T-BET+ B-CELL DEVELOPMENT IN MS: ASSOCIATION WITH BRUTON'S TYROSINE KINASE ACTIVITY AND TARGETING BY EVOBRUTINIB

Liza Rijvers (1), Marie-José Melief (1), Jamie van Langelaar (1), Annet F. Wierenga-Wolf (1), S. Marieke van Ham (2), Ursula Boschert (3), Roland Grenningloh (4), Joost Smolders (1, 5), Marvin M. van Luijn (1)

(1) Department of Immunology, MS Center ErasMS, Erasmus MC, Rotterdam, The Netherlands

(2) Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

(3) Ares Trading SA, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany

(4) EMD Serono Research & Development Institute, Inc., Billerica, Massachusetts, USA; a business of Merck KGaA, Darmstadt, Germany
(5) Department of Neurology, MS Center ErasMS, Erasmus MC, Rotterdam, The Netherlands

Disclosure

EMD Serono Research & Development Institute, a business of Merck KGaA

- *Research funding*
- *Inhibitory compound*

Background

- B-cell depletion is an efficacious treatment in both relapsing and progressive MS

  Hauser, S.L. et al N Engl J Med 2008 Feb;14;358(7):676–88.

  Montalban, X. et al. N Engl J Med 2017 Jan;19;376(3):221–34

  Hauser, S.L. et al N Engl J Med 2017 Jan;19;376(3):221–34

- Bruton's tyrosine kinase (BTK) inhibitor evobrutinib reduces disease activity in MS patients (phase II trial, NCT02975349)

  Montalban, X. et al. N Engl J Med. 2019 Jun;380(25):2406-2417

- T-bet(CXCR3)$^{+}$ B cells preferentially infiltrate the CNS of MS patients and are induced under IFN-γ- and TLR9-stimulating, germinal center-like conditions, such as those found in leptomeningeal infiltrates

  van Langelaar, J. and Rijvers, L. et al. Ann Neurol. 2019 Aug;86(2):264-278

Aim 1

To study BTK levels and activation in distinct B cell subsets, comparing different types of MS

Methods 1

Ex vivo screen BTK and phosphorylated BTK (pBTK) levels

- Blood B cells, 13-color FACS
  - Subsets: Transitional, naive mature, class-switched and non class-switched B cells
  - Conditions: Unstimulated and α-IgM stimulated B cells
- Clinical subgroups
  - Age- and gender matched HC (n=30)
  - CIS (n=30), RRMS (n=30), SPMS (n=15) and PPMS (n=15) → all treatment-naive

BTK is more activated in B cells from RRMS and SPMS patients

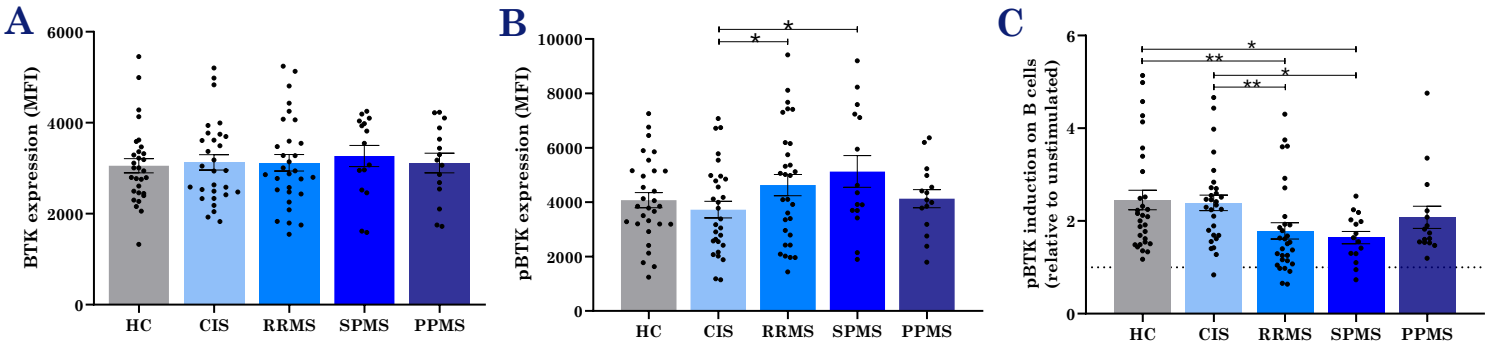


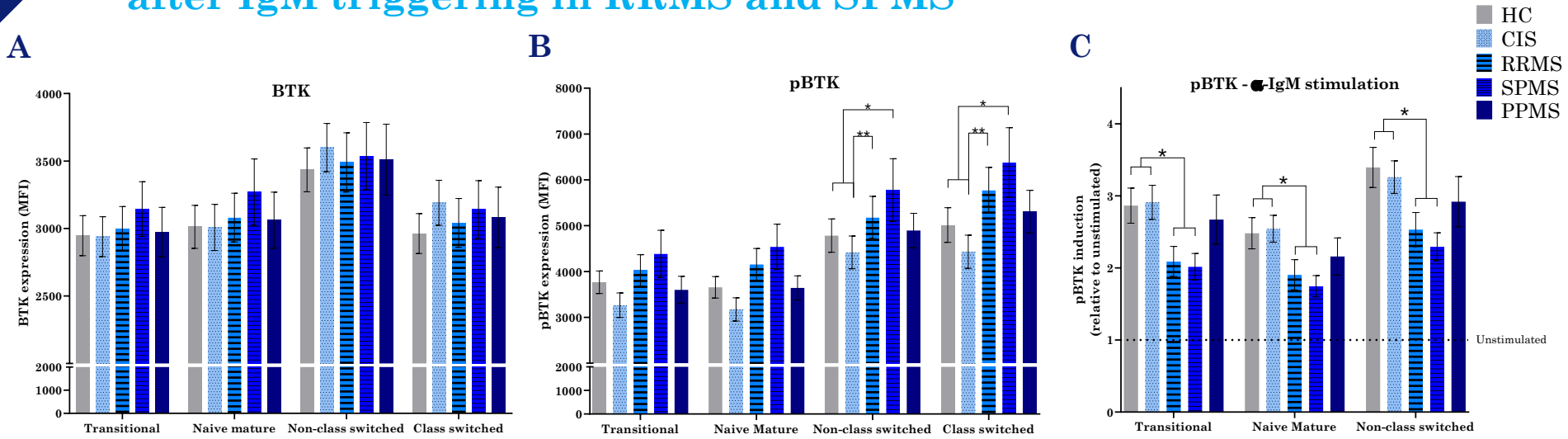
***Figure 1**: BTK and pBTK levels were measured for ex vivo unstimulated (**A-B**) and a-IgM stimulated (5 min; **C**) B cells by flow cytometry. In **C** data are presented as fold induction, relative to un-stimulated pBTK levels (dotted line). In **A-C**, untreated HC (n=30), CIS (n=30), RRMS (n=30), SPMS (n=15) and PPMS (n=15) patients were analyzed.*

A: BTK levels are not elevated in B cells from MS patients

B: BTK activation, measured as Y223 phosphorylation, is elevated in B cells from RRMS and SPMS patients at baseline

C: B cells of all cohorts activate BTK in response to BCR triggering. However, the higher baseline BTK activation in RRMS and SPMS patients leads to a lower stimulatory index

pBTK levels are the highest in memory B cells and less induced after IgM triggering in RRMS and SPMS



A: BTK levels are the highest in non-class switched memory B cells

B: BTK activation, measured as Y223 phosphorylation, is the highest in both non-class switched and class switched memory B cells, and is increased in RRMS and SPMS patients at baseline

C: The higher baseline BTK activation in RRMS and SPMS patients leads to a lower stimulatory index in all IgM+ B cell subsets

Figure 2: *BTK and pBTK levels were measured for ex vivo unstimulated (**A-B**) and α-IgM stimulated (5min; **C**) blood B cells of HC, CIS, RRMS, SPMS and PPMS patients. Transitional ($CD38^{hi}CD27^{-}$), naive mature ($CD38^{dim}IgM^{+}$ $CD27^{-}$), non class-switched ($IgM^{+}CD27^{+}$) and class-switched ($IgM^{-}CD27^{+}$) B cells were analyzed. In **C**, data are presented as fold induction relative to unstimulated pBTK levels (dotted line).*

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Correlation of pBTK with CXCR3 and VLA-4 surface levels in *ex vivo* blood B cells

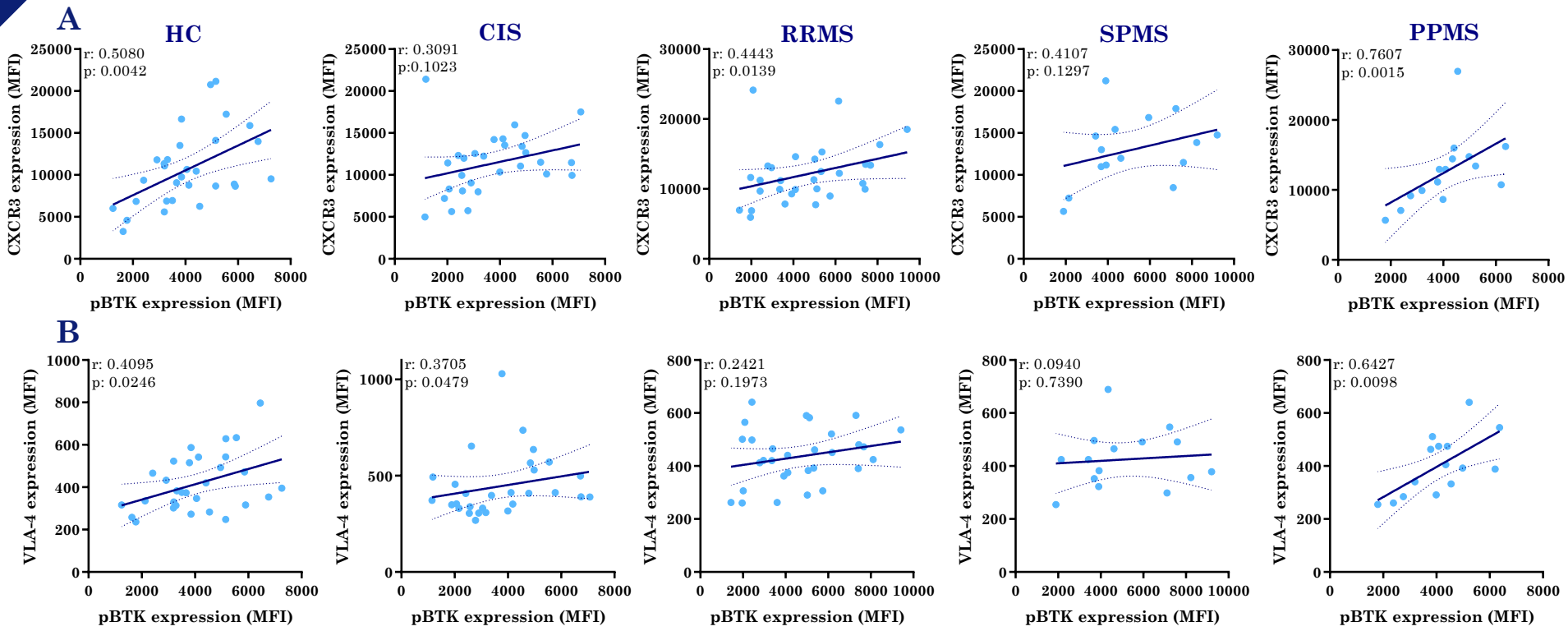

Figure 3: *Levels of pBTK, CXCR3* ***(A)*** *and VLA-4* ***(B)*** *levels were measured for ex vivo unstimulated B cells of HC, CIS, RRMS, SPMS and PPMS patients by flow cytometry. Linear regression was plotted with 95% confidence intervals. Pearson's correlation coefficients were determined.*

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Aim 2

To study the relation between BTK activity and the development of MS brain-infiltrating T-bet^{+} B-cells

Methods 2

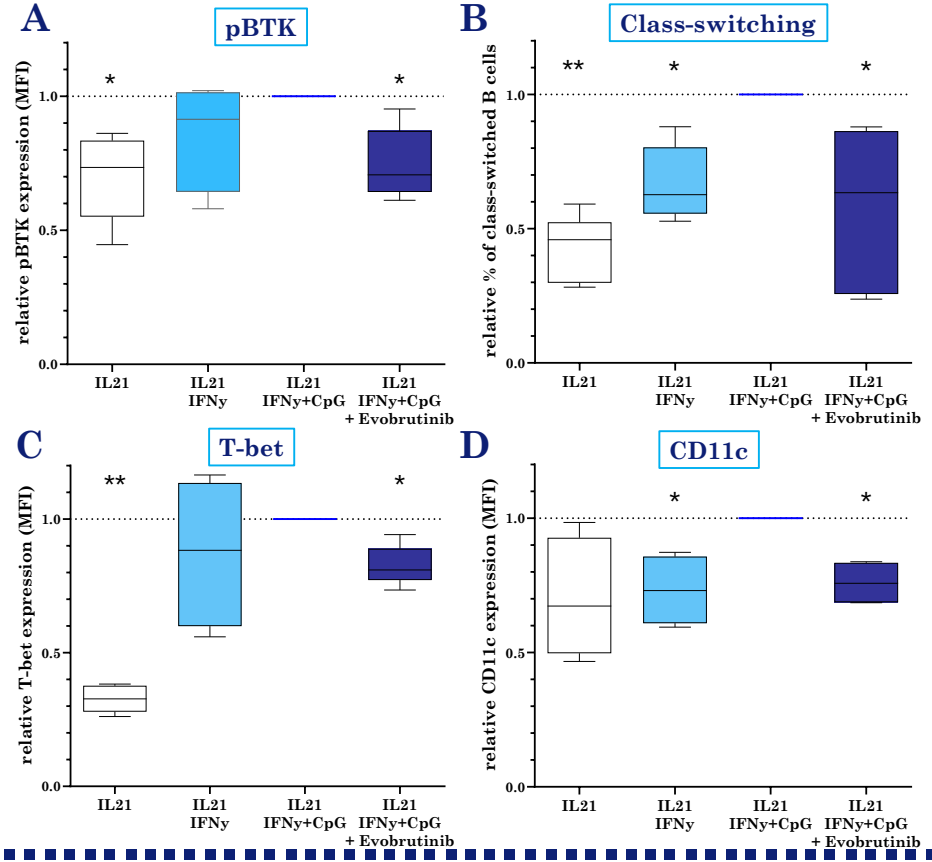
Germinal center-like i*n vitro* B-cell differentiation

- Sorted naive mature (CD38^{dim}CD27^{-}) and memory (CD27^{+}) B cells
- CD40L- expressing 3T3 cells + recombinant IL-21
  - Recombinant IFN-γ and TLR9 ligand CpG
- BTK inhibitor evobrutinib (1µM)

Readouts:

- Class switching and plasmablast formation (FACS)
- IgG and IgM secretion in supernatants (ELISA)

Evobrutinib inhibits both IFN-γ- and CpG-induced pBTK expression and T-bet+ memory B cell differentiation



A: Evobrutinib inhibits IFN-γ- and CpG-induced pBTK expression

B: CpG-induced class-switching of naive mature B cells is inhibited by evobrutinib

C-E: Evobrutinib interferes with the differentiation of T-bet+CD11c+CD21low pathogenic memory B cells

This argues that BTK inhibition interferes with CpG/TLR9 signaling in IFN-γ-induced B cells

Figure 4: *Sorted naive mature B cells (CD38dimCD27-) were cultured in germinal center-like conditions for 3 (A) or 11 (B-D) days with IFN-γ, and IFN-γ+CpG (TLR9), with and without evobrutinib. pBTK expression* ***(A)*** *as well as B cell class switching (CD27+IgM-)* ***(B),*** *T-bet* ***(C)*** *and CD11c* ***(D)*** *expression was analyzed by FACS. Data are normalized to the IL21+IFN-γ+CpG condition in each donor (dotted line).*

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In vitro germinal center-like differentiation of memory B cells into IgG-secreting cells is attenuated by evobrutinib

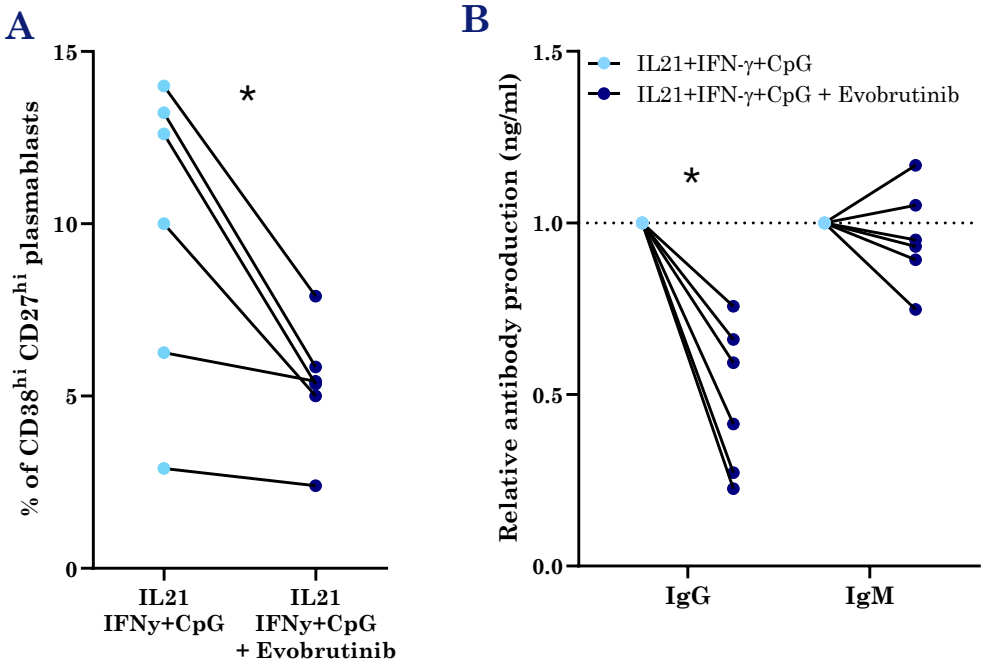


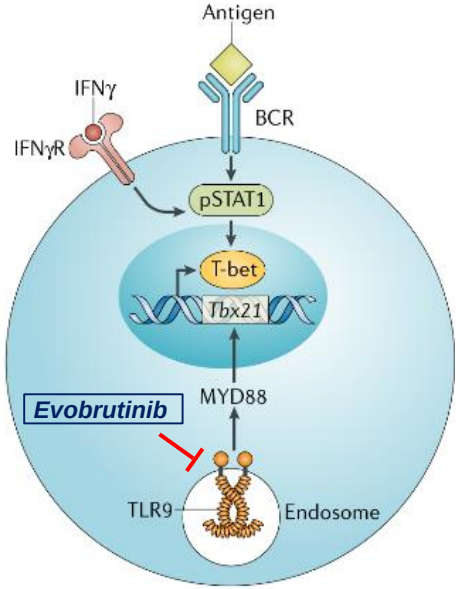
Figure 5: *Sorted memory B cells ($CD38^{dim}CD27^{+}$) were cultured under germinal center-like conditions for 6 days with IFN-γ, and IFN-γ+CpG (TLR9), with and without evobrutinib. B cell differentiation into IgM- or IgG-secreting plasmablasts (**A**; FACS; **B**; ELISA) was analyzed. Data are normalized to the IL21+IFN-γ+CpG condition for each donor (dotted line).*

A: IFN-γ+CpG induced plasmablast differentiation is inhibited by evobrutinib

B: B cell class-switching towards IgG and subsequent antibody secretion is inhibited by evobrutinib

Conclusion

- Within various B cell populations, BTK activation is strongest in memory B cells
- BTK is more activated in B cells of RRMS and SPMS patients, which positively correlates to CXCR3 and VLA-4 surface expression
- Evobrutinib suppresses IFN-γ- and TLR9-driven class switching of T-bet^{+} memory B cells *in vitro* (see illustration)
- Class-switching and IgG-secreting plasma cell differentiation is attenuated by evobrutinib



Adapted from:
Pritchard, G.H. et al. Nat. Rev. Immunol. 2019;19;398–410

❖ This study provides new mechanistic insights into how evobrutinib intervenes in pathogenic human B-cell differentiation and can modulate the clinical course of MS