# P4162 − Expression of BTK in leptomeningeal B-cell rich infiltrates in two models of progressive multiple sclerosis and its inhibition by Evobrutinib underscores BTK as a target for compartmentalized neuroinflammation

H. Kebir[1], G. Ceja[1], M. C. Miller[1], C. Li[1], M. J. May[2], C. H. Vite[3], M. E. Church[1], R. Grenningloh[4], U. Boschert[5], J. I. Alvarez[1]

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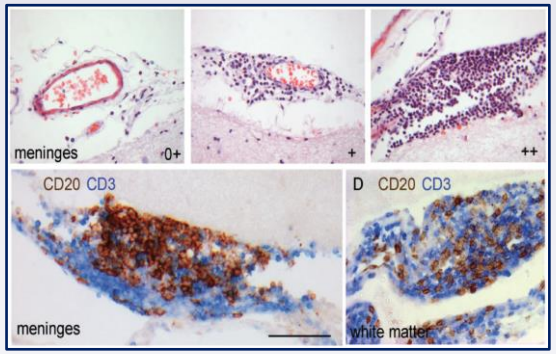
## Progressive multiple sclerosis (MS)

- Multiple sclerosis (MS) is the most common chronic inflammatory disease of the CNS.
- Most patients are initially diagnosed with relapsing-remitting MS, but the disease typically transitions to a progressive course with steady increase in disability over time.
- Compartmentalized CNS inflammation may be a source of progressive disease in MS:

  **Meningeal inflammation** consisting of B cell-rich aggregates with adjacent subpial cortical pathology, is predictive of an aggressive disease with rapid, unrelenting **progression of disability** (Howell *et al.*, 2011, Magliozzi *et al.*, 2018).




*Choi et al., Brain 2012*

- Challenges in the treatment of progressive MS:
  - Few animal models recapitulate the pathology of progressive MS.
  - CNS B cells are protected from the direct effects of anti-CD20 therapies.

    While B-cell depleting antibodies effectively eliminate circulating B cells, they do not remove those in the CNS, leaving them able to act as a source of underlying progressive disease activity (Komori et al. ACTN 2016).

## Bruton's tyrosine kinase (BTK)

- Alternative approach: **Bruton's tyrosine kinase (BTK)** inhibition:
  - BTK is expressed by B cells, macrophages/microglia, but not by T cells (Lopez-Herrera *et al.*, 2014).
  - BTK is critically involved in B cell and myeloid cell activation and survival (Lopez-Herrera *et al.*, 2014).
  - The BTK inhibitor Evobrutinib has been described to enter the CNS (Boschert *et al.*, 2017, ECTRIMS).

## Hypothesis and Objectives

**Hypothesis –** BTK represents a potential candidate to target compartmentalized neuroinflammation and reduce MS disability progression.

**Objectives**

- **Aim 1 –** Evaluate BTK expression in the CNS of two animal models recapitulating determining aspects of progressive MS:
  - A canine model: **GME** (granulomatous meningoencephalomyelitis).
  - A murine model: **pEAE** (progressive experimental autoimmune encephalomyelitis).
- **Aim 2 –** Test the efficacy of the BTK inhibitor Evobrutinib *in vivo* in the pEAE model.

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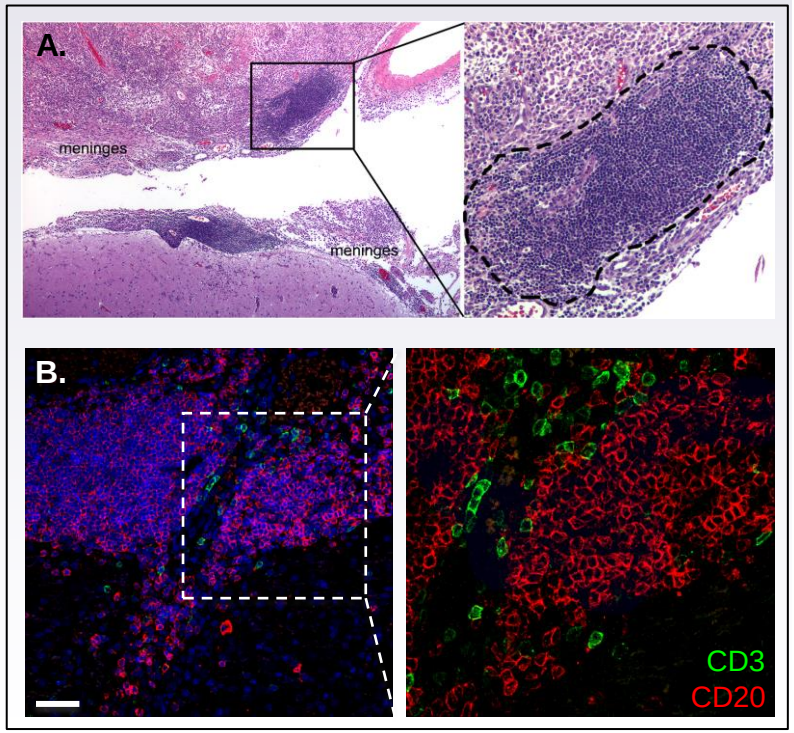
## 1. Canine model of progressive MS-like disease (GME)

- Granulomatous meningoencelphalomyelitis (**GME**) is the most common idiopathic neuroinflammatory disease in dogs that prevails in **young females** (Coates and Jeffery, 2014).

**Figure 1. Neuropathology of GME.**

(**A**) Hematoxylin & eosin (H&E) staining showing heavy immune cell infiltration in the meninges of GME dogs.

(**B**) $CD20^+$ B cells (red) are the predominant leukocyte population within the meningeal infiltrate, as compared to $CD3^+$ T cells (green). Nuclei are stained in blue. Scale bar: 50 μm.



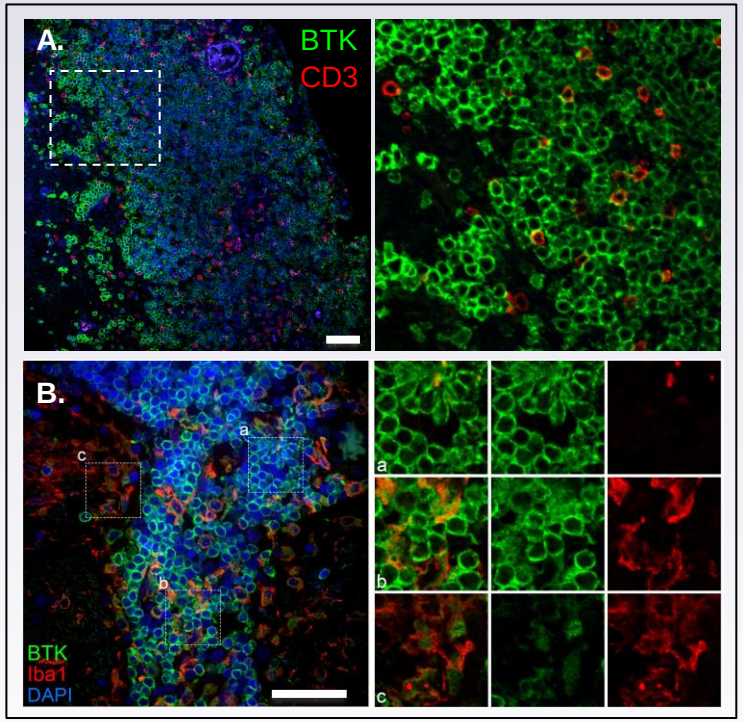

## 2. Expression of BTK within meningeal infiltrates in the GME model

**Figure 2. Expression of BTK in the GME brain.**

(**A**) Immunofluorescent staining revealing a lack of co-localization between BTK (green) and CD3 T cells (red) staining in a meningeal infiltrate.

(**B**) Myeloid cells (Iba-$1^+$, red) express BTK within meningeal infiltrates. The majority of $BTK^+$ cells are most likely B cells. Nuclei are stained in blue. Scale bar: 50 μm.

*Primary antibodies directed against canine CD20 and BTK are both raised in the same species, preventing double immunofluorescent staining.*




**Results**:

- GME is marked by leukocyte infiltration in the parenchyma and **meninges** (Vite, 2005; Cherubini *et al.*, 2006).
- Meningeal infiltrates in GME express high levels of BTK.

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## 3. Mouse model of progressive MS-like disease (pEAE model)

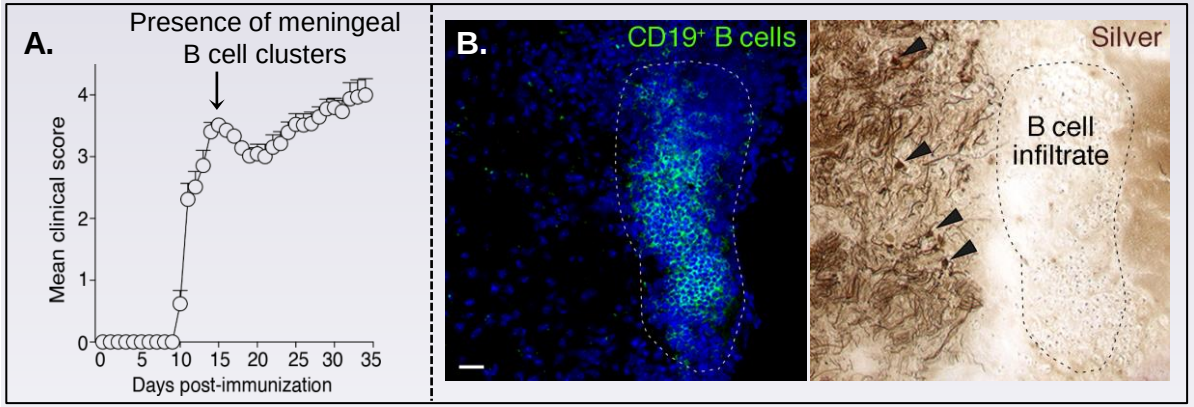


**Figure 3. EAE in a model of progressive MS-like disease (pEAE).** 10 wk-old C57BL/6×FVB mice were immunized with $MOG_{35-55}$ peptide. (**A**) Representative disease course with mean clinical score (± SEM). *n* = 21. Scoring scale: 0, no clinical signs; 1, limp tail; 2, impaired righting reflex; 3, hindlimb weakness; 4, paralysis of both hindlimbs; 5, moribund/death. (**B**) Meningeal inflammation consisting of $CD19^+$ B cell-rich clusters (green) that persist until day 35 and are found adjacent to areas of subpial neuronal damage (silver staining) that involve axonal swelling and blebbing (arrowheads). Scale bar: 50 μm.

**Results**:

- Aggressive EAE course that gradually worsens after the first acute episode.
- Meningeal inflammation with B cell-rich clusters, adjacent to areas of subpial neuropathology.

## 4. Expression of BTK within meningeal infiltrates in the pEAE model

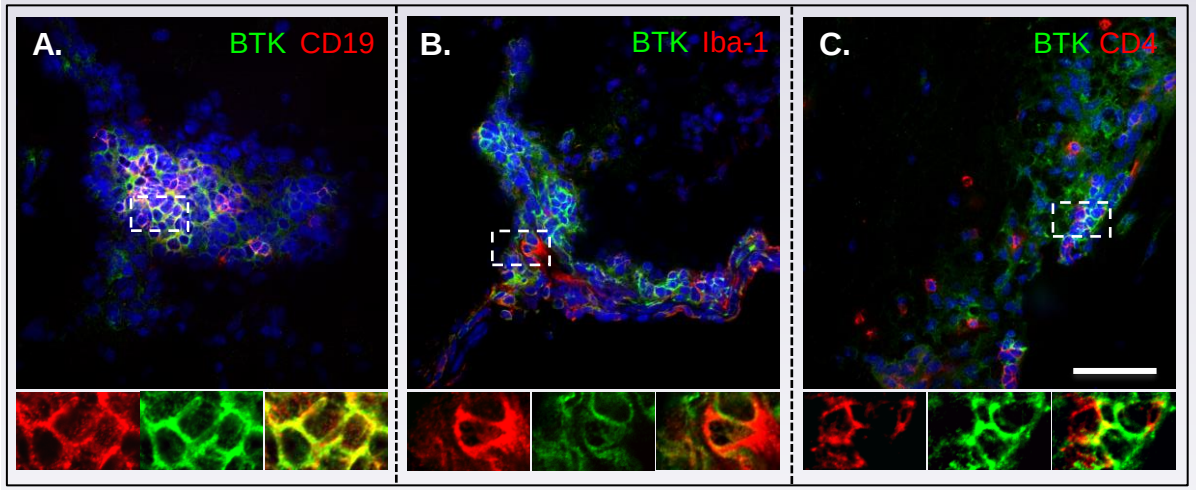


**Figure 4. Expression of BTK in the CNS of pEAE mice at day 16.** (**A**) $CD19^+$ B cells (red) and (**B**) Iba-$1^+$ myeloid cells (red) express BTK (green) within meningeal infiltrates in the brain of pEAE mice. (**C**) Lack of co-localization between BTK (green) and CD4 T cells (red) in a spinal cord meningeal infiltrate.
Nuclei are stained in blue. High power views (dashed rectangle) are shown to the right. Scale bar: 50 μm.

**Results**:

- $CD19^+$ B cells and Iba-$1^+$ microglia/macrophages express BTK.
- $CD4^+$ T cells are BTK negative.

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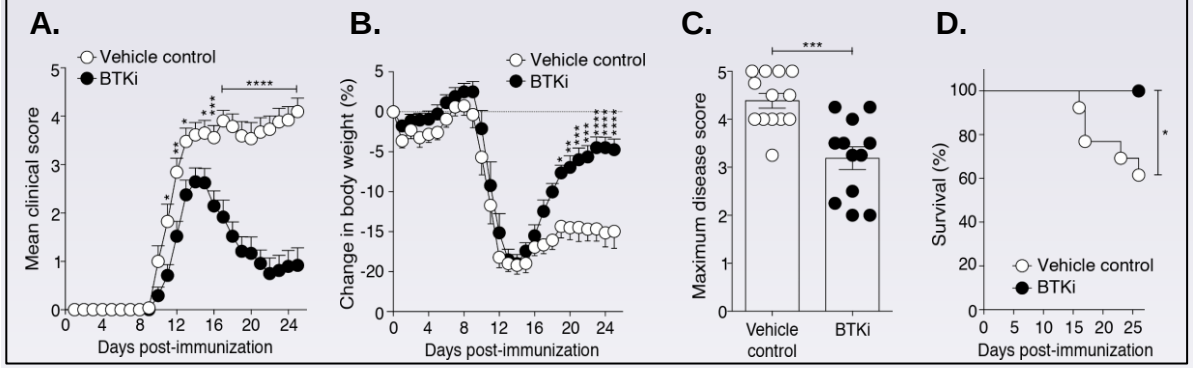
## 5. Treatment with the BTK inhibitor Evobrutinib in the pEAE model

**Prophylactic Regimen**




**Therapeutic Regimen**

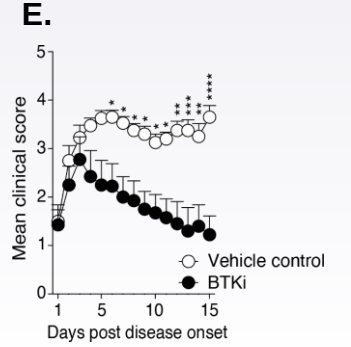
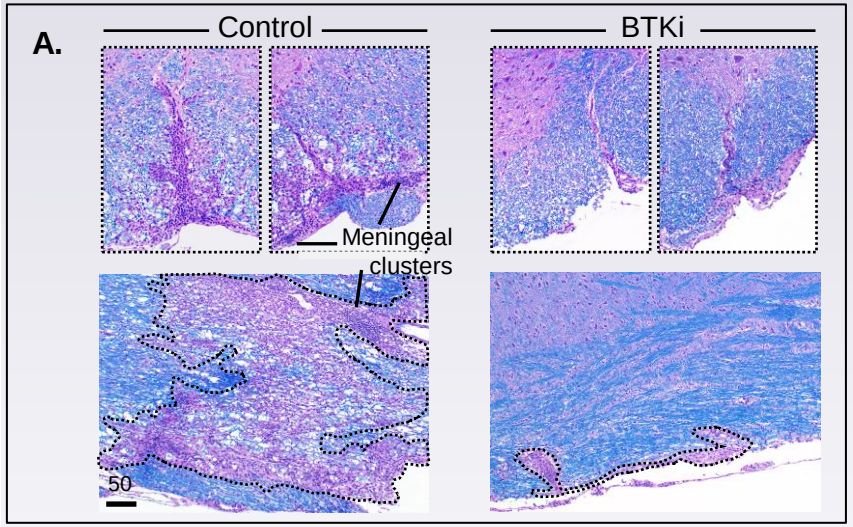



**Figure 5. Effect of the BTK inhibitor Evobrutinib on pEAE.** (**A**) Clinical disease score (mean ± SEM), (**B**) percentage body weight change, (**C**) maximum disease score, and (**D**) percentage survival rate in control and Evobrutinib (BTKi)-treated mice. Evobrutinib (10mg/kg - orally) was given daily starting the day of immunization. *n* = 13. (**E**) Clinical disease score (mean ± SEM) in control and Evobrutinib (BTKi)-treated mice. Evobrutinib was given daily starting the day of disease onset. *n* = 10. Statistics: (A, B, E) Two-way ANOVA with repeated measures and Bonferroni's multiple comparisons test, (C) Mann-Whitney, (D) Logrank (Mantel-Cox) test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

## 6. Histopathology in Evobrutinib-treated pEAE mice



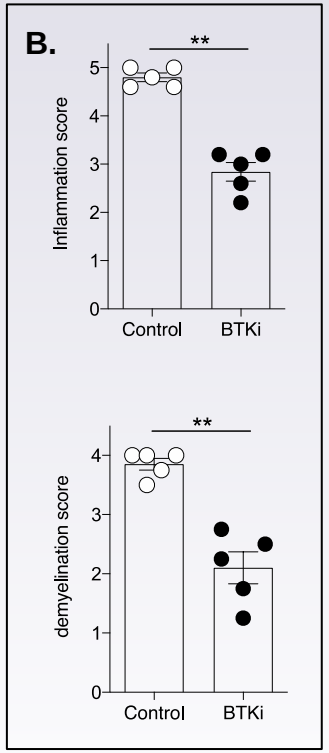




**Figure 6. The BTK inhibitor Evobrutinib reduces inflammation and demyelination in pEAE.** (**A**) Luxol fast blue/H&E staining of spinal cords from control (*n* = 5) and Evobrutinib (*n* = 5) pEAE mice at day 25. Evobrutinib was given in a prophylactic regimen. (**B**) Quantification of inflammation and demyelination in both groups. ***P* < 0.01.

**Results**:

- BTK inhibition with Evobrutinib (prophylactic and therapeutic regimens) reduces disease severity in the pEAE model and reduces the associated neuropathology.

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## Conclusions & Acknowledgments

- Both the GME and the pEAE models are characterized by prominent B cell infiltrates in the leptomeninges, associated with subpial neuronal damage in the adjacent tissue, reminiscent of the meningeal inflammation seen in cases of human MS.
- We found robust BTK expression within B cell-rich leptomeningeal infiltrates in both the GME and the pEAE model.
- Iba1$^+$ cells (macrophages/microglia) express BTK.
- BTK inhibition with Evobrutinib (prophylactic and therapeutic regimens) reduces disease severity of pEAE.
- BTK inhibition with Evobrutinib (prophylactic regimen) reduces the neuropathology of pEAE, including immune cell infiltration in the meninges and parenchyma and the extent of demyelination.

**Acknowledgements**

We wish to thank **EMD Serono Inc.** (an affiliate of Merck KGaA, Darmstadt, Germany).

We are grateful to the **American Academy of Neurology (AAN).**

We also acknowledge the Comparative Pathology Core of the School of Veterinary Medicine at the University of Pennsylvania.

## Disclosures & Funding

- Hania Kebir holds a postdoctoral fellowship from the Fonds de Recherche du Québec — Santé (FRQS).
- Guadalupe Ceja, Miles C. Miller, Cen Li, Michael J. May, Charles H. Vite, and Molly E. Church have nothing to disclose.
- Roland Grenningloh is an employee of EMD Serono Inc., USA (an affiliate of Merck KGaA, Darmstadt, Germany) and Ursula Boschert is an employee of Merck KGaA, Darmstadt, Germany.
- Jorge I. Alvarez receives research support from EMD Serono, the National Institutes of Health (NIH)-National Institute of Neurological Disorders and Stroke (NINDS), and the Institute for Translational Medicine and Therapeutics (ITMAT) of the University of Pennsylvania.
- This study is supported by funding from EMD Serono Inc. (an affiliate of Merck KGaA, Darmstadt, Germany).

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