Evobrutinib, a highly selective BTK inhibitor, prevents antigen-activation of B cells and ameliorates experimental autoimmune encephalomyelitis

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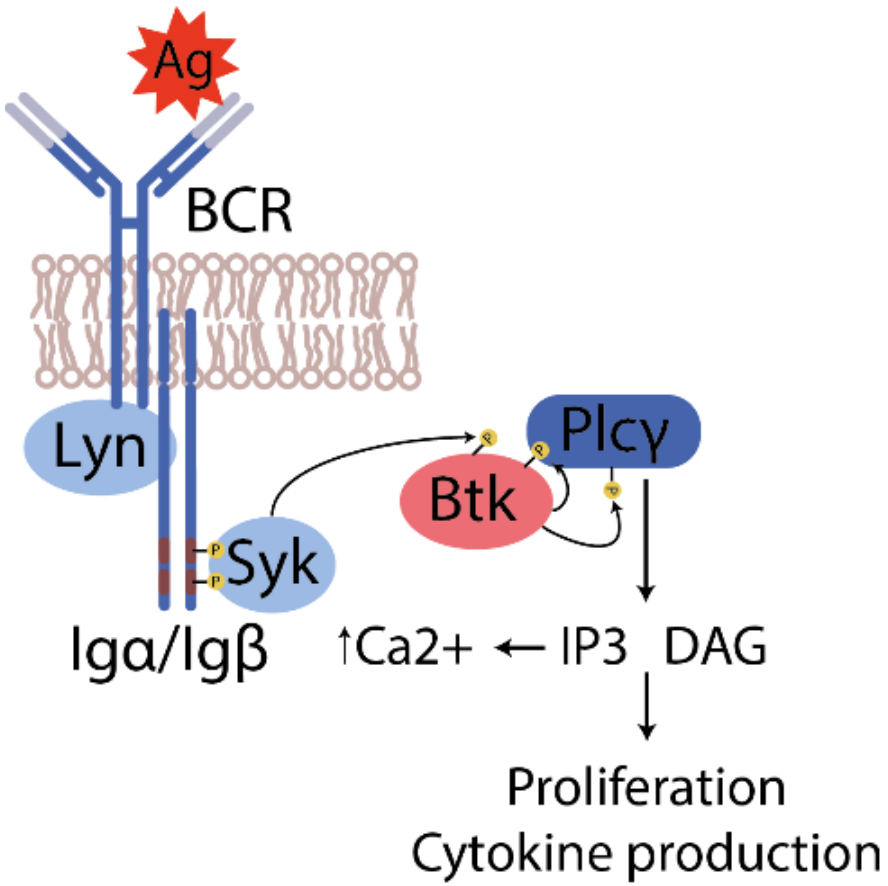
Disclosures

- Sebastian Torke: received travel support from EMD Serono.
- Roxanne Pretzsch: receives a medMS doctoral stipend from the Hertie foundation.
- Darius Häusler: Nothing to disclose.
- Roland Grenningloh: Employee of EMD Serono (an affiliate of Merck KGaA, Darmstadt, Germany).
- Ursula Boschert: Employee of Merck Serono (an affiliate of Merck KGaA, Darmstadt, Germany).
- Wolfgang Brück: Received honoraria for lectures by Bayer Vital, Biogen, Merck Serono, Teva Pharma, Genzyme, Sanofi-Aventis and Novartis. Member of scientific advisory boards for Teva Pharma, Biogen, Novartis, Celgene, Medday and Genzyme. Receives research support from Teva Pharma, Biogen, Medday, Genzyme and Novartis. Serves on the editorial boards of Neuropathology and Applied Neurobiology, Therapeutic Advances in Neurological Diseases and Multiple Sclerosis International.
- Martin S. Weber: Editor for PLoS One. Received travel funding and/or speaker honoraria from Biogen-Idec, EMD Serono, Novartis, Roche and Bayer. Receives research support from the National Multiple Sclerosis Society (NMSS; PP1660), the Deutsche Forschungsgemeinschaft (DFG; WE 3547/4-1), from Novartis, EMD Serono, TEVA, Biogen-Idec, Roche and the ProFutura Programm of the Universitätsmedizin Göttingen.
- This study was supported by EMD Serono SRDI, a business of Merck KGaA, Germany.

Background

- B cells are key mediators of inflammatory processes in multiple sclerosis (MS).
- Anti-CD20-mediated B-cell depletion effectively reduces acute MS flares.
- Recent data shows that antibody-mediated extinction of B cells harbours the risk of developing humoral deficiencies[1,2].
- A potentially more selective approach may be the therapeutic abrogation of pro-inflammatory B cell functions by Bruton´s tyrosine kinase (BTK) inhibition, affecting functional properties but without immunoglobulin depletion[3].
- BTK is centrally involved in B cell receptor (BCR) signaling and the subsequent activation and differentiation of B cells.
- The BTK inhibitor evobrutinib shows efficacy as a monotherapy in relapsing-remitting MS[4].
- The aim of this poster is to investigate B cell inhibition by evobrutinib in an animal model of MS and to evaluate BTK as a target in healthy human subjects.



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Methods

Daily treatment with the BTK inhibitor evobrutinib started 7 days prior to immunization with conformational myelin oligodendrocyte glycoprotein (MOG) 1-117 protein, a B cell-mediated model of experimental autoimmune encephalomyelitis (EAE).

EAE severity was assessed using a standard scale (0–5).

Flow cytometric analysis of the B cell maturation was performed 12 days post immunization.

Intracellular calcium flux was performed using the calcium-sensitive dyes Fluo-3 and Fura Red and aIgM or aCD3/aCD28 stimulation for B and T cells, respectively.

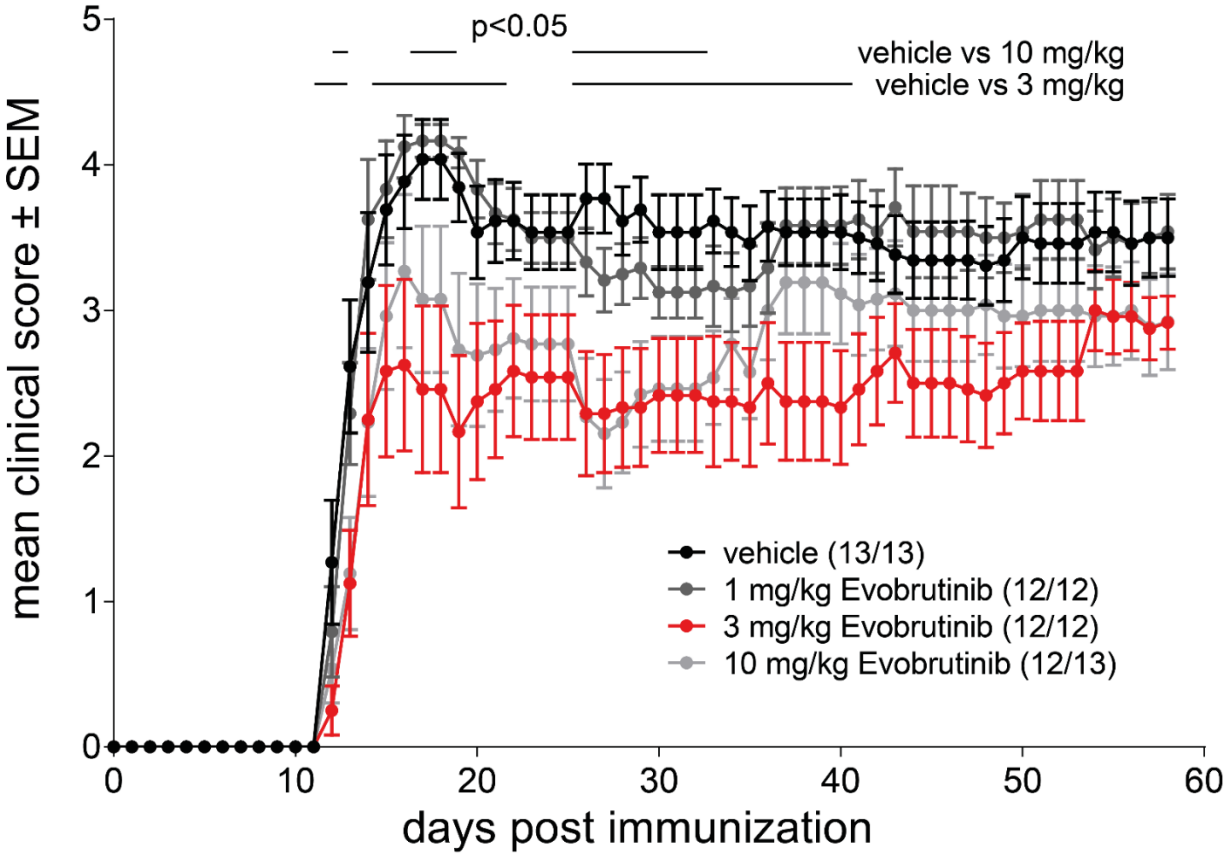
Evobrutinib treated B cells were co-cultured with naïve MOG-reactive 2D2 T cells for 72h and T cell proliferation and differentiation were assessed via CFSE dilution or positivity for IFNγ, IL17 or FoxP3.

Peripheral blood mononuclear cells (PBMCs) of healthy donors were directly used after preparation or thawed from frozen storage, stained for surface markers and stimulated using aIgM.

Evobrutinib ameliorates B cell-mediated EAE.

- Daily, oral treatment started 7 days prior to immunization with conformational MOG_{1-117} protein, a B cell-mediated animal model of MS.

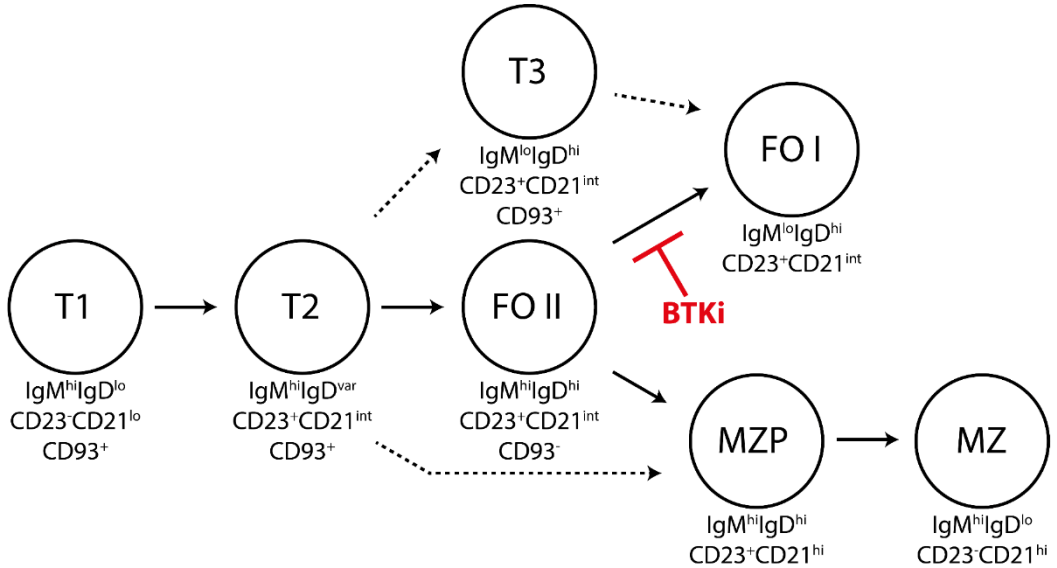
0 = no clinical signs
1 = tail paralysis
2 = righting reflex disturbance
3 = beginning hind limb paresis
4 = paralysis of both hind limbs
5 = moribund / death.

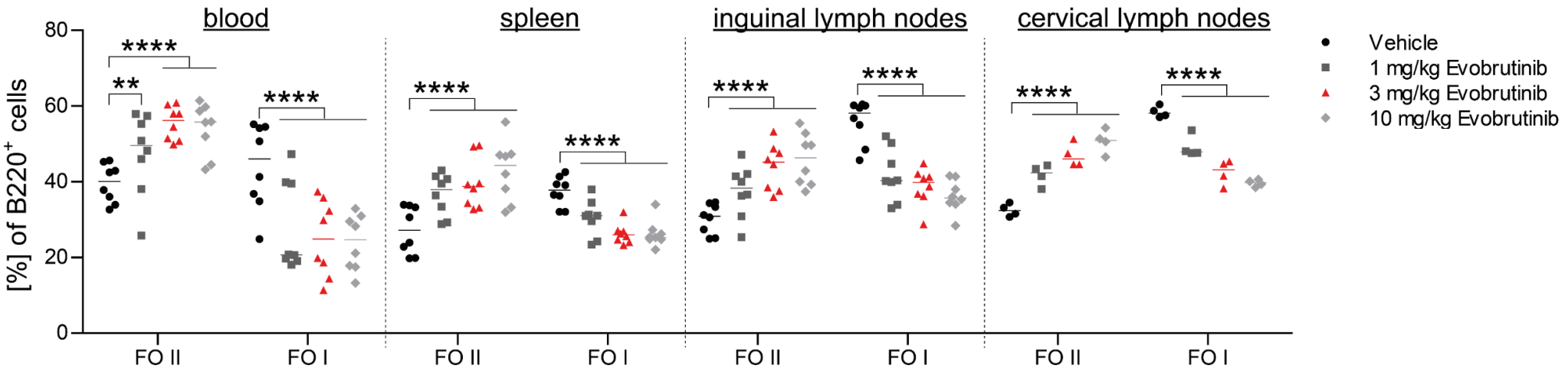


Evobrutinib blocks the differentiation of FO II to FO I B cells.

- B cell maturation was assessed 12 days after MOG protein immunization.

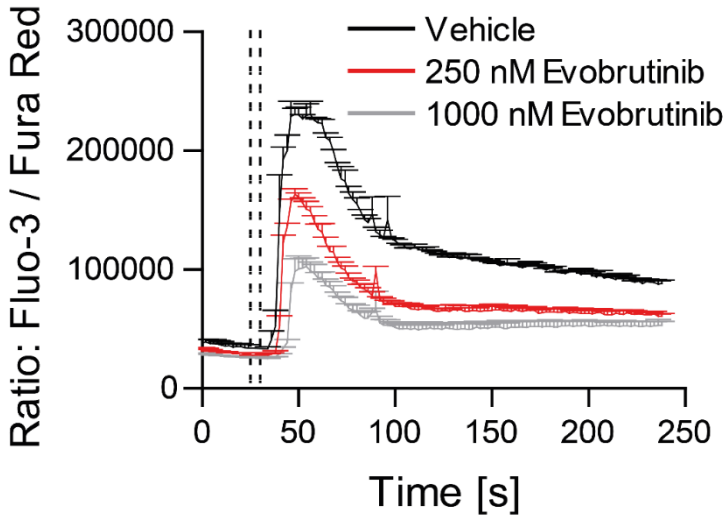
Transitional B cells (T1-T3)
Follicular B cells (FO)
Marginal zone precursor cells (MZP)
Marginal zone cells (MZ)

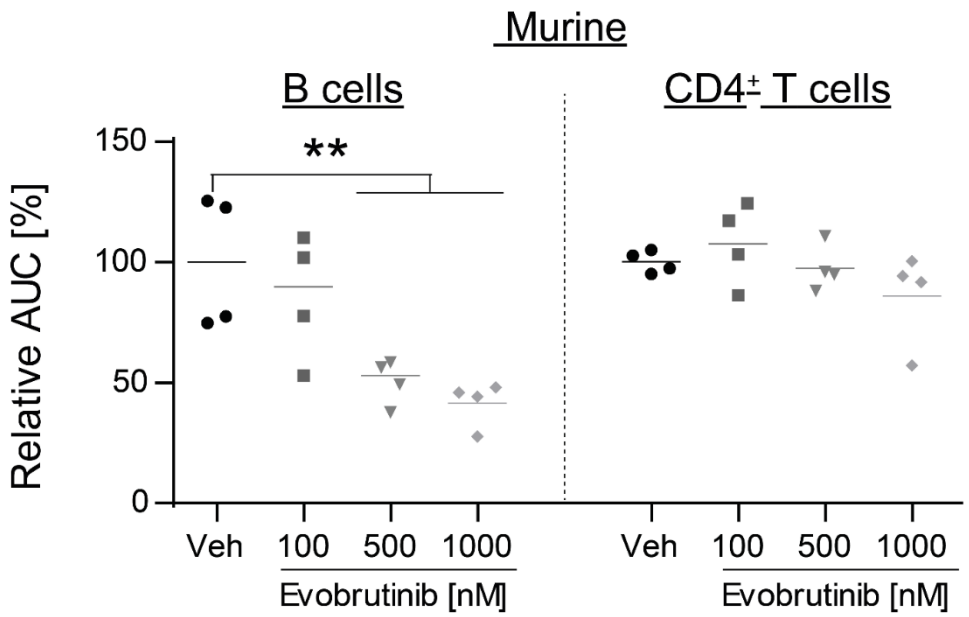


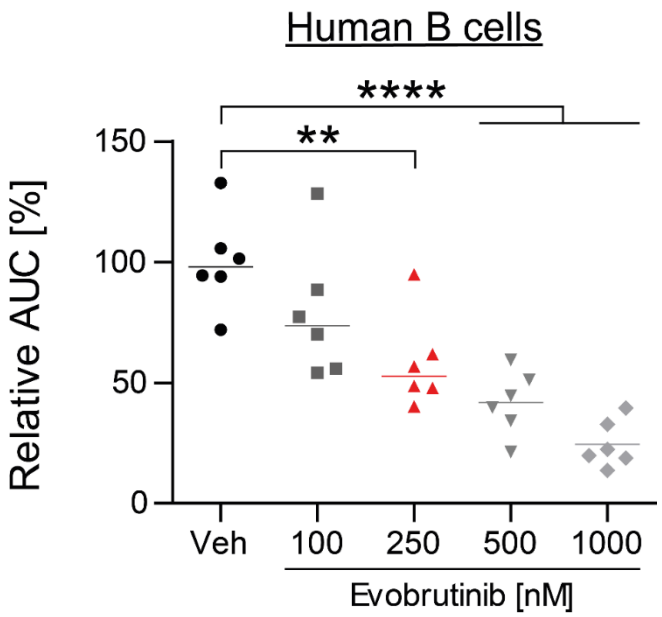


Evobrutinib inhibits calcium mobilization in murine and human B cells after BCR stimulation.

- Calcium flux analysis was performed on murine or human B cells using anti-IgM BCR stimulation and on T cells using CD3 / CD28 crosslinking and is displayed as area under the curve (AUC) relative to vehicle control.

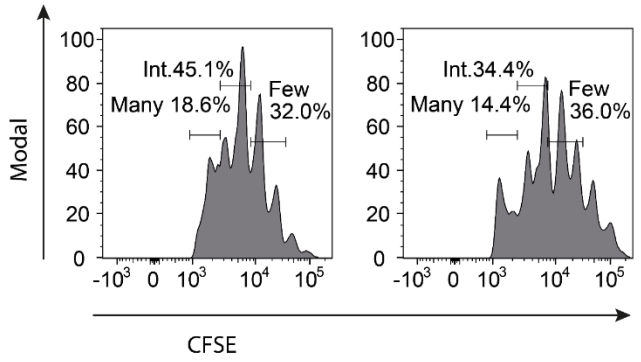


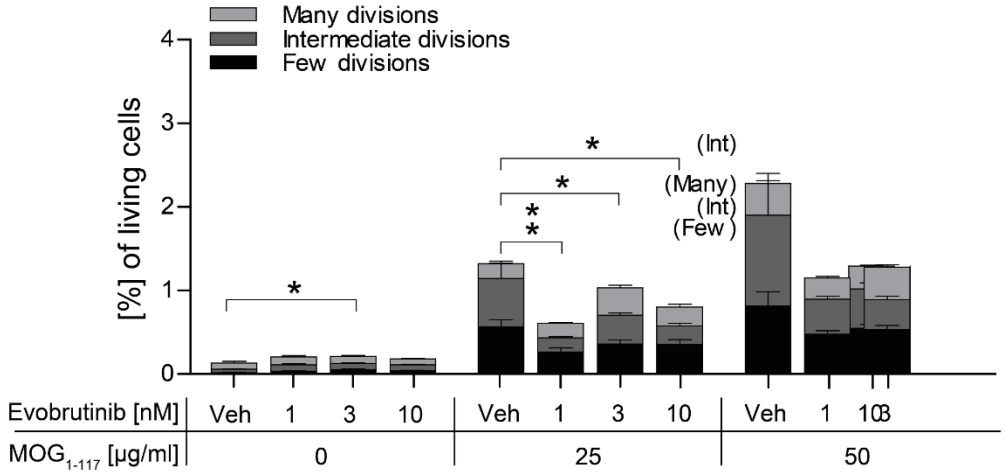


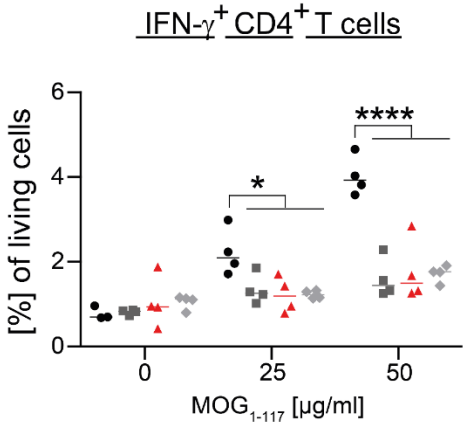


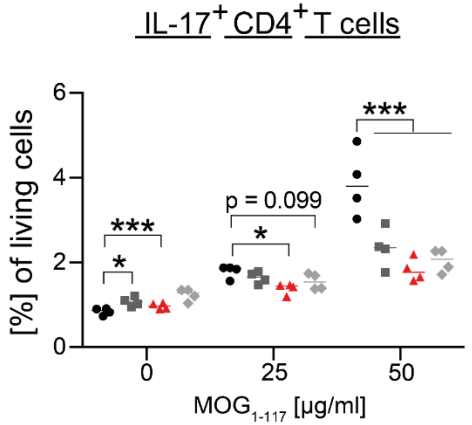
Evobrutinib inhibits B cell APC function and pro-inflammatory T cell cell differentiation.

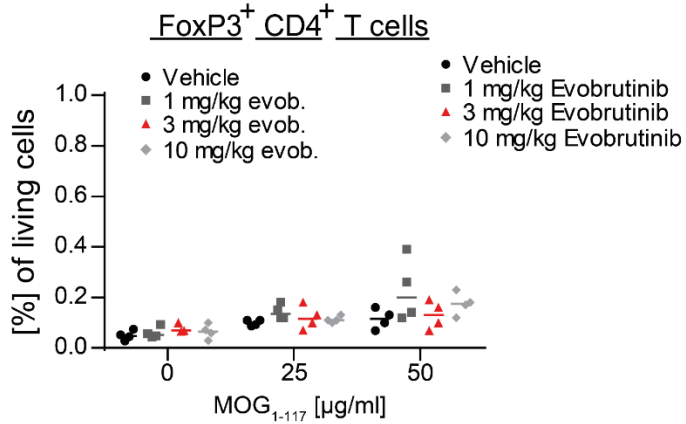
- Splenic B cells from evobrutinib-treated mice 12 days after MOG protein immunization or T cells from unimmunized 2D2 mice were isolated by magnetic separation and co-cultured for 72h.
- T cell proliferation was analyzed by CFSE dilution; T cell differentiation was analyzed by intra-cellular flow cytometry for the production of IFN-γ, IL-17 and FoxP3.









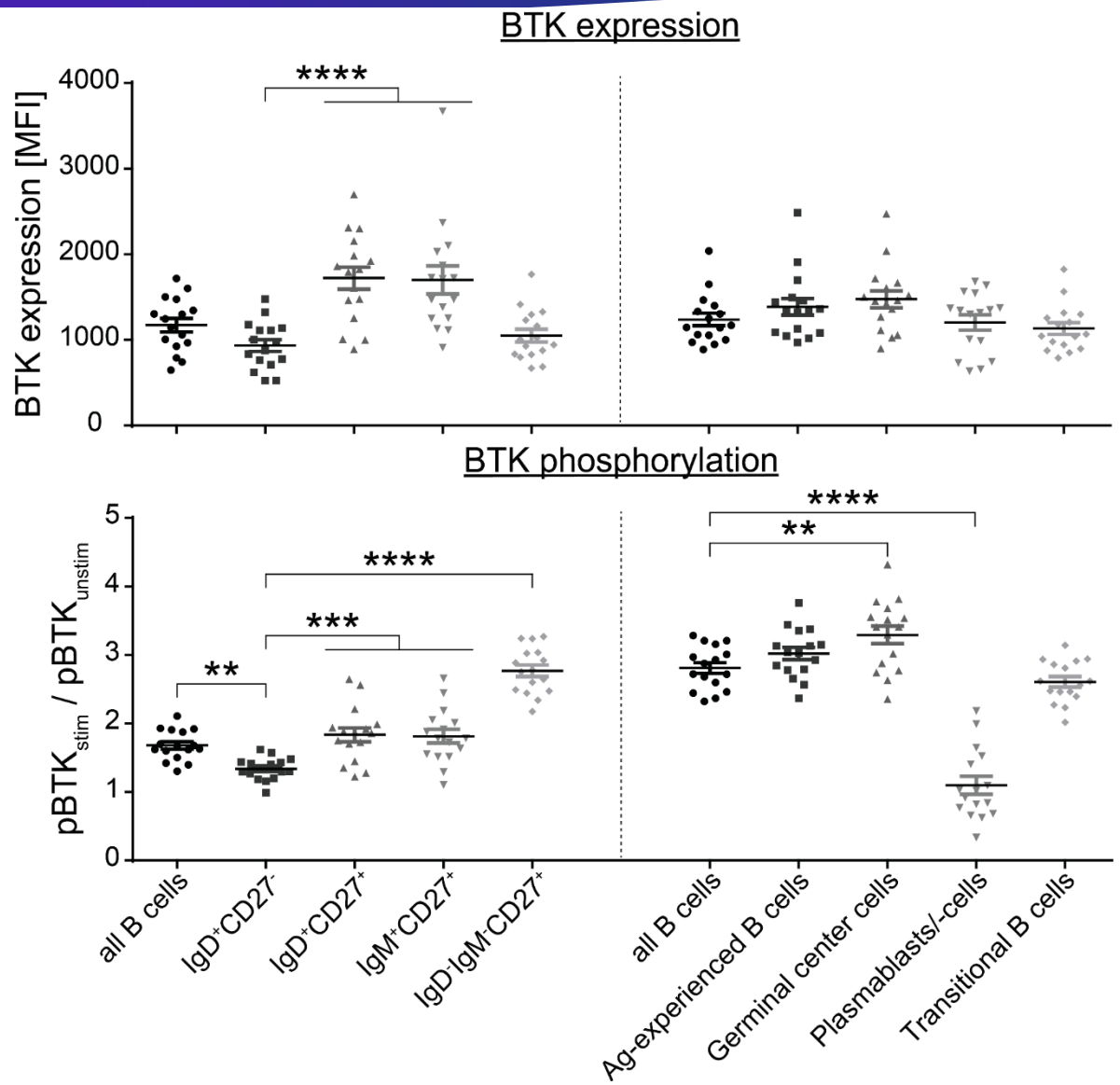


BTK expression and phosphorylation depend on B cell maturation and activation.

PBMCs from healthy individuals were thawed from -80°C storage, stained for surface markers using 2 different panels (left and right side) and stimulated for 30s using anti-IgM. After immediate fixation and permeabilization, intra-cellular antibodies for BTK and pBTK were incubated for 1h.

- BTK expression is increased in activated B cells ($CD27^+$).
- BTK phosphorylation is increased in activated B cells and strongest in fully class-switched B cells ($IgD^-IgM^-CD27^+$).

Naïve B cells (IgD^+CD27^-)
Activated B cells (IgD^+CD27^+ or IgM^+CD27^+)
Fully class-switched B cells ($IgD^-IgM^-CD27^+$)



Conclusion and Outlook

Evobrutinib

- Impairs the influx of excitatory calcium in B cells upon BCR stimulation and prevents activation and maturation of B cells.
- Diminishes B-cellular cytokine production and antigen-presentation, impairing the development of encephalitogenic T cells.
- Ameliorates disease severity in a B cell-accentuated EAE model.

Taken together with the increased expression of BTK and the enhanced inducibility of BTK phosphorylation in activated and matured human B cells, these findings highlight BTK as a promising new target in inflammatory CNS disease.

We are currently investigating the potential of evobrutinib to control pathogenic activity of reappearing B cells in a sequential therapeutic approach after pan B cell depletion.