the compound
CD25	Activation	no effect
CD69	Activation	no effect
CD80	Activation/ Costimulation	↑ Baseline, ↑ GM-CSF
CD11a/LFA1 a chain	Adhesion	↓ Baseline, ↓ GM-CSF
CD11b/Mac1	Adhesion	↓ GM-CSF, ↑ LPS
CD49d/VLA4	Adhesion	↑ LPS, ↑ IL1, ↑ GM-CSF
CD163	Phagocytosis	↑ Baseline, ↑ LPS, ↑ IL1, ↑ GM-CSF

BTKi supports expression of VLA4/CD49d, an integrin directing immune cell migration towards the central nervous system, and CD163, a well known M2 marker potentially involved in removal of myelin debris. This effect is maintained under all tested inflammatory settings.

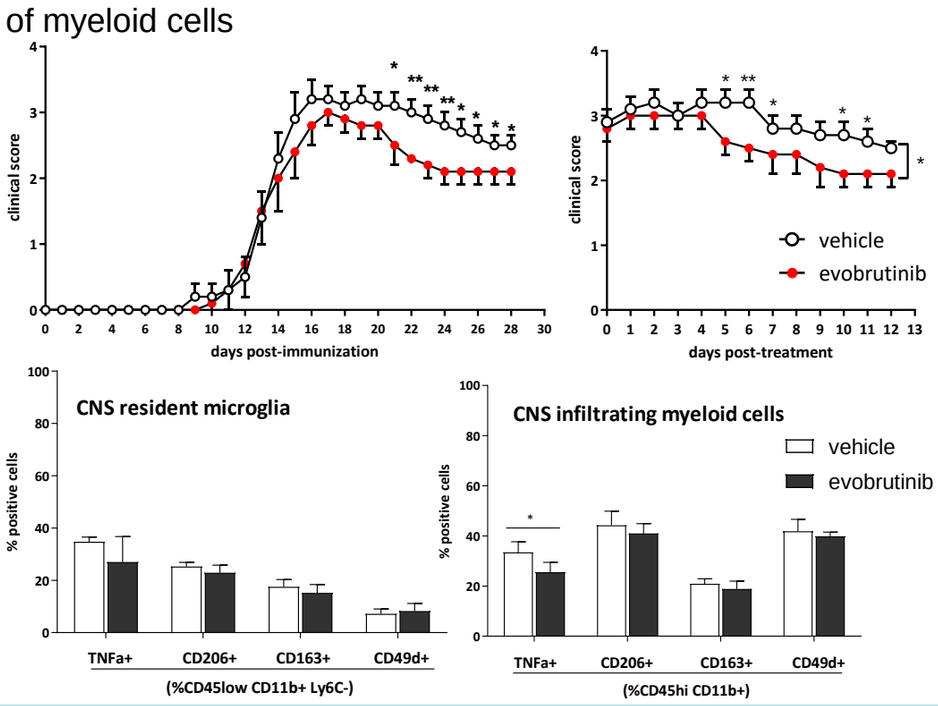


In vitro exposure to BTKi enhances the expression of CD49d and CD163 also in MS monocytes, suggesting higher propensity to CNS seeding and phagocytosis.

2 Oral administration of evobrutinib ameliorates EAE and reduces the frequency of CNS-infiltrating TNFa-producing myeloid cells

The experimental system

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, clinical observation, sacrifice at day 28 for flow cytometry analysis of myeloid cells

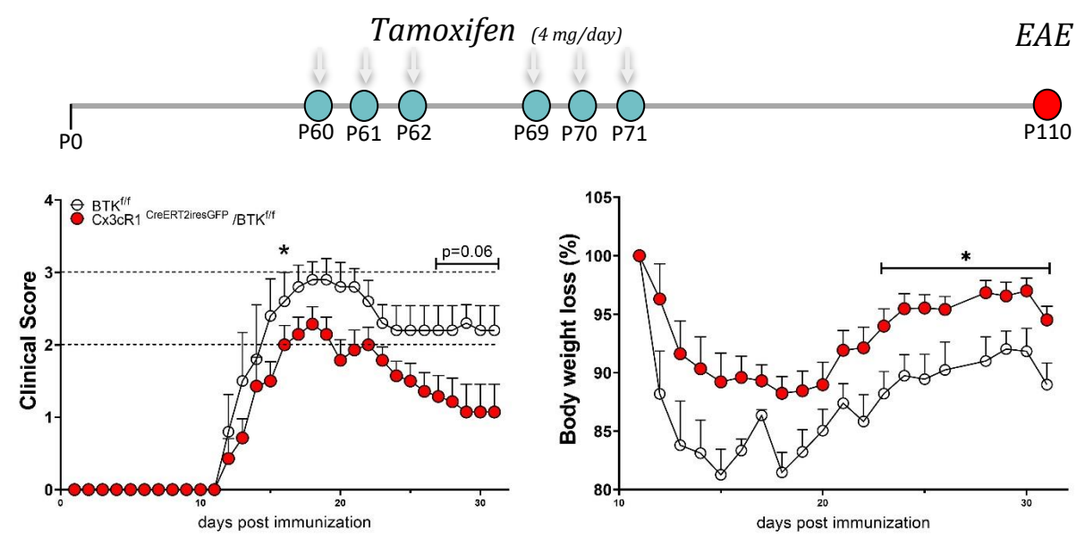


Clinical efficacy of evobrutinib is associated with a lower load of CNS-infiltrating inflammatory myeloid cells

3 BTK deletion in Cx3CR1⁺ cells ameliorates EAE

The experimental system

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).

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.

Disclosures:
Roland Grenningloh was an employee of EMD Serono Research & Development Institute Inc., USA, an affiliate of Merck KGaA, 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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