ACTRIMS
March 1, 2024

A Selective, Allosteric TYK2 Small-molecule Inhibitor Modulates Immune Cell Functions and Ameliorates Experimental Autoimmune Encephalomyelitis.

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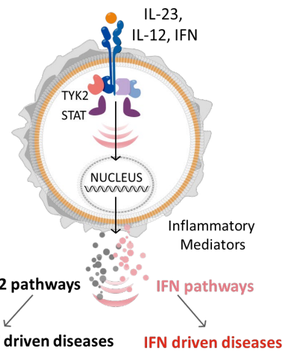
Disclosures:
Commercial support was provided by Alumis Inc.
All authors are employed by Alumis. The authors have no other relationships or conflicts of interest to disclose.

Background

Tyrosine kinase 2 (TYK2) is a member of the Janus kinase (JAK) family of proteins. Expressed by cells of the immune system, TYK2 mediates signaling responses to several cytokines, including interleukin (IL)-23, IL-12 and interferon-alpha (IFNα). Clinical validation of TYK2 inhibitors in peripheral autoimmune conditions has been established, with a recent approval for psoriasis, and trials are ongoing with TYK2 inhibitors in other indications [1]. Human genetic evidence suggests that TYK2 loss-of-function variants are associated with protection from developing Multiple Sclerosis (MS[2,3]). TYK2 inhibition may therefore represent a novel approach to treating MS.



- TYK2 mediates signaling from key proinflammatory cytokines, including IL-23, IL-12, and IL-17 downstream of IL-23, plus type I IFNs
- Selectively targeting TYK2 avoids safety liability of JAK inhibitors
- TYK2 biology may be relevant in CNS microglial immune responses

Objectives

The objective of this study was to evaluate the potency, selectivity, and cellular pharmacology of A-005, a novel, allosteric, brain-penetrant TYK2 small molecule inhibitor. The compound was also evaluated for exposure in the central nervous system (CNS), as well as efficacy in experimental autoimmune encephalomyelitis (EAE).

Methods

We determined the affinity of A-005 binding for the TYK2 regulatory (JH2) and kinase (JH1) domains in a biochemical assay. Potency and selectivity of the compound were evaluated in a commercial kinase panel. The effect of the compound on immune cell activity was evaluated in human peripheral blood mononuclear cells (PBMC), whole blood, and microglial cells. Cerebrospinal fluid (CSF) exposure was measured and microdialysis was performed in rats to evaluate the potential of the compound to cross the blood-brain barrier. Finally, we assessed the impact of the compound on clinical signs in mouse EAE.

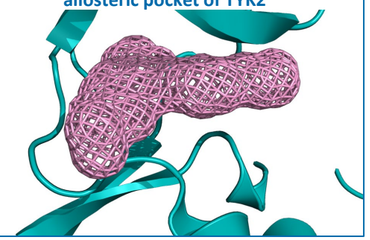
References

1. Jensen et al. EBioMedicine, 97: 104840
2. Couturier et al. Brain, 134: 693
3. Ban et al. Eur J Hum Genet, 17: 1309
4. Gorman et al. Front Immunol, 10: 44.

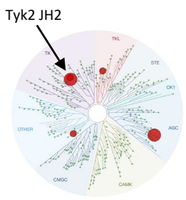
Results

- Pseudokinase/regulatory JH2 domain
- Kinase JH1 domain
- Allosteric binding pocket
- Typical kinase inhibitor binding pocket
- Intrinsic selectivity versus JAK inhibitors
- Selectivity vs JAK inhibitors is challenging

Model depicting A-005 binding in the allosteric pocket of TYK2



A-005 allosteric binding minimizes off-target activity, with > 100-fold selectivity over other kinase panel hits



Target	Kd (nM)	Selectivity
TYK2 JH2*	0.017	-
JAK1 JH2*	2.5	149-fold
JAK1 JH1	>10,000	>595,000
JAK2 JH1	737	43,869
JAK3 JH1	737	43,869
TYK2 JH1	>30,000	>1,786,000
BMPR2*	80	4,762
GCN2*	88	5,238
RSK3*	1,700	>100,000

*Eurofins kinase panel hits >90% at 1 μM A-005

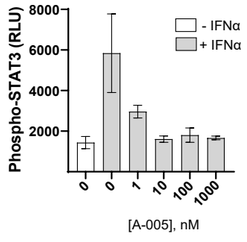
A-005 exhibits no measurable inhibition of JAK1 pathways in human PBMCs and whole blood

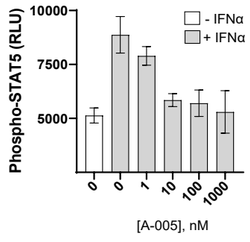
Stimulus	Readout	Pathway	Human whole blood IC_{50} (nM)	Human PBMC IC_{50} (nM)	HMC3 microglia IC_{50} (nM)
IFNα	pSTAT3	TYK2	30	3.7	5.4
IFNα	pSTAT5		31	3.3	4.8
IL-12	pSTAT4		56	8.1	N/A
IL-2	pSTAT5	JAK1/JAK3	>1,000	>1000	N/A
TPO	pSTAT5	JAK1/JAK2	>1,000	N/A	N/A

TPO, thrombopoietin; pSTAT, phosphorylated signal transducer and activator of transcription

A-005 potently inhibits TYK2-mediated pathways in human immune cells and microglia

A-005 inhibits IFNα-stimulated STAT phosphorylation in iPSC-derived microglia



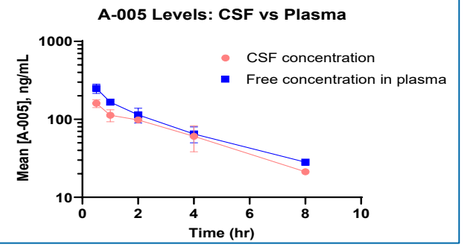


Human iPSC-derived microglia were stimulated with recombinant human IFNα in the absence or presence of A-005. After a 15-minute stimulation period, cells were lysed and levels of phosphorylated STAT3 (left) or STAT5 (right) were measured. Data are presented as mean relative luciferase units (RLU) +/- S.D. Results shown are representative of two independent experiments (biological replicates) with similar results.

A-005 achieves comparable exposures in rat CSF and in the periphery

	CSF (Free)	Plasma (Free Fraction)	Ratio (CSF/Plasma)
T_{max} (hr)	0.5	0.5	---
C_{max} (ng/mL)	160	235	**0.68**
AUC_{last} (ng•hr/mL)	536	640	**0.84**

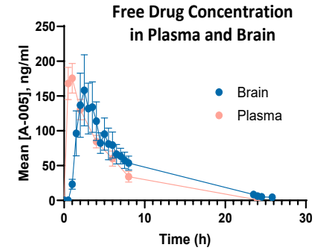
A-005 dosed at 10 mg/kg to female CD Rats (n=3): Rat PPB = 94.35%
Study design: Single PO dose; samples collected up to 8 h



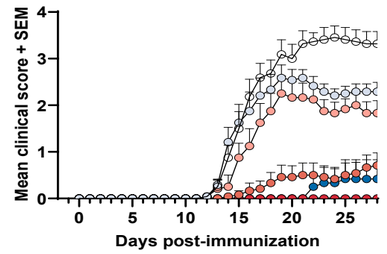
Results

Rat microdialysis: Pharmacologically relevant brain exposures achieved (Kp_{uu} = 1.3)

Free Drug Concentration in the Matrix	Tmax (h)	Cmax (ng/mL)	AUCt (ng*h/mL)	AUCinf (ng*h/mL)
Brain				
Geometric mean	2.9	137	864	886
Geometric mean %CV	23	74.9	54.2	49
Plasma				
Geometric mean	0.66	151	637	679
Geometric mean %CV	39	25.4	24	22



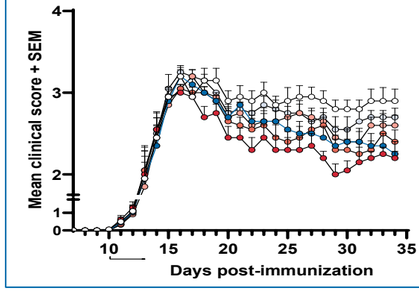
Dose-dependent attenuation of MOG_{35-55}-induced EAE by A-005: Prophylactic dosing



- Vehicle (n=12)
- 1 mg/kg A-005 (n=12)
- 3 mg/kg A-005 (n=12)
- 10 mg/kg A-005 (n=12)
- 30 mg/kg A-005 (n=12)
- 3 mg/kg Fingolimod (n=6)

A-005 recapitulates TYK2 human loss of function variant knock-in (P1104A) mouse EAE data [4].
QD PO dosing initiated 1 day prior to EAE induction. Mann-Whitney U-test vs vehicle, p<0.05 for 1mg/kg (day 20-28); 3mg/kg (day 20-28); 10mg/kg (day 14-28); 30mg/kg (day 14-28)

Dose-dependent attenuation of MOG_{35-55}-induced EAE by A-005: Therapeutic dosing



- Vehicle (n=10)
- 1 mg/kg A-005 (n=10)
- 3 mg/kg A-005 (n=10)
- 10 mg/kg A-005 (n=10)
- 30 mg/kg A-005 (n=10)
- 3 mg/kg Fingolimod (n=10)

QD PO dosing initiated at the onset of EAE clinical signs (enrollment period, days 10-13). Experiment continued until each mouse had been dosed for at least 21 days (day 34). Mann-Whitney U-test vs vehicle, p<0.05 for 10 mg/kg (day 21, 25-27, 29-32, 34) ; 30 mg/kg (day 18-34)

Conclusions

- A-005 is a highly potent and selective allosteric small-molecule TYK2 inhibitor expected to enter human clinical trials in early 2024.
- A-005 inhibits TYK2 pathway activation in human whole blood, PBMCs and microglial cells.
- A microdialysis study in rats shows the ability of A-005 to cross the blood brain barrier.
- A-005 reduces EAE clinical scores when administered prophylactically or therapeutically.
- Dose levels in Ph1 clinical trials are anticipated to achieve full target inhibition in both the CNS and the periphery.

References

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