Fenebrutinib, a Noncovalent, Reversible, Bruton's Tyrosine Kinase Inhibitor, Potently Blocks Neuroinflammation Induced by FcγR Activation in Human Microglial Systems: Implications for MS Treatment

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OBJECTIVE

To evaluate the pharmacological and functional properties of the BTKi FEN in human microglial systems.

CONCLUSIONS

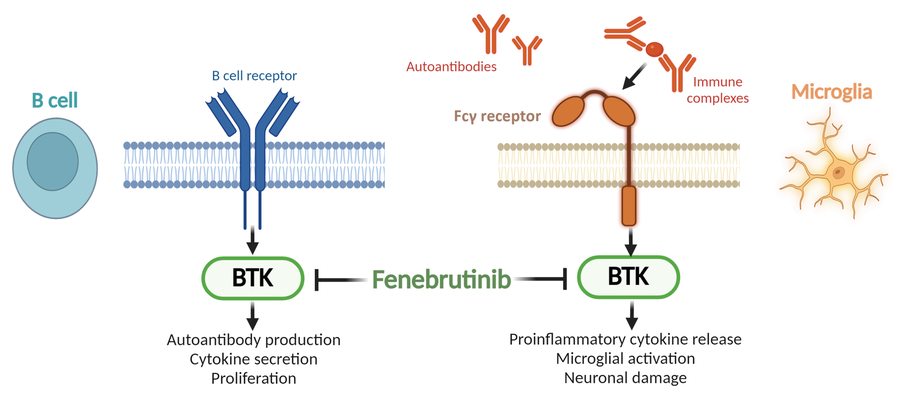
FEN acts on microglia to ameliorate pathogenic neuroinflammation resulting from FcγR activation. In people with MS, this effect may reduce progressive neurodegeneration resulting from chronic CNS inflammation in MS.

Further biomarker and efficacy assessments in clinical trials may help to confirm the clinical relevance of this observed effect on microglia.

INTRODUCTION

- Neuroinflammation driven by detrimental microglial activation may contribute to progressive disease biology in **multiple sclerosis** (MS)
- In the periphery, **Bruton's tyrosine kinase** (BTK) is expressed in both **adaptive** (B cells) and **innate** (monocytes, macrophages) immune cells,[1] whereas in the central nervous system (CNS), BTK is expressed primarily in **microglia**[2]
- **Fenebrutinib (FEN) is a potent, highly selective, noncovalent, reversible BTK inhibitor** (BTKi)[3] that may address MS disease progression by targeting multiple pathogenic mechanisms in both the peripheral and CNS arms of the immune system

Figure 1. Fenebrutinib Proposed Mechanism of Action

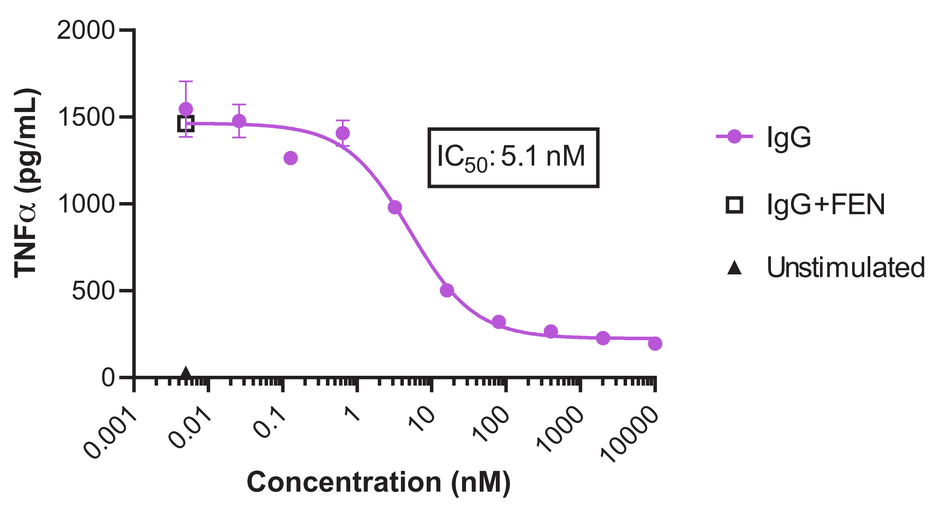


- Much research to date has investigated the effects of BTKis using rodent disease models. Here, we characterise the impact of FEN in blocking BTK-mediated, **disease-associated Fcγ receptor (FcγR) activation** in **human microglia and complex human brain cell systems** to enhance translatability to people with MS

RESULTS

Figure 2. FEN Potently Blocked FcγR-Induced Activation in Human Microglia

- FEN showed similar high potency in microglia to B cells and monocytes



FcγR, Fcγ receptor; FEN, fenebrutinib; IC_{50}, half maximal inhibitory concentration; IgG, immunoglobulin G; TNF-α, tumour necrosis factor α.

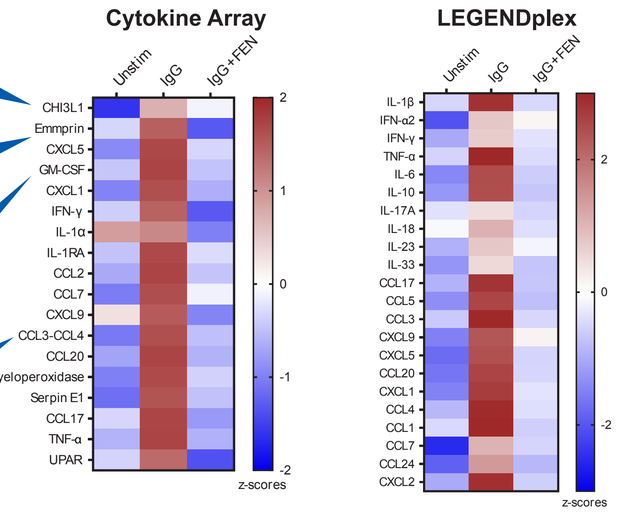
Figure 3. FEN Acts on Human Microglia to Attenuate the Release of Multiple Pro-Inflammatory Cytokines and Chemokines Linked to MS Pathogenesis

CHI3L1 (YKL-40) levels in CSF are associated with disability in progressive MS.[4]

Emmprin/BSG is elevated in CSF of people with MS and regulates leucocyte transmigration.[5,6]

Pro-inflammatory cytokines like GM-CSF and TNF-α induce loss of neurite integrity.[7]

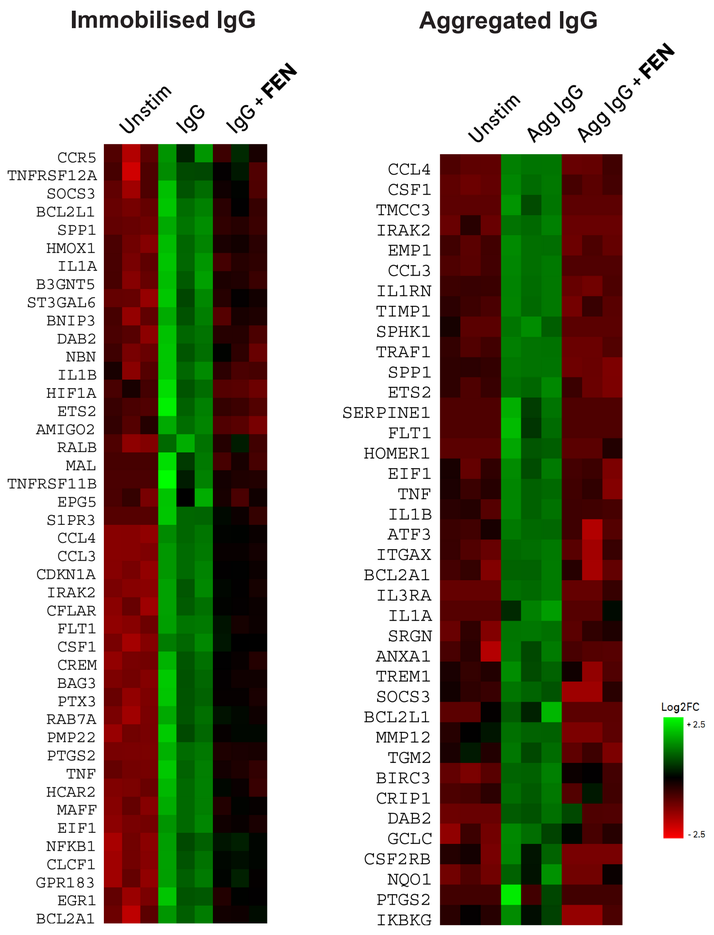
Chemokines (e.g. CCL3, CCL4) mediate leucocyte migration and activation.[8]

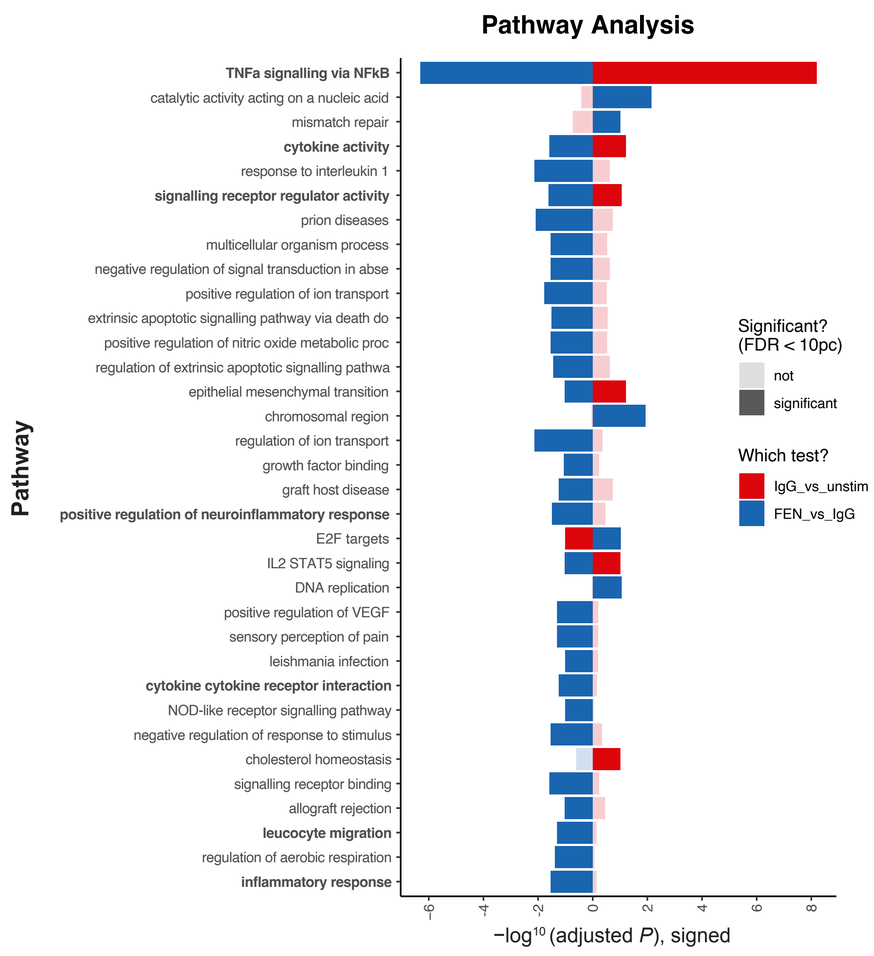


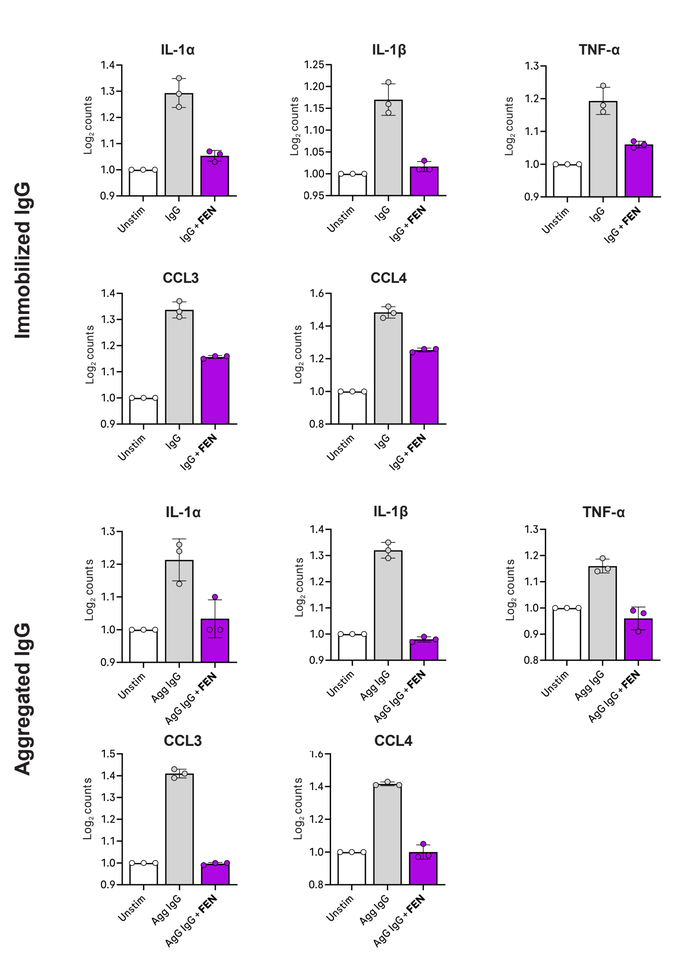
BSG, basigin; CCL, C-C motif chemokine ligand; CHI3L1, chitinase 3 like 1; CSF, cerebrospinal fluid; FEN, fenebrutinib; CXCL, C-X-C chemokine ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN, interferon; IgG, immunoglobulin G; IL, interleukin; MS, multiple sclerosis; TNF-α, tumour necrosis factor α; UPAR, urokinase plasminogen activator receptor.

Figure 4: Gene Expression Analyses Identified Microglial Genes and Pathways Linked to Neuroinflammation and Cytokine Signalling Modulated by FEN

- FEN reversed gene expression changes induced by FcγR signalling in microglia
- Transcriptional signatures of FEN activity included pathways linked to neuroinflammation and cytokine signalling
- Consistent effects of FEN on cytokine (IL-1α, IL-1β, TNF-α) and chemokine (CCL3, CCL4) genes were observed



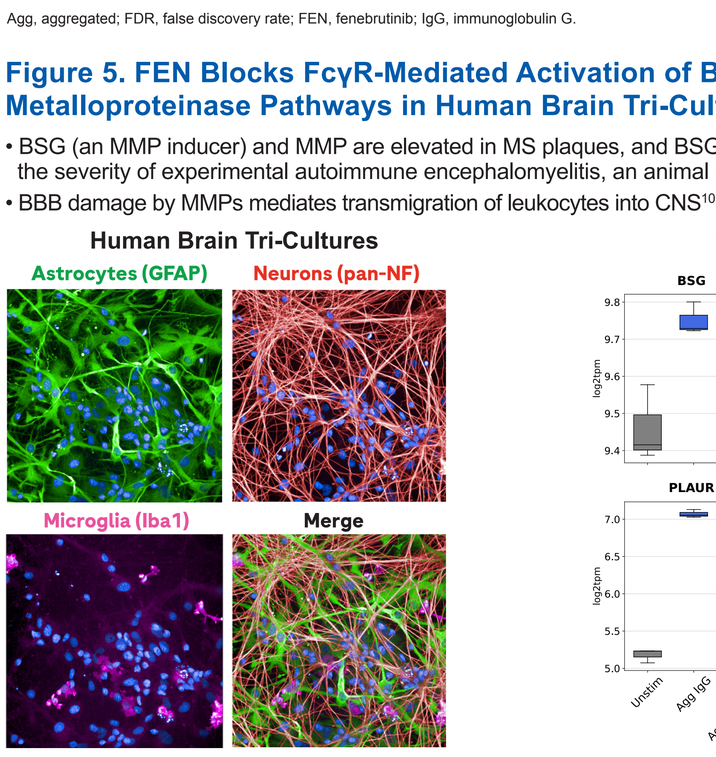


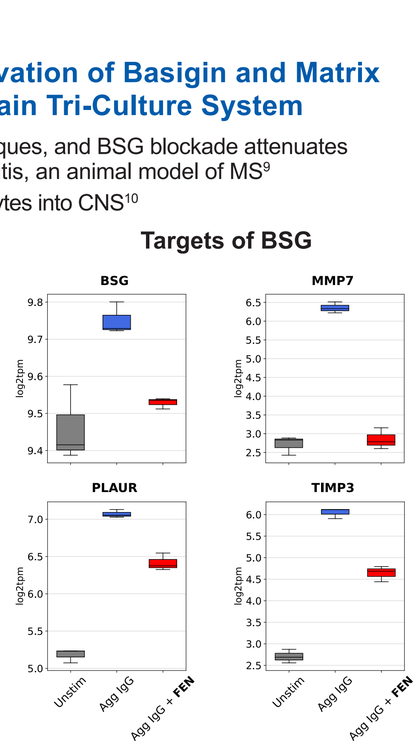


Agg, aggregated; FDR, false discovery rate; FEN, fenebrutinib; IgG, immunoglobulin G.

Figure 5. FEN Blocks FcγR-Mediated Activation of Basigin and Matrix Metalloproteinase Pathways in Human Brain Tri-Culture System

- BSG (an MMP inducer) and MMP are elevated in MS plaques, and BSG blockade attenuates the severity of experimental autoimmune encephalomyelitis, an animal model of MS[9]
- BBB damage by MMPs mediates transmigration of leukocytes into CNS[10]

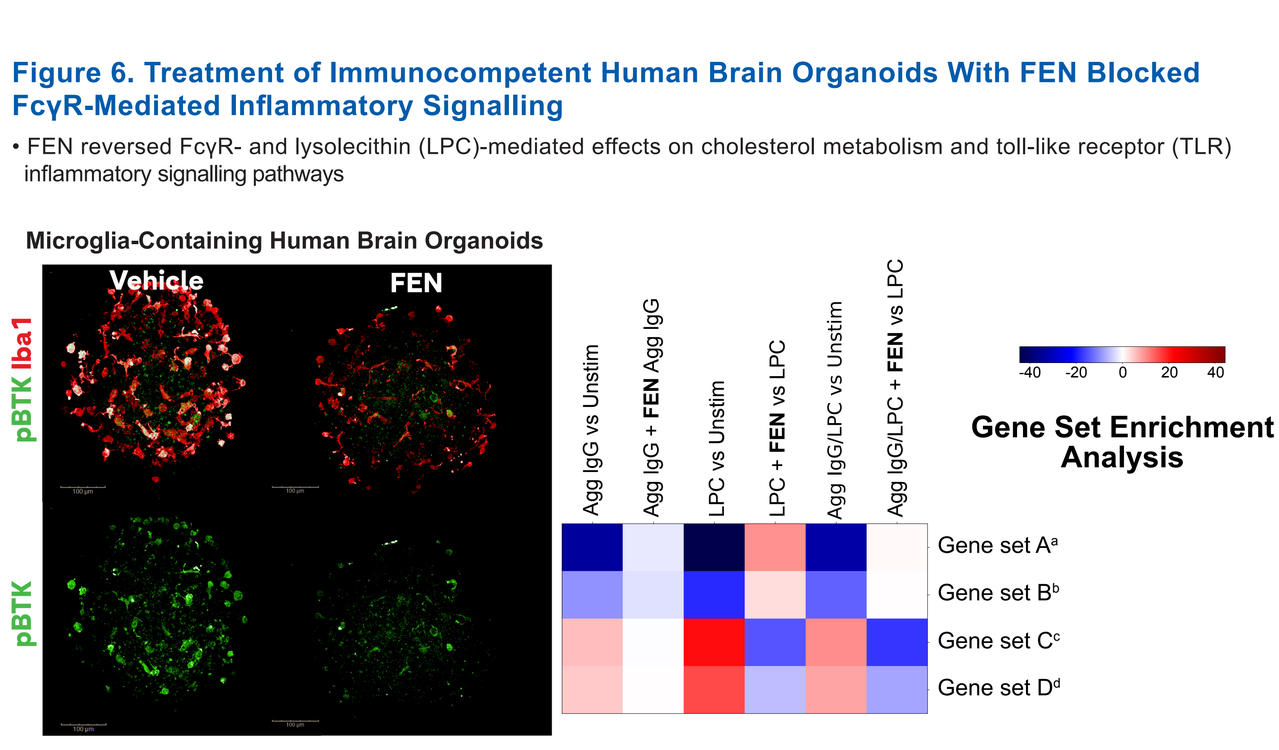




Agg, aggregated; BSG, basigin; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adaptor molecule 1; IgG, immunoglobulin G; MMP, matrix metalloproteinase; NF, neurofilament; PLAUR, plasminogen activator, urokinase receptor; TIMP3, TIMP metallopeptidase inhibitor 3.

Figure 6. Treatment of Immunocompetent Human Brain Organoids With FEN Blocked FcγR-Mediated Inflammatory Signalling

- FEN reversed FcγR- and lysolecithin (LPC)-mediated effects on cholesterol metabolism and toll-like receptor (TLR) inflammatory signalling pathways



Agg, aggregated; BTK, Bruton's tyrosine kinase; FEN, fenebrutinib; Iba1, ionized calcium binding adaptor molecule 1; IgG, immunoglobulin G.
[a]Gene set A = cholesterol_biosynthesis_REACTOME. [b]Gene set B = activation_of_gene_expression_by_SREBF_SREBP_REACTOME. [c]Gene set C = enriched_in_monocytes_(II)_(M11.0). [d]Gene set D = TLR_and_inflammatory_signalling_(M16).

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DISCLOSURES
J Langlois, S Lange, J DeGeer, W Macnair, M Ebeling, R Schmucki, L Collin and **J Keaney** are employees and shareholders of F. Hoffmann-La Roche Ltd. **C Harp** and **Y-A Shen** are employees of Genentech, Inc. and shareholders of F. Hoffmann-La Roche Ltd. **JA Nicholas** has received research grants from Biogen, Novartis, PCORI, Genentech, Inc., University of Buffalo; she has received compensation for consulting from EMD Serono, Genentech, Inc., Greenwich Biosciences, Novartis, TG Therapeutics and Sanofi and speaking honoraria from BMS, EMD Serono, Horizon, TG Therapeutics. **J Oh** has received personal compensation for serving as a consultan from Biogen, Bristol-Myers Squibb, Eli Lilly, Novartis AG, EMD Serono, F. Hoffmann-La Roche Ltd and Sanofi. The institution of Dr Oh has received research support from Biogen, EMD Serono and F. Hoffmann-La Roche Ltd.

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Supplementary Material

Methods

- Efficacy and potency of fenebrutinib (FEN) was assessed in human-induced pluripotent stem cell (iPSC)–derived microglia by measuring tumour necrosis factor α (TNF-α) release following Fcγ receptor (FcγR) activation
- Two different immunoassay methods were used to detect a broad range of cytokines and chemokines modulated by FEN treatment
- Two different methods to activate FcγR signaling in human microglia were used: immobilized immunoglobulin G (IgG) and aggregated IgG
- NanoString gene expression profiling and RNA-sequencing was used to capture transcriptional signatures associated with FEN treatment in iPSC-derived microglia, human brain tri-cultures and human brain organoids