The BTKi Evobrutinib Demonstrates Superior Efficacy in Targeting Compartmentalized Neuroinflammation Compared to Anti-CD20 Treatment

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Disclosure statement

Disclosure: Jorge I Alvarez, Ph.D.

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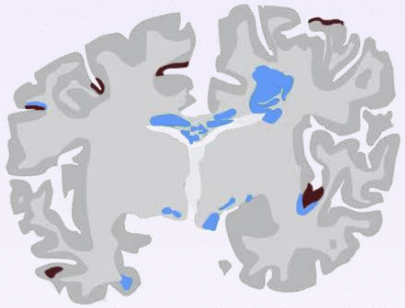
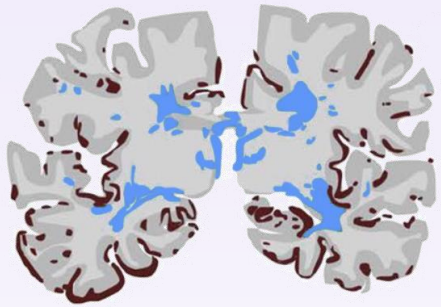
Compartmentalized CNS inflammation in progressive MS

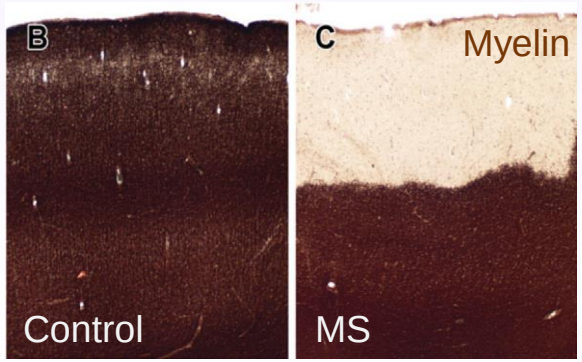
Relapsing-remitting MS



Progressive MS

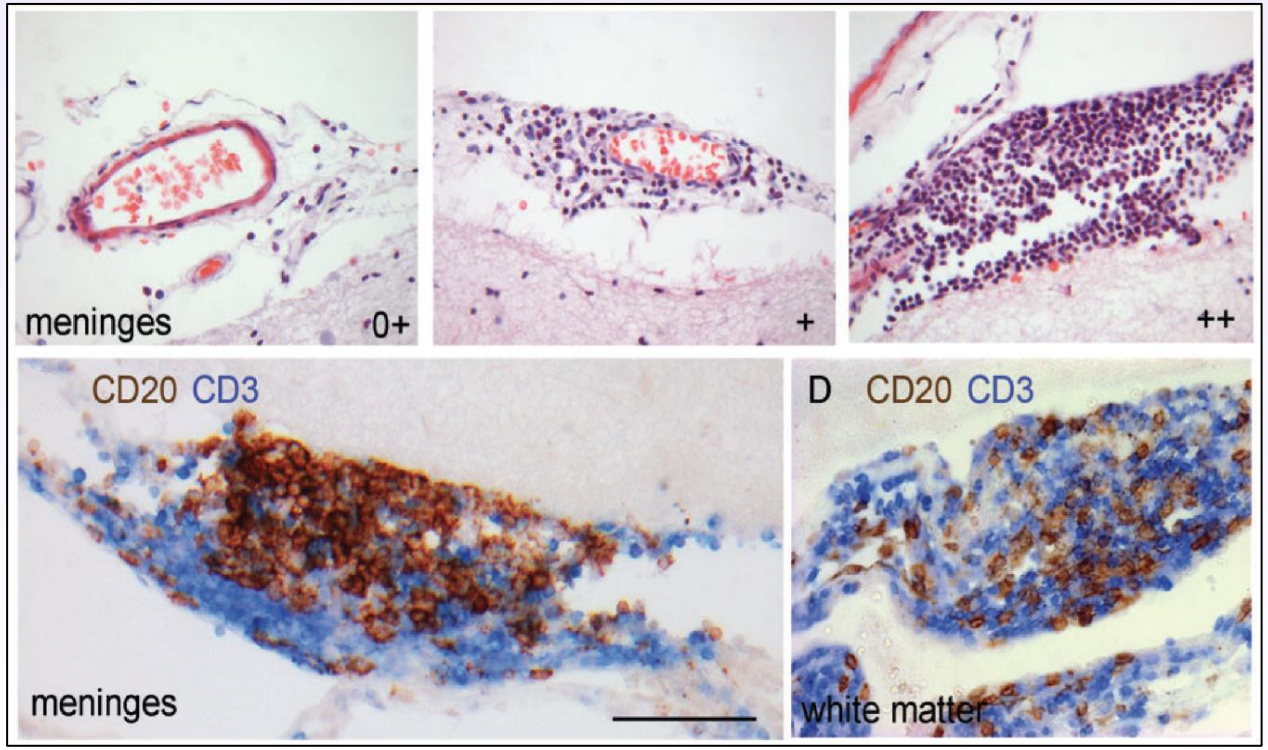


demyelination
submeningeal pathology



Chang et al Ann Neurol 2012

Meningeal B cell aggregates



Choi et al., Brain 2012

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

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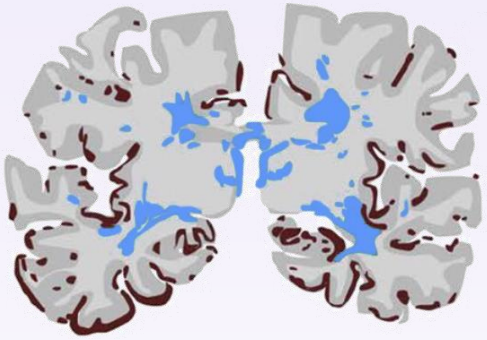
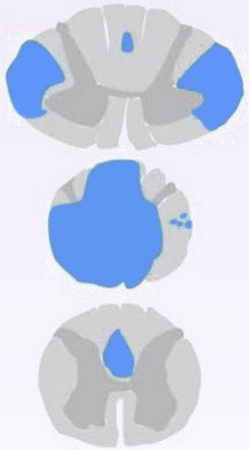
Challenge in modeling the pathology of progressive MS

MS (Human)

Brain

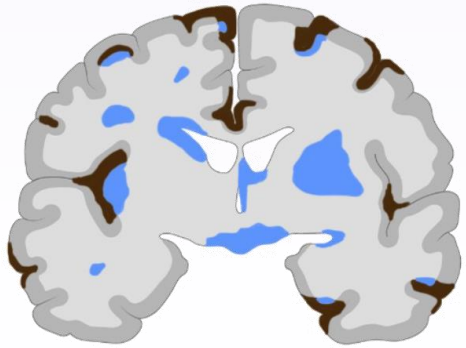


Spinal cord



demyelination
submeningeal pathology

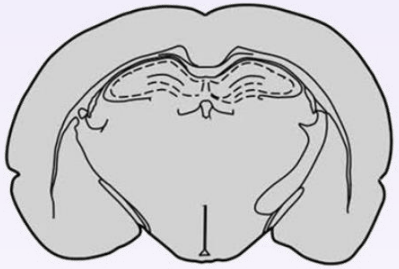
GME (Dog) *Church et al, J Immunol 2021*



GME: Granulomatous meningoencephalomyelitis

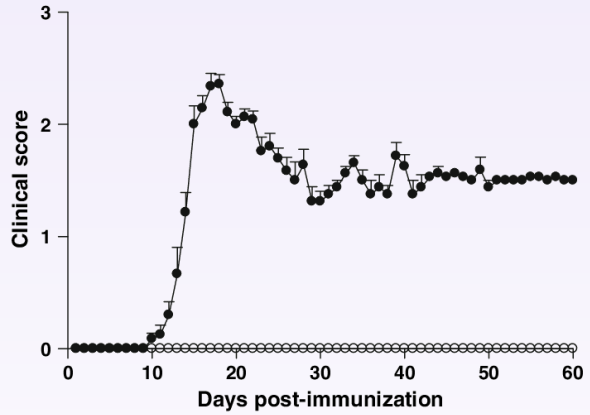
EAE (mouse) MOG-induced in C57BL/6

Brain



Spinal cord

Clinical disease course



Major limitations of MOG-induced EAE

- Pathology largely confined to the spinal cord with little inflammation in the brain.
- Disease course: acute monophasic.
- Low involvement of B cells.

EAE: Experimental autoimmune encephalomyelitis

Modified from *Lassmann and Bradl, Acta Neuropathol 2016*

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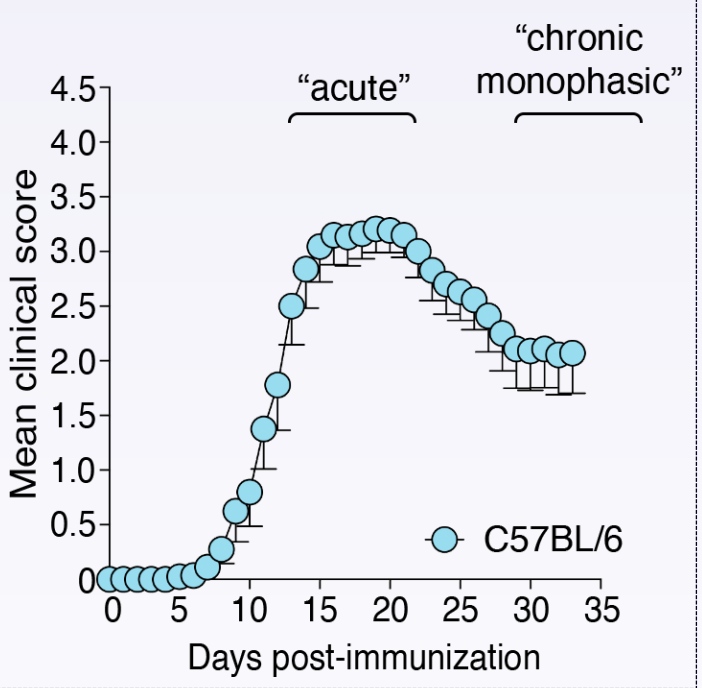
Progressive EAE model (pEAE)

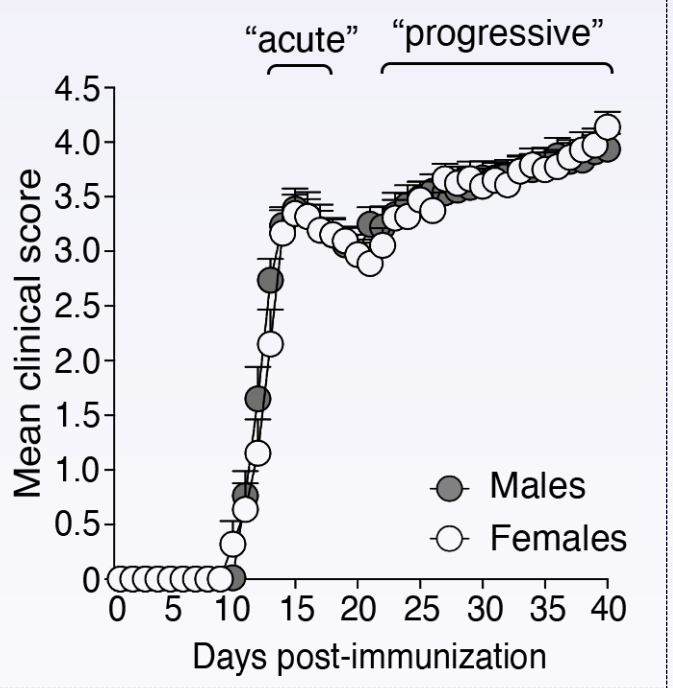
"classical EAE" (in C57BL/6)



EAE induction
- MOG_{35-55}/CFA
- PTX (d0, d2)

C57BL/6 *vs* **C57BL/6 × FVB mice**

pEAE (in C57BL/6 × FVB mice)



EAE Clinical Score: 0= No clinical signs; 1= Limp tail; 2= Impaired righting reflex; 3= hindlimb weakness; 4= Paralysis of both hindlimbs; 5= Moribund/death

n = 38 mice (20 males, 18 females).
4 independent experiments.
Statistics (♂ vs ♀♀ Females): ns, Two-way ANOVA with Šídák's multiple comparisons test.

- EAE course that gradually worsens after the first acute episode.
- 100% disease incidence with no significant differences in clinical course between males and females.

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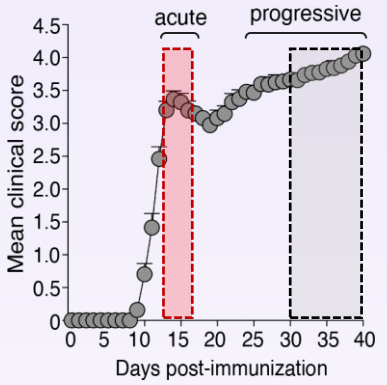
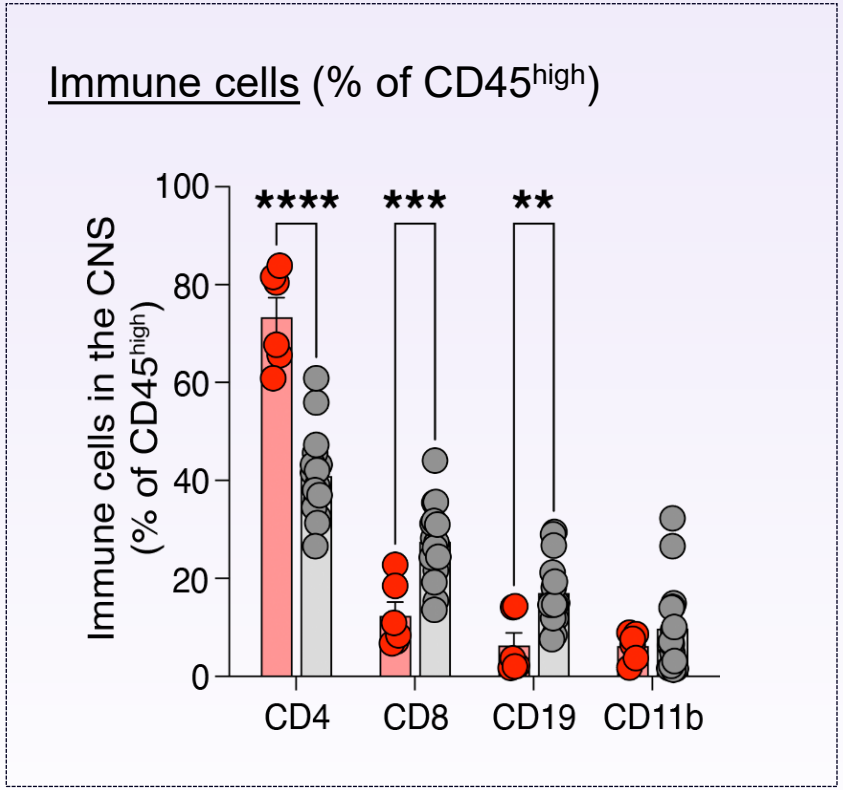
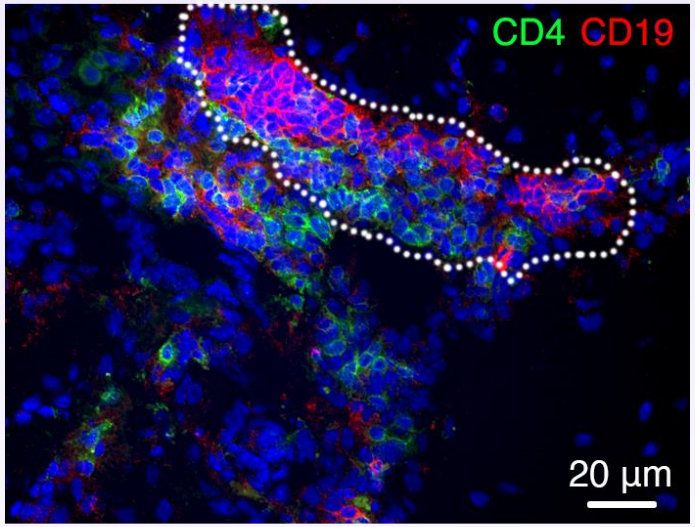
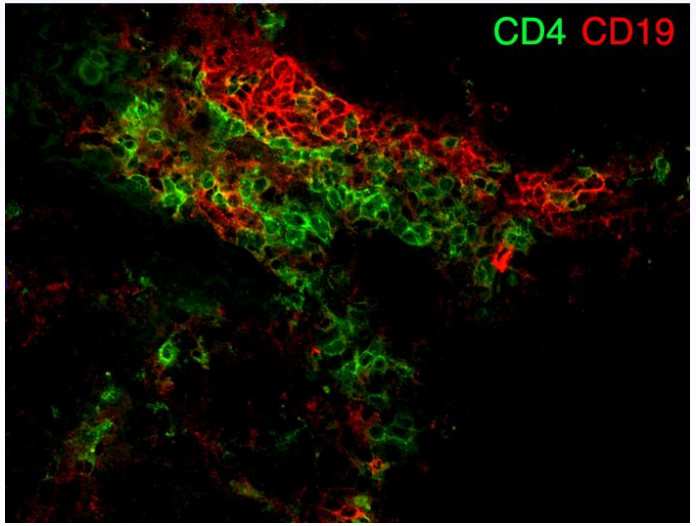
Composition of immune cell infiltrates in the pEAE CNS



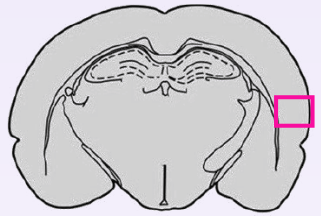


n = 6 mice at d14; *n* = 17 mice between d30-40.
Gating: Leukocytes/ Single cells/Live cells/ CD45^{high}
Statistics: Unpaired Student's *t*-test.
***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.





Representative of *n* = 5 mice at d30.



- Higher percentage of B cells and CD8 T cells, as well as lower CD4 T cells during the progressive phase of the disease.

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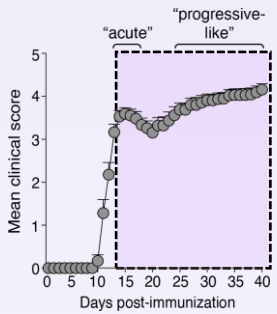
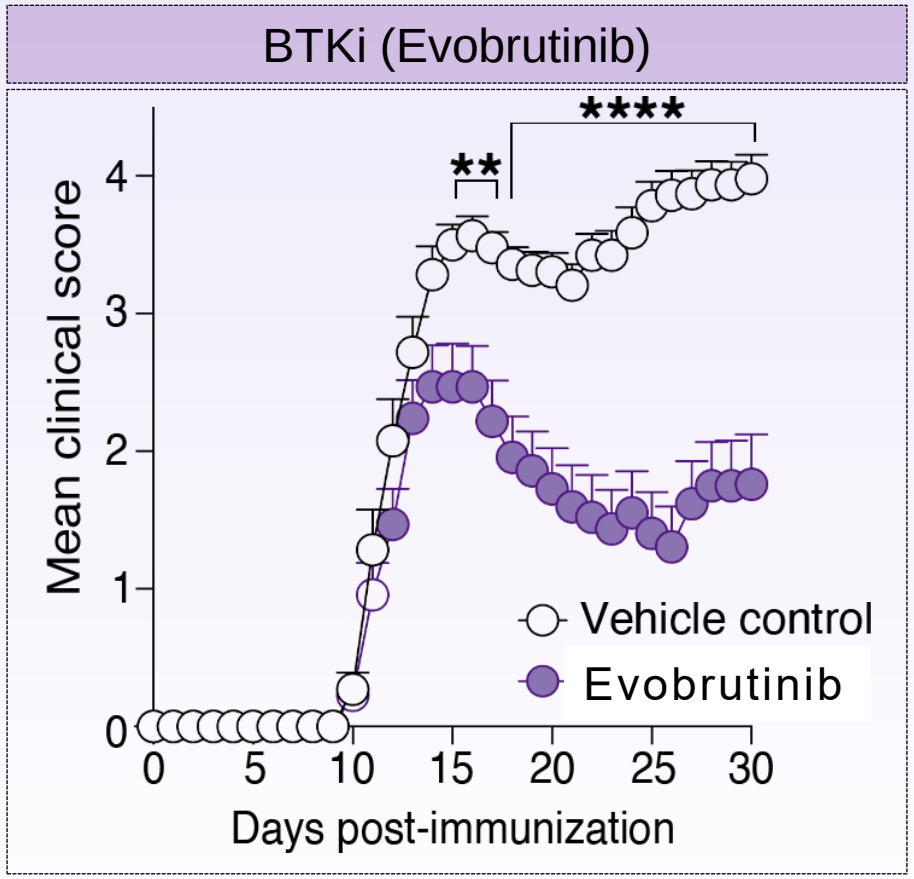
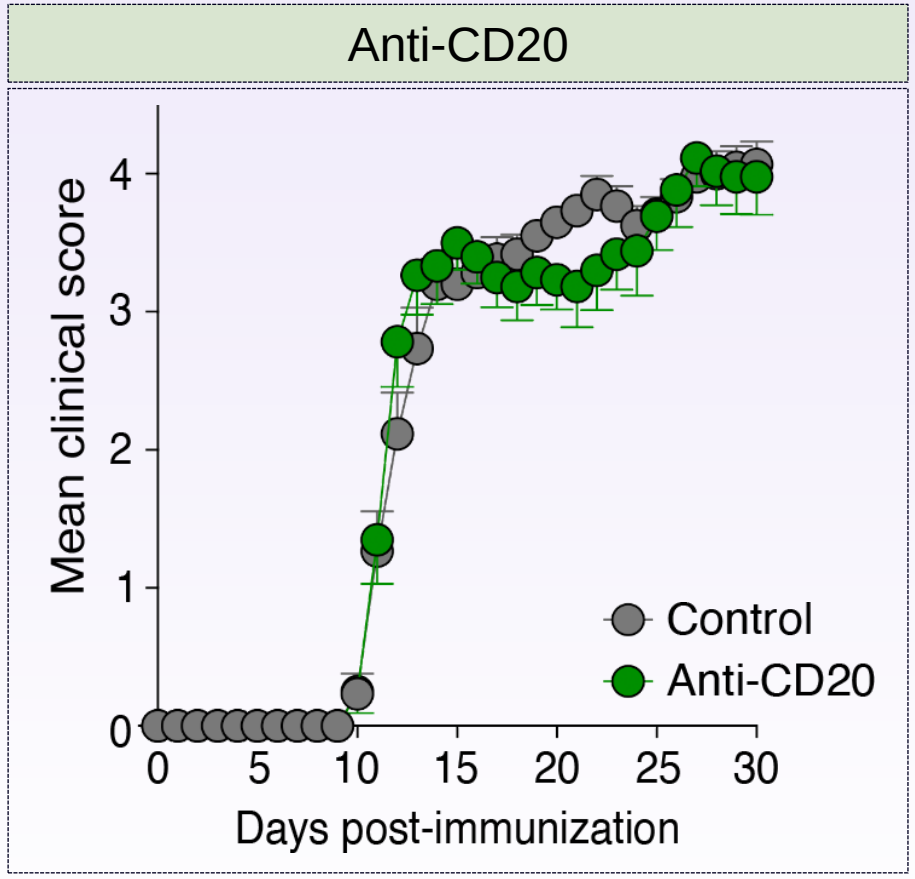
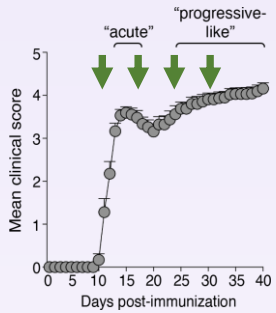
Therapeutic treatment with the BTKi Evobrutinib and anti-CD20









n = 15-25 mice per group. From 2 independent experiments.
Evobrutinib (10 mg/kg) was given orally daily starting when each individual mouse reached a clinical score ≧1.
Anti-CD20 (clone SA271G2 - 100 μg/mouse) was administered intraperitoneally starting when each individual mouse reached a clinical score ≧1, and injected again every 7 days.
Statistics: Two-way ANOVA with Šidák multiple comparisons test; $^{**}P < 0.01$; $^{****}P < 0.0001$.

- BTK inhibition but not anti-CD20 significantly reduces disease severity in the pEAE model.

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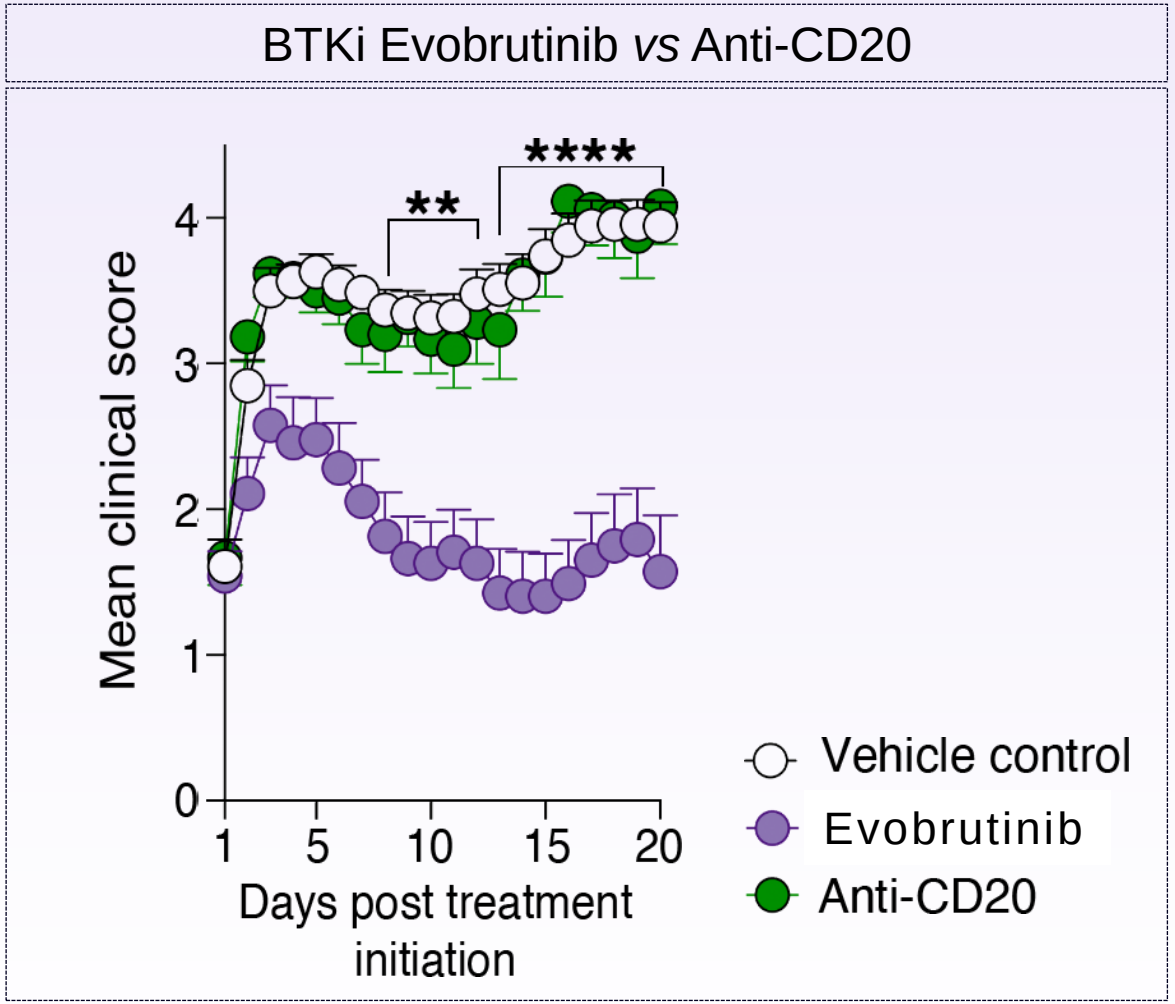
Therapeutic treatment with the BTKi Evobrutinib and anti-CD20



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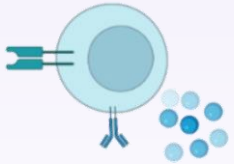
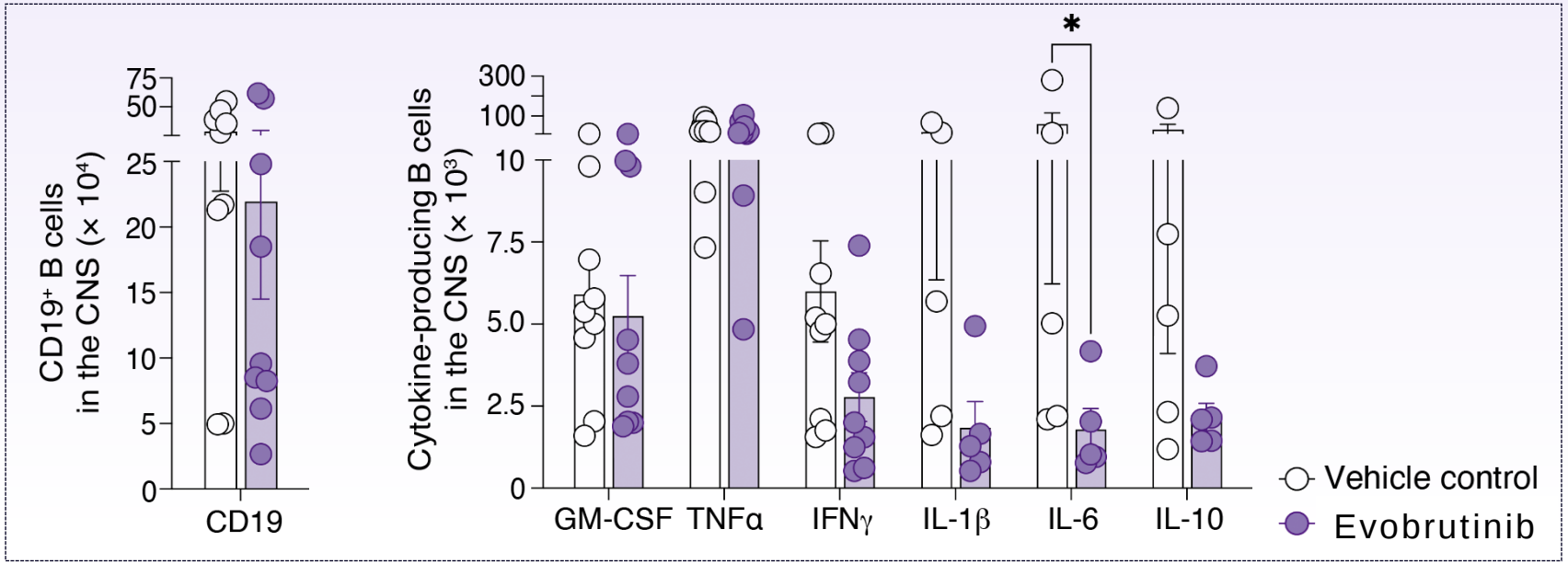
B cells in the CNS after treatment

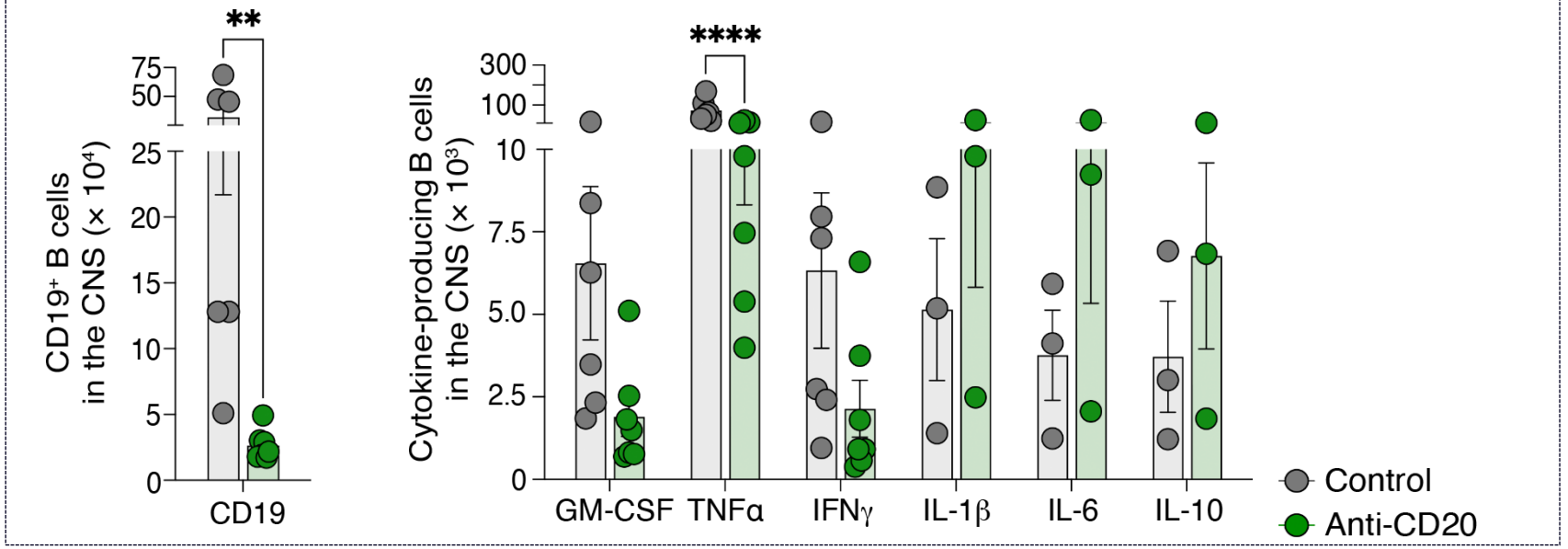
B cell



BTKi Evobrutinib



Anti-CD20



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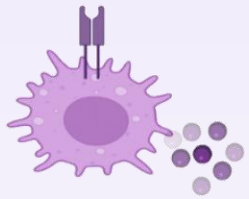
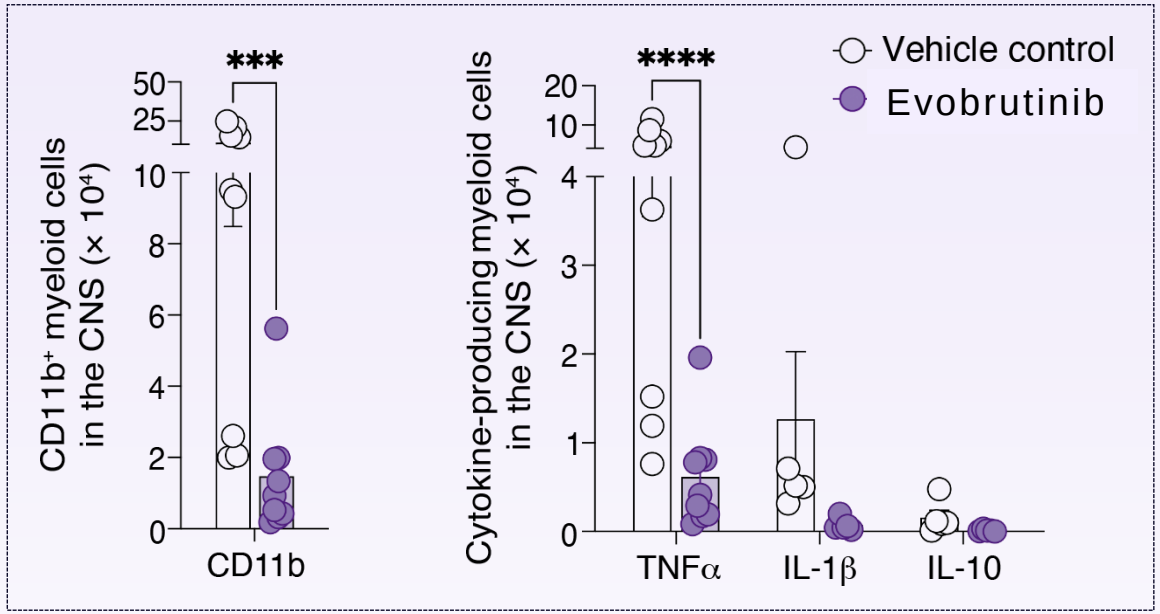
Myeloid and T cells in the CNS after treatment

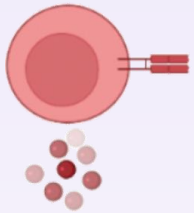
Myeloid cell



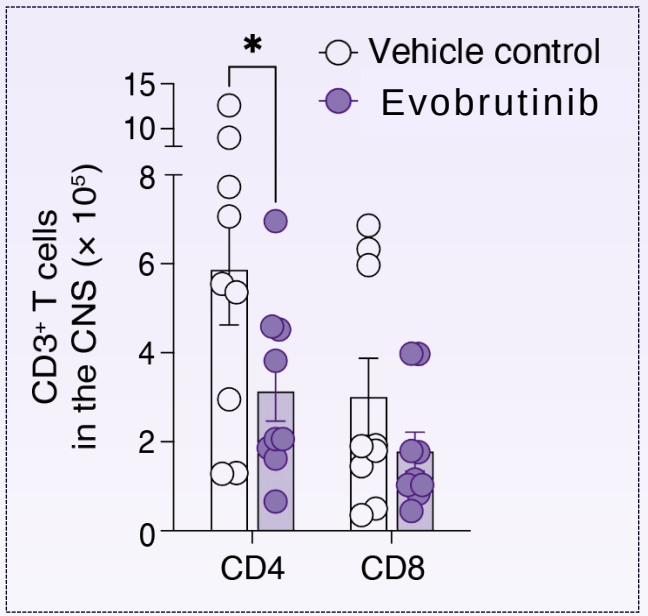
BTKi - Evobrutinib



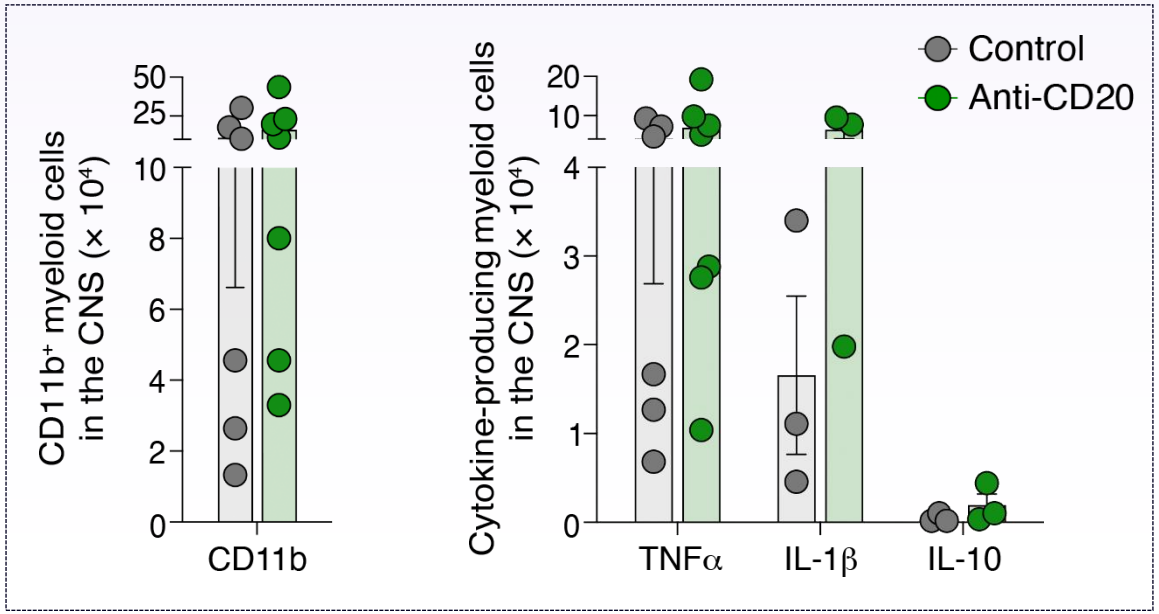
T cell



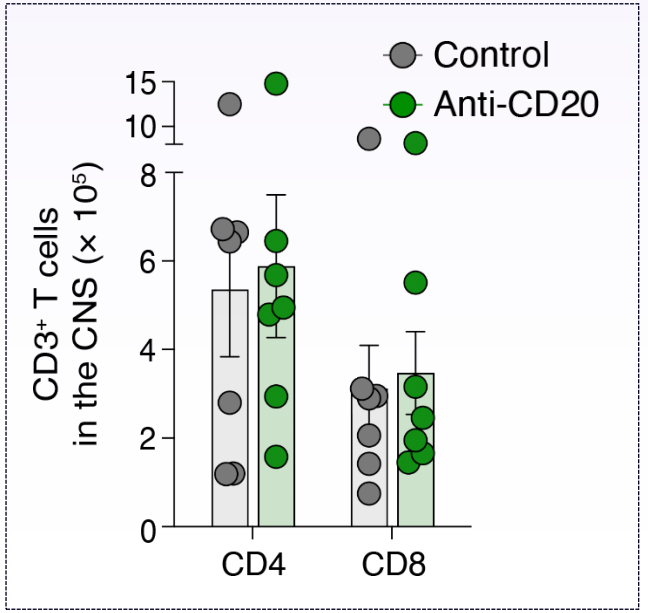
BTKi - Evobrutinib



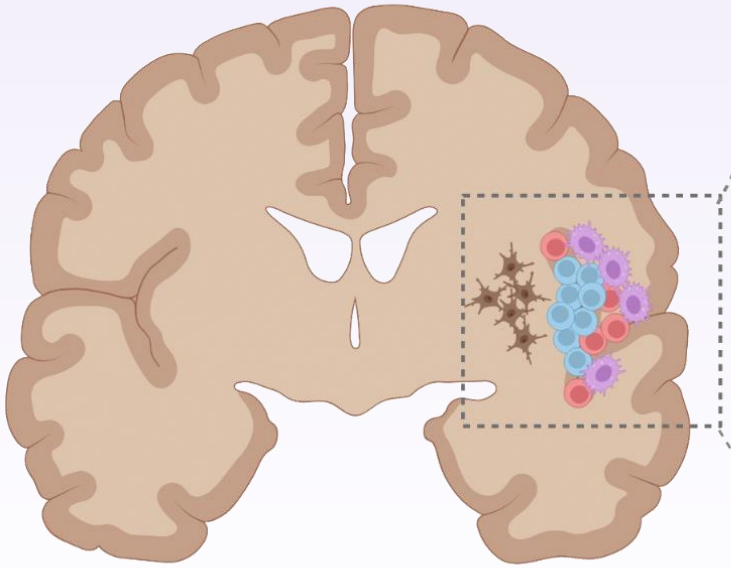
Anti-CD20

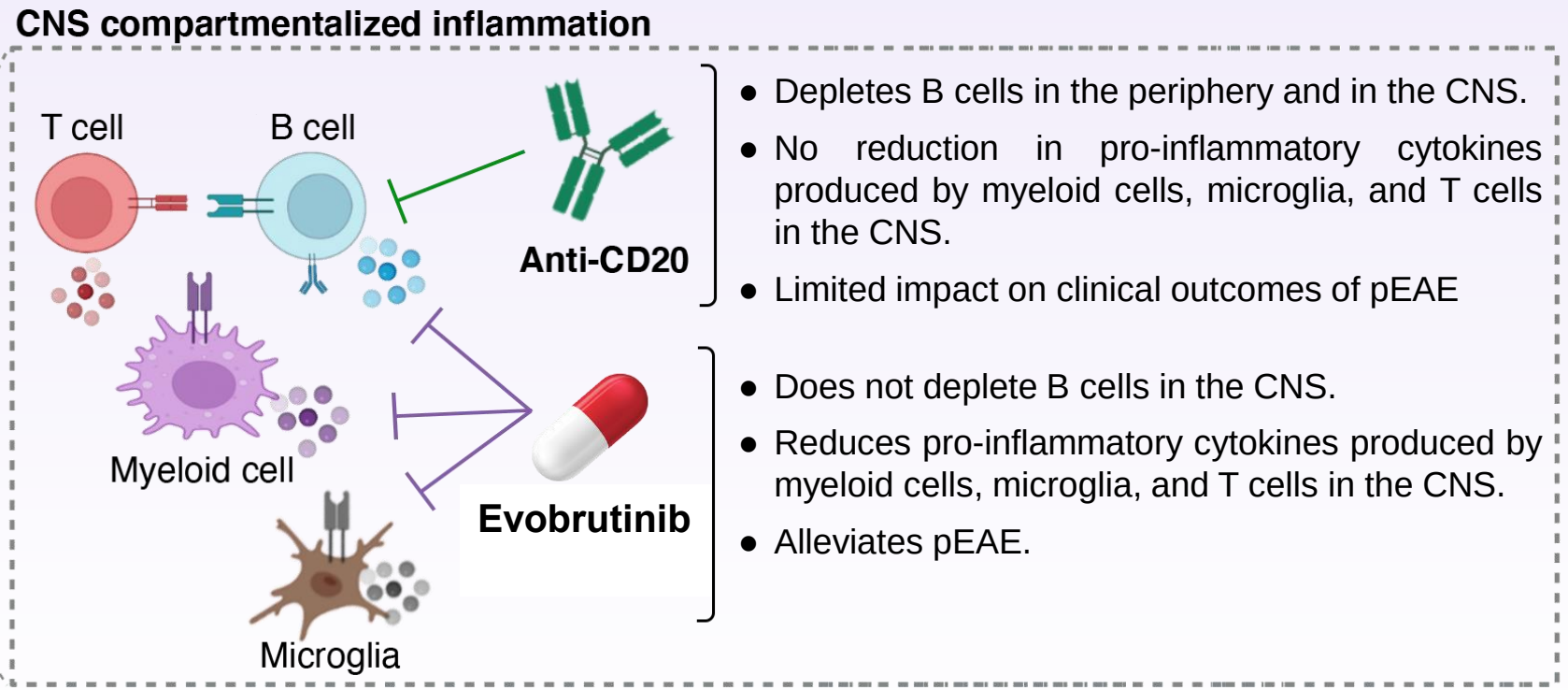


Anti-CD20



Conclusions & Working Model





- Depletes B cells in the periphery and in the CNS.
- No reduction in pro-inflammatory cytokines produced by myeloid cells, microglia, and T cells in the CNS.
- Limited impact on clinical outcomes of pEAE

- Does not deplete B cells in the CNS.
- Reduces pro-inflammatory cytokines produced by myeloid cells, microglia, and T cells in the CNS.
- Alleviates pEAE.

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