B cells infiltrating the multiple sclerosis brain: local maturation and targeting by evobrutinib

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Introduction & purpose

Background:

- In multiple sclerosis (MS) patients, peripheral EBV-infected B cells are probably triggered by IFN-γ producing T_{FH} cells to differentiate into CNS-directed T-bet$^+$ memory B cells [1,2,3,4].
- T-bet regulates IgG class switching and promotes CXCR3 expression, enabling these class-switched (CS) memory B cells to infiltrate the CNS [1,2,5,6,7].
- Within the CNS, B-cell clones are likely reactivated in meningeal tertiary lymphoid structures and white matter perivascular spaces [1,2,8,9].

Questions:

- How do CXCR3$^+$ B cells further evolve in the CNS of MS patients to contribute to local pathology?
- What is the impact of BTK inhibition on the effector program of this pathogenic B-cell subset?

Aim:

- To define B-cell maturation in the CNS of MS patients and obtain new insights into the mechanism of action of the BTK inhibitor evobrutinib

Methods

- *Ex vivo* flow cytometry was used to analyze the transition of B cells to antibody-secreting cells (ASCs) in postmortem blood, CSF, meninges and white matter from MS (n=28) and control (n=10) donors *(NBB)*.
- Laser capture microdissection was applied to obtain perilesional areas and rims of MS lesions (*NBB*; n=15) and IgG gene expression was determined using microarrays [10,11].
- IgG index and CSF unique oligoclonal bands (OCBs) were analyzed in MS donor samples using nephelometry, isoelectric focusing and immunoblotting.
- Immunofluorescence staining and confocal imaging were used to assess CD19, CD3 and CXCR5 expression in MS white matter lesions (*NBB*; n=6).

- *Ex vivo* flow cytometry was performed on blood from patients in the MS and control groups (n=15-30) to determine phosphorylated BTK levels.
- Human B-cell differentiation (under T_{FH}-like conditions) and migration through the blood-brain barrier were assessed using *in vitro* assays with/without evobrutinib.

Results: CXCR3$^+$ B-cell to ASC transition in the MS brain

Figure 1: Increased ASC (CD38^{high}CD27^{high}) versus B-cell ratios in the CNS of MS and not control donors

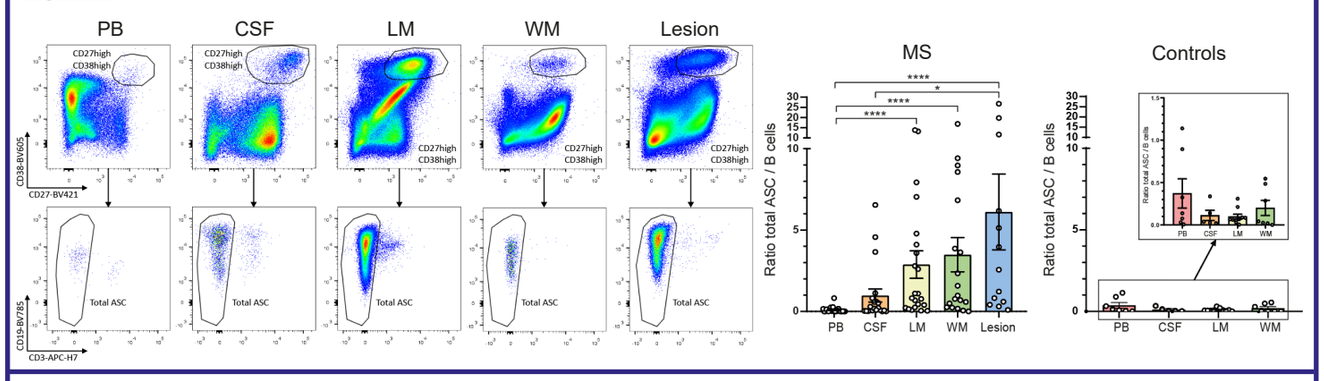


Figure 2: ASCs in the MS CNS correspond to both local IgG gene expression and intrathecal IgG production

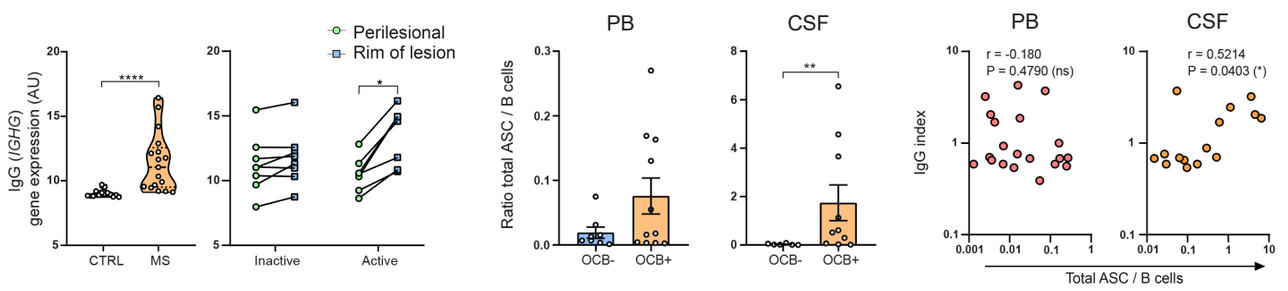


Figure 3: CXCR3 levels correlate with intrathecal IgG

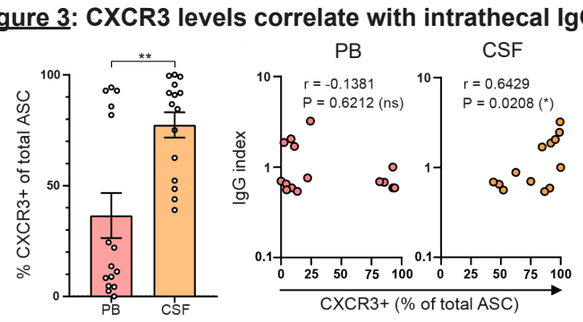


Figure 4: Lesional ASCs correlate with CD4$^+$ T cells

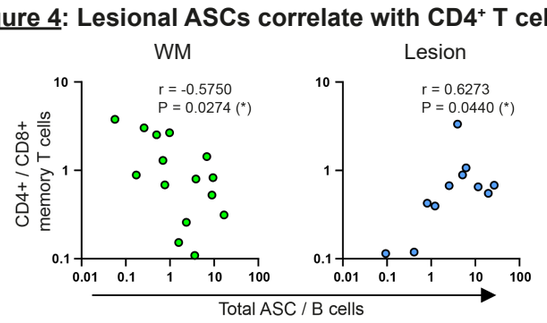
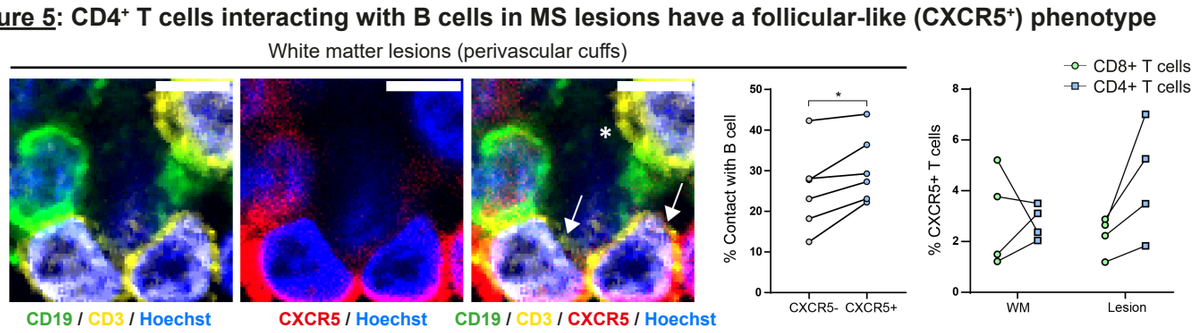


Figure 5: CD4$^+$ T cells interacting with B cells in MS lesions have a follicular-like (CXCR5$^+$) phenotype



Results: BTK inhibition in CXCR3$^+$ B cells by evobrutinib

Figure 6: pBTK is elevated in CS B cells in MS

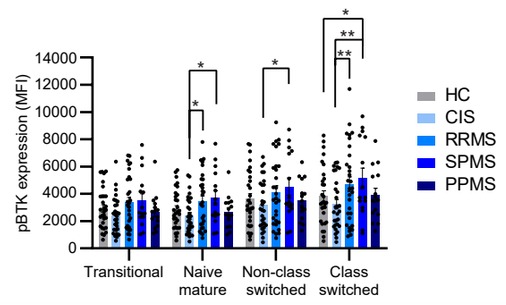


Figure 7: BTK activity correlates with CXCR3 levels

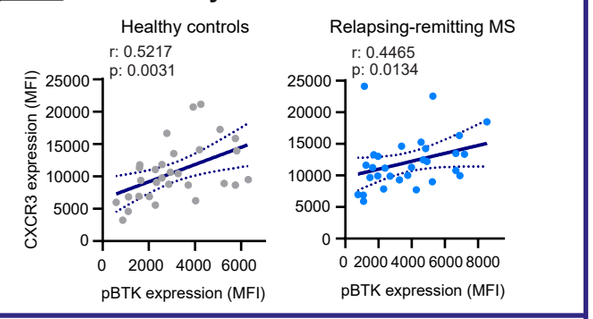


Figure 8: Evobrutinib inhibits differentiation of naive B cells to T-bet+ CS memory B cells *in vitro*

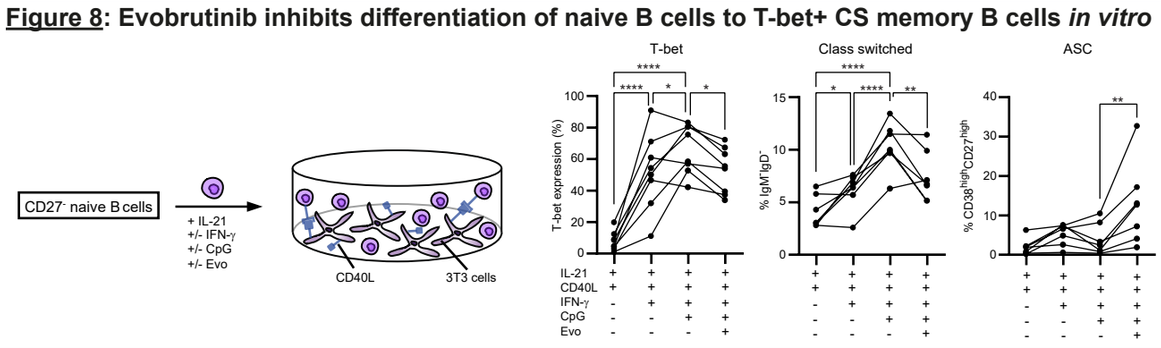


Figure 9: Evobrutinib affects CXCL10-mediated migration of CXCR3$^+$ class-switched B cells

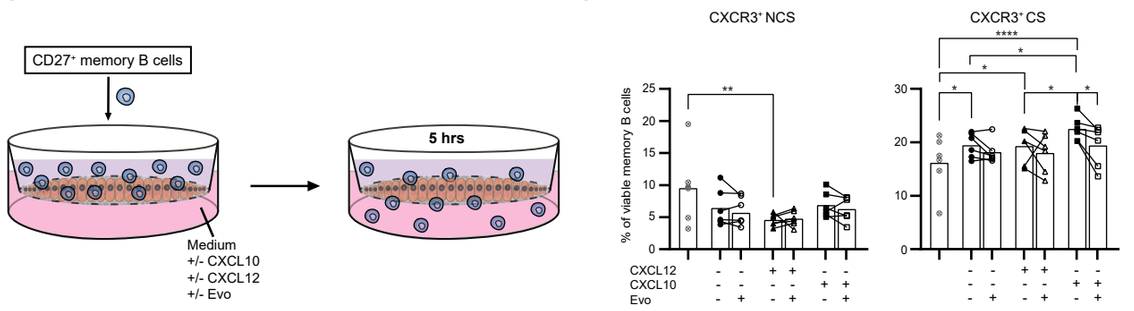
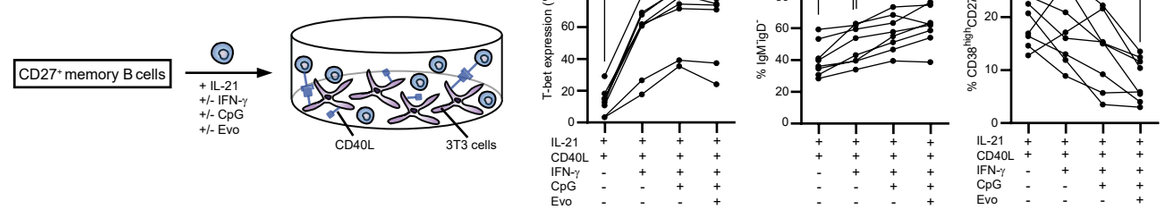


Figure 10: Evobrutinib inhibits maturation of memory B cells to ASCs *in vitro*



Conclusions

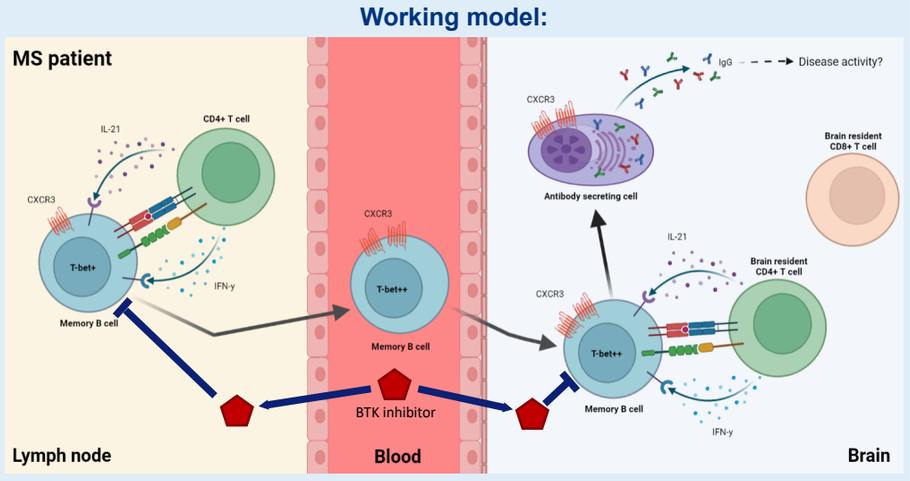
- Increased transition of CXCR3$^+$ B cells to ASCs in MS brain tissues [12]
- ASC formation in MS lesions correlates with local CD4$^+$ memory T cells [12]

→ ASCs potentially contribute to disease activity through both intrathecal and local IgG production in MS [13]

- BTK activity is increased and corresponds to CXCR3 expression in B cells of MS patients [14]
- Evobrutinib targets CXCR3$^+$ B cells *in vitro* by hindering T_{FH}-dependent class-switching, transmigration and maturation towards ASCs [14]

→ Next-generation BTK inhibitor evobrutinib is a promising drug to target CXCR3$^+$ B-cell to ASC maturation in the MS brain [14]

Working model:



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- *All other authors have no conflicts of interest to disclose.*

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