Fenebrutinib Reduces Disease Activity in a Mouse Model of Inflammatory Multiple Sclerosis Associated With Reduced Microglial Activation

Martin S. Weber,[1,2] Christopher Harp,[3] Alexandra Goodyear,[3] Tracy J. Yuen,[3] Matthew R. Durk,[3] Ludwig Kappos[4]

[1]Institute of Neuropathology, University Medical Center, Göttingen, Germany; [2]Department of Neurology, University Medical Center, Göttingen, Germany; [3]Genentech, Inc., South San Francisco, CA, USA; [4]Research Center for Clinical Neuroimmunology and Neuroscience Basel (RC2NB), University Hospital and University of Basel, Basel, Switzerland.

INTRODUCTION

T cells, B cells and myeloid lineage cells may drive acute and chronic inflammation in multiple sclerosis (MS)

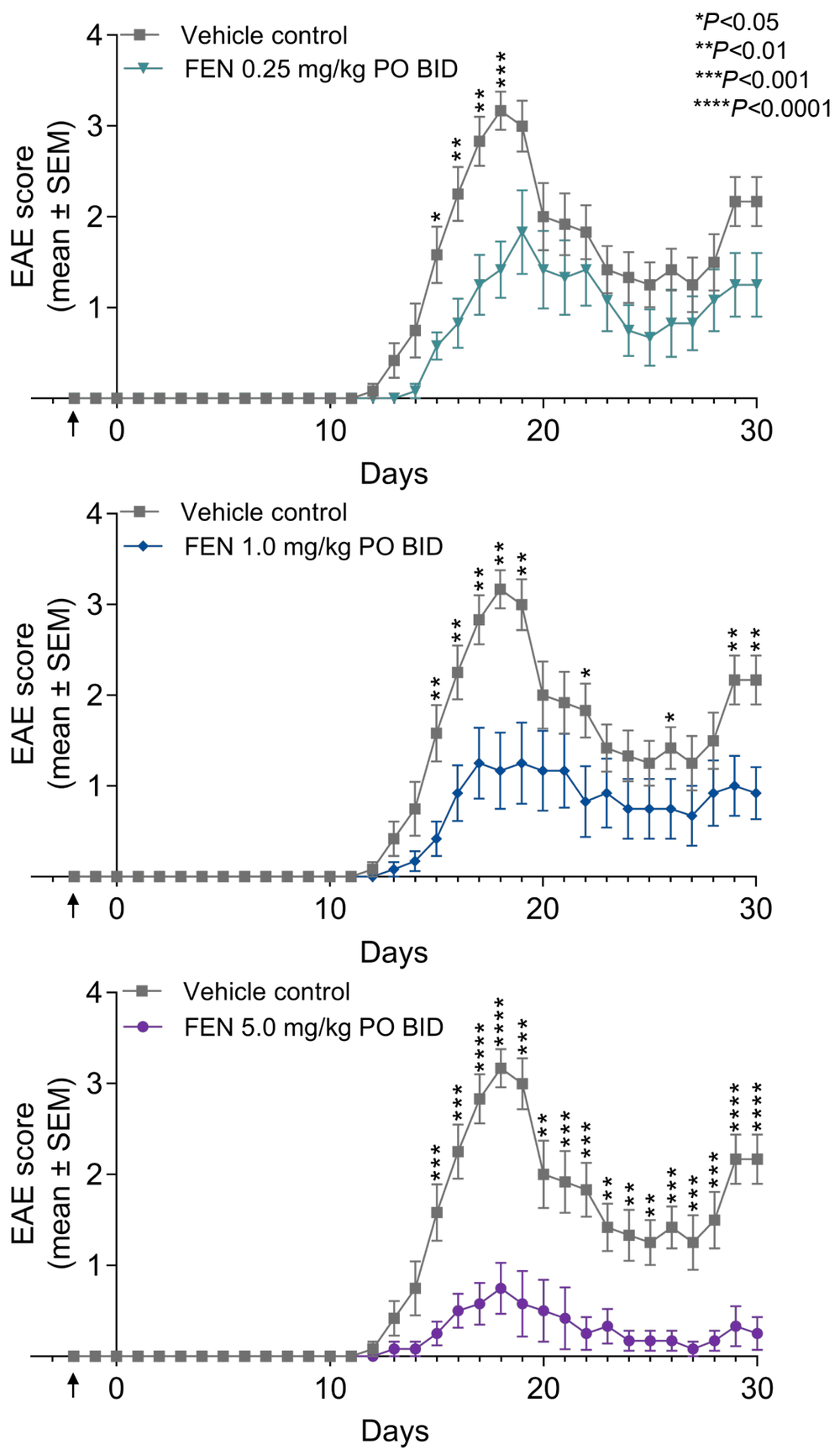
Bruton tyrosine kinase (BTK) regulates B-cell development and B-cell and myeloid cell activation; fenebrutinib (FEN) selectively inhibits BTK

Experimental autoimmune encephalitis (EAE) is a mouse model of MS; mice (n=12 per group) were treated prophylactically with vehicle or FEN from 2 days prior to EAE induction (via immunization with peptide MOG35-55) through 30 days after

We investigated treatment with FEN in an EAE model with minimal B-cell involvement

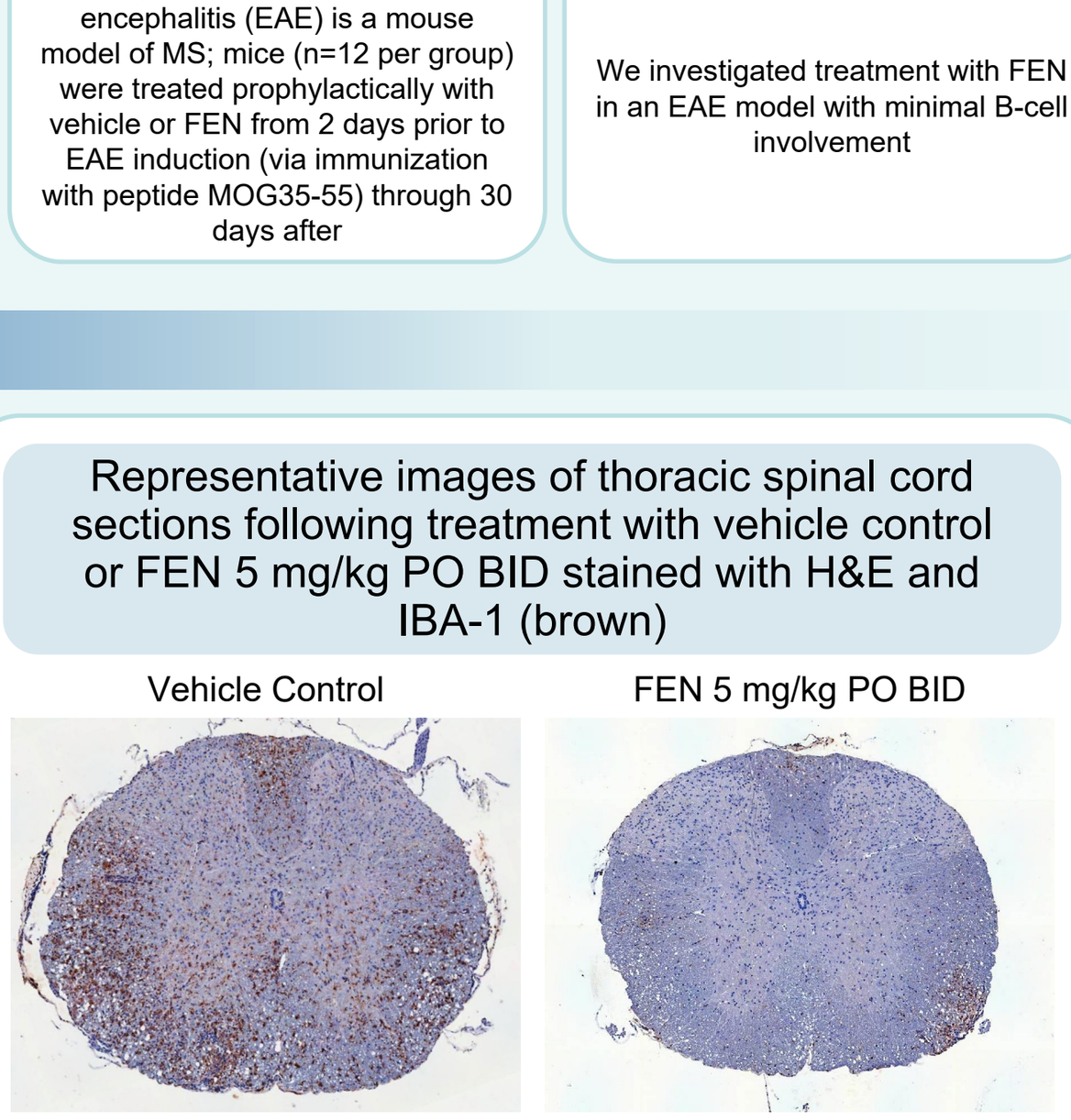
KEY FINDINGS

EAE clinical scores by FEN dose over time[a]



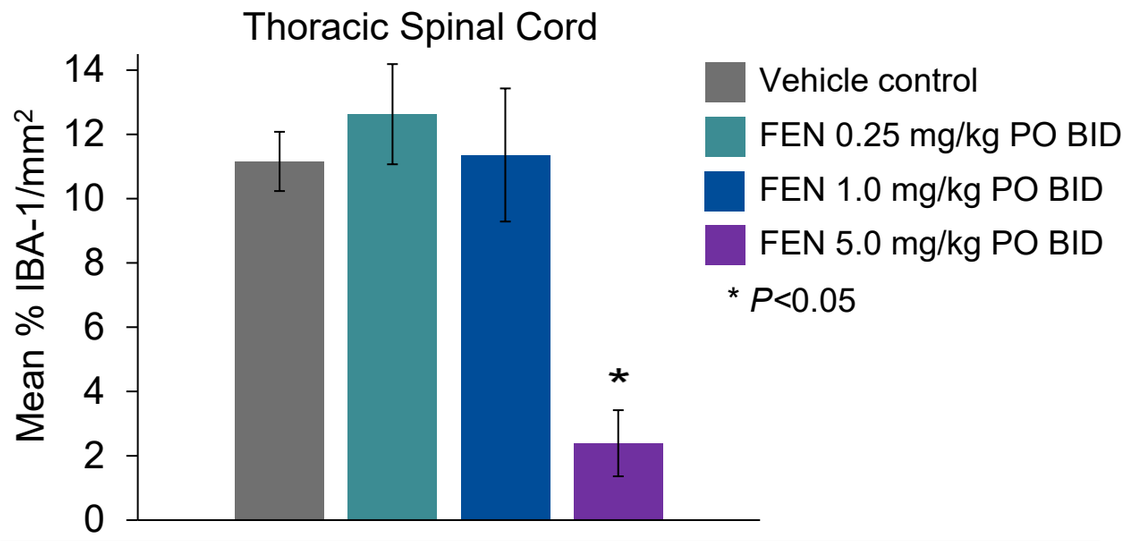
BID, twice daily; EAE, experimental autoimmune encephalitis; FEN, fenebrutinib; PO, orally.
[a]Significance determined with Mann-Whitney U test. ↑ indicates start of dosing with vehicle or FEN.

Representative images of thoracic spinal cord sections following treatment with vehicle control or FEN 5 mg/kg PO BID stained with H&E and IBA-1 (brown)



BID, twice daily; FEN, fenebrutinib; H&E, hematoxylin and eosin; IBA-1, ionized calcium-binding adapter molecule 1; PO, orally.

At the highest dose, FEN-mediated suppression of EAE severity was associated with significantly reduced microglial activation, as assessed by IBA-1 IHC in the thoracic spinal cord[a]



BID, twice daily; EAE, experimental autoimmune encephalitis; FEN, fenebrutinib; IBA-1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemistry; PO, orally.
[a]Significance determined with ordinary one-way analysis of variance (ANOVA) with Dunnett post hoc test. Error bar is standard error of the mean (SEM).

Scan QR code or click link to download a PDF of the poster and supplemental content
https://bit.ly/3kbWtPA

Presented at the 37th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), 13–15 October 2021, Virtual Congress

Sponsored by F. Hoffmann-La Roche Ltd; writing and editorial assistance was provided by Health Interactions, Inc.

Supplemental Content

Background

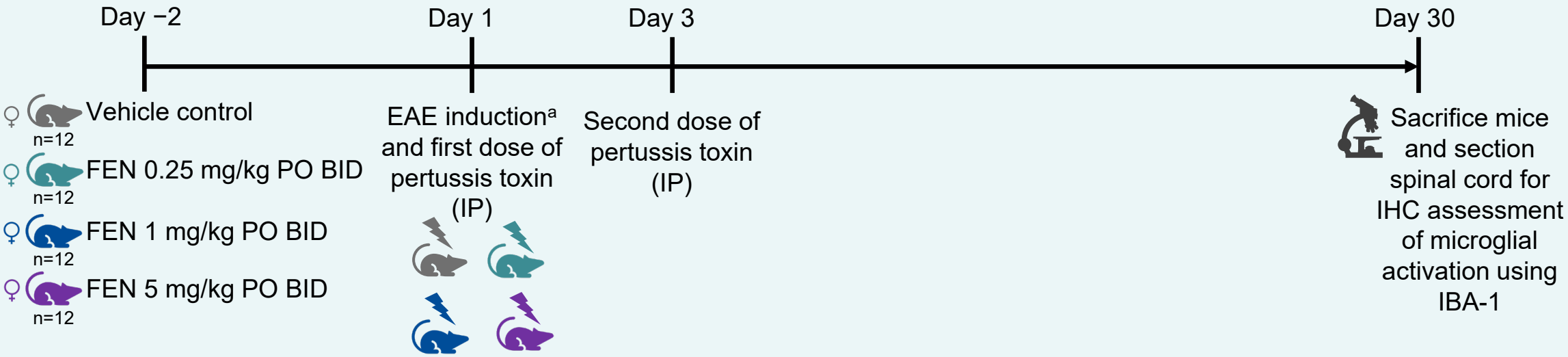
- B-cell–depleting therapies have been successful in treating MS[1]
- BTK is expressed in B cells, macrophages and microglia; clinical and nonclinical studies have demonstrated beneficial results of BTK inhibition in treating MS[2]
- EAE, induced by MOG peptide, is a $CD4^+$ T-cell–mediated autoimmune disease commonly used to model the human immune-mediated demyelinating disease MS[3]
- While the precise contributions of B cells and macrophages to EAE initiation and progression remain unclear, recent studies have suggested that inhibition of BTK substantially alters EAE disease course[4]
- Here, we investigated treatment with the BTK inhibitor FEN in a preclinical EAE model of chronic inflammatory MS with minimal B-cell involvement

BTK, Bruton tyrosine kinase; EAE, experimental autoimmune encephalomyelitis; FEN, fenebrutinib; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis.

1. Lee DSW, et al. *Nat Rev Drug Discov* 2021;20:179–199. 2. Piehl F. *J Intern Med* 2021;289:771–791. 3. Constantinescu CS, et al. *Br J Pharmacol* 2011;164:1079–1106.
4. Torke S, et al. *Acta Neuropathol* 2020;140:535–548.

Methods
Study design

Treatment with vehicle control or FEN PO BID from Day −2 to 30
Body weight and EAE clinical scores of mice (C57BL/6) assessed daily

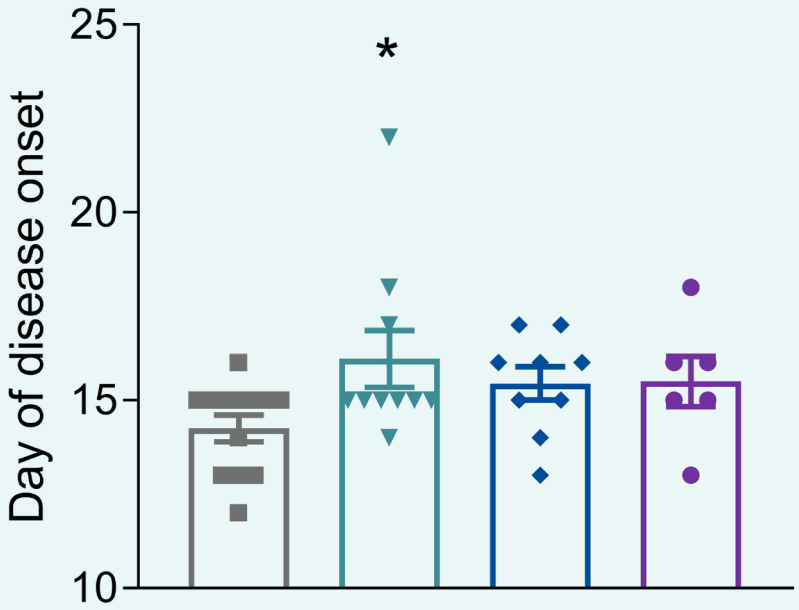


BID, twice daily; EAE, experimental autoimmune encephalomyelitis; FEN, fenebrutinib; IBA-1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemical; IP, intraperitoneal; MOG, myelin oligodendrocyte glycoprotein; PO, orally.
[a]EAE induced by immunization with peptide MOG35-55 in complete Freund adjuvant.

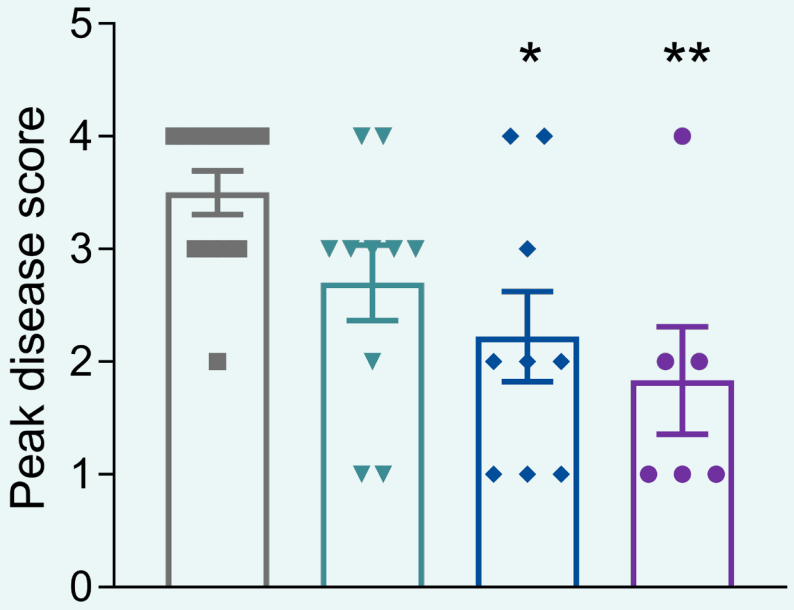
Results

Day of disease onset and peak disease score

Day of Disease Onset Following EAE Induction by Treatment Group[a]



Peak Disease Score Following EAE Induction by Treatment Group[a]



Vehicle control
FEN 0.25 mg/kg PO BID
FEN 1.0 mg/kg PO BID
FEN 5.0 mg/kg PO BID

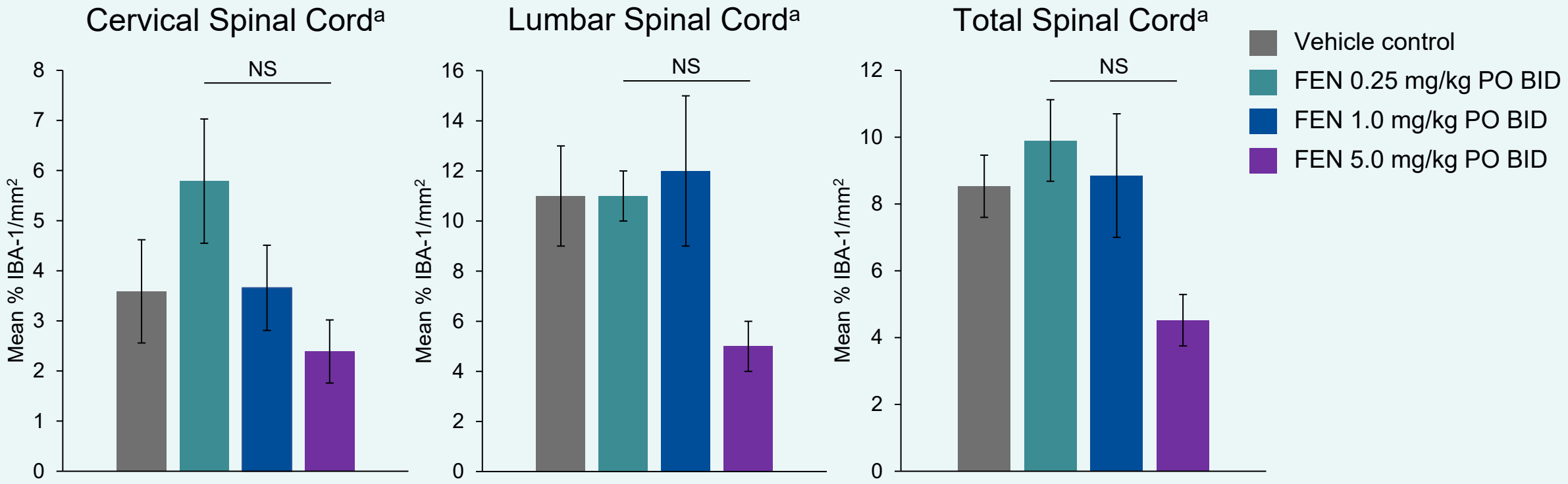
*P<0.05
**P<0.01

BID, twice daily; EAE, experimental autoimmune encephalomyelitis; FEN, fenebrutinib; PO, orally.
[a]Significance determined with ordinary one-way ANOVA with Dunnett post hoc test. Error bar is SEM.

Results

IBA-1 IHC staining in additional spinal cord regions

IBA-1 is a cytoplasmic protein expressed in microglia[1]



BID, twice daily; FEN, fenebrutinib; IBA-1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemistry; NS, not significant; PO, orally.

[a]Significance determined with ordinary one-way ANOVA with Dunnett's post hoc test. Error bar is SEM.

1. Imai Y, et al. *Biochem Biophys Res Commun* 1996;224:855–862.

Future directions

- To explore whether FEN also reverses established EAE
- To determine to what extent FEN exerts a direct effect on chronically activated microglia

EAE, experimental autoimmune encephalomyelitis; FEN, fenebrutinib.