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A-005, a Selective Oral Brain Penetrant TYK2 Inhibitor, Modulates Astrocytes and Microglia

Poster No. P352

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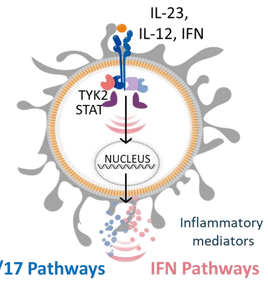
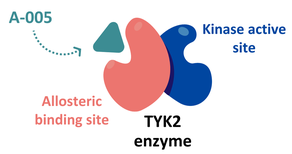
Background





- A-005 is an oral, central nervous system (CNS) penetrant, selective, allosteric tyrosine kinase 2 (TYK2) inhibitor for the treatment of neuroinflammatory and neurodegenerative diseases.

- TYK2 mediates signaling from key proinflammatory cytokines, including IL-23, IL-12, and IL-17 downstream of IL-23, plus type I interferons (IFNs).
- TYK2 is expressed by cells of the immune system, as well as by CNS-resident astrocytes and microglia.
- Pathogenic TYK2 signaling is associated with immune-mediated diseases in both the periphery and CNS.
- Astrocyte and microglial activation is believed to contribute to the 'smoldering inflammation' that is a hallmark of progressive forms of Multiple Sclerosis (MS).[1]
- Clinical validation of TYK2 inhibitors in peripheral autoimmune conditions has been established, and loss-of-kinase-function TYK2 genetic variants are protective for an array of immune-mediated diseases, including MS.[2,3]
- TYK2 inhibition may, therefore, represent a novel approach to treating CNS inflammation that is associated with neurodegeneration and consequent disability in MS.

Objectives

- This study aimed to further evaluate the *in vitro* activity and pharmacology of A-005 in CNS cells - ie, induced pluripotent stem cell (iPSC)-derived astrocytes and microglia - as well as in peripheral B cells.
- In addition, the efficacy of the compound was determined in experimental autoimmune encephalomyelitis (EAE), a widely studied mechanistic model of neuroinflammation.

Methods

- Evaluate A-005 for cellular activity in human iPSC-derived astrocytes and microglia, and in *in vitro* models of human B cell activation.
- Assess the impact of TYK2 inhibition on pro-inflammatory cytokine induction *in vivo*, and on clinical outcomes in a B-cell dependent model of EAE.

References

[1] *Bittner et al. Nat Rev Neurol, 2023.*
[2] *Couturier et al. Brain, 2011.*
[3] *Ban et al. Eur J Hum Genet, 2009.*
[4] *Lyons et al. Eur J Immunol, 1999.*
[5] *Graham et al. ACTRIMS Forum 2024, Poster No. P400*
[6] *Sharma et al. ACTRIMS Forum 2025, Poster No. P335*

Results

A-005 limits TYK2 pathway activation in astrocytes, microglia, and B cells

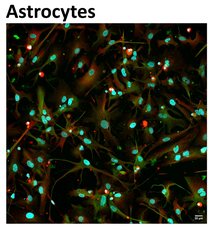
		IC_{50} (nM)		
Stimulus	**Readout**	**Astrocyte**	**Microglial cell**	**B cell**
IFNα	pSTAT3	9.9	2.2	3.4
	pSTAT5	4.9	2.8	1.7
IFNα	IP-10	5.8	1.7	-
CpG-B + IFNα	IP-10	-	-	25.4

Numbers represent the mean IC_{50} value for the indicated readout.
Number of discrete donors used for these studies: Astrocytes = 3; Microglia = 2; B cells = 3-4.
STAT, signal transducer and activator of transcription.

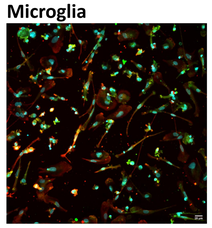
Human iPSC-derived astrocytes, microglia, or primary human B cells were treated with the indicated stimuli. Levels of phosphorylated STAT (pSTAT) proteins were measured by Alpha-LISA and levels of secreted Interferon Gamma-induced Protein 10 (IP-10) were measured by MSD (Meso Scale Discovery) Assay Kit.

TYK2 protein is expressed in iPSC-derived glial cells

Astrocytes **Microglia**



Hoechst/S100B/TYK2

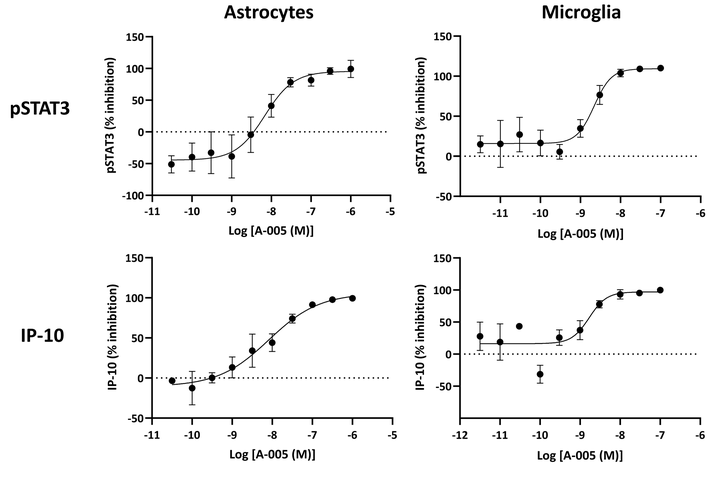


Hoechst/Iba1/TYK2

Human iPSC astrocytes (left) were stained with anti-S100B and anti-TYK2 antibodies, followed by incubation with species-specific AF488- and AF647-conjugated secondary antibodies.
Human iPSC microglia (right) were stained with anti-Iba1 and anti-TYK2 antibodies, followed by incubation with fluorophore-conjugated secondary antibodies.
Astrocytes and microglia were stained with Hoechst as a nuclear marker.

Magnification, 20X; Scale bar, 25 µm.

A-005 suppresses TYK2 pathway activation (IFNα-induced STAT phosphorylation and IP-10 secretion) in iPSC astrocytes and microglia, with nanomolar potency

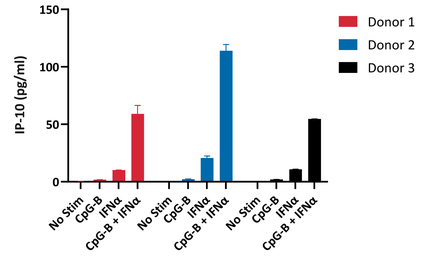


Human iPSC-derived astrocytes (left column) or microglia (right column) were stimulated *in vitro* with IFNα. Levels of pSTAT3 were measured by Alpha-LISA (top row) and levels of secreted IP-10 were measured by MSD Assay Kit (bottom row).

Data are presented as mean percent inhibition ± SD.
Representative graphs are shown.

A-005 inhibits functional responses in B cells

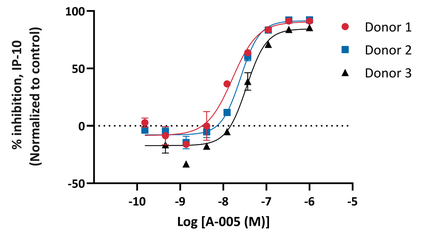
B cells stimulated with a TLR9 agonist + IFNα produce IP-10



Purified B cells from 3 independent human donors were incubated in the presence of media (No stim), CpG-B, IFNα, or CpG-B + IFNα. IP-10 levels in supernatants were measured by MSD Assay Kit.

Bars represent the mean + range of duplicate wells.

A-005 suppresses CpG-B + IFNα-stimulated IP-10 production by B cells



Purified human B cells were stimulated with CpG-B + IFNα in the absence or presence of a concentration range of A-005. IP-10 levels in supernatants were measured by MSD Assay Kit.

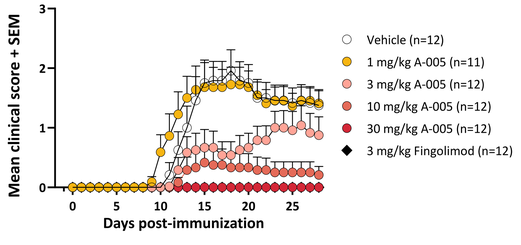
Data are presented as percent inhibition, which was calculated based on the level of IP-10 measured after stimulation with CpG-B + IFNα plus Vehicle.

Symbols represent the mean ± range of duplicate wells.

A-005 exhibits significant and dose-dependent suppression of clinical signs in a B cell-dependent model of EAE

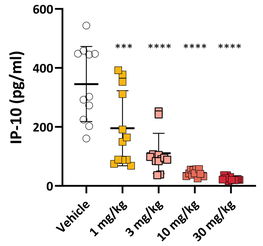
Mice were immunized with full length MOG protein (MOG_{1-125}), an EAE model with pathology that is dependent on B cells.[4] Once daily oral A-005 dosing was initiated 1 day prior to EAE induction.

A-005 achieves complete suppression of clinical EAE



Mann-Whitney U test vs Vehicle:
- p<0.05 for 3 mg/kg (Day 15-22)
- p<0.05 for 10 mg/kg (Day 14-28)
- p<0.05 for 30 mg/kg (Day 13-28)

A-005 suppresses IP-10 induction in vivo



After 7 days of dosing, IP-10 plasma levels were measured by MSD Assay Kit.

p<0.005 (***), p<0.001 (****) vs Vehicle. ANOVA followed by Dunnett's multiple comparisons test.

Conclusions

These data demonstrate that A-005 potently targets microglia and astrocytes, key drivers of chronic neuroinflammation, as well as TYK2 signaling and effector readouts within peripheral B cells.

Consistent with these observations, A-005 exhibits robust efficacy in a B cell-dependent model of EAE, which aligns with previously demonstrated efficacy for A-005 in T cell-dependent EAE models.[5]

- A-005 has successfully completed a Phase 1 (Ph1) study[6], and we plan to initiate a Ph2 proof-of-concept study in MS in 2025.
- TYK2 inhibition also has the potential to ameliorate other human neuroinflammatory and neurodegenerative diseases.