Potent and Selective Tyk2 Inhibitor is Highly Efficacious in Rodent Models of Inflammatory Bowel Disease and Psoriasis

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BACKGROUND

Tyk2, a member of the JAK family kinases, is a key mediator of IL-12, IL-23, and Type I Interferon signaling. These cytokines have been implicated in the pathogenesis of multiple inflammatory and autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis and inflammatory bowel diseases (IBD). Supported by compelling data from human genetic association studies, Tyk2 inhibition is an attractive therapeutic strategy for autoimmune diseases.

METHODS

We utilized cutting edge proprietary structure-based drug design tools to identify highly selective Tyk2 inhibitors. These inhibitors were characterized for their drug-like properties, potency and selectivity in enzyme and cell-based assays, and in mouse models of IBD and psoriasis.

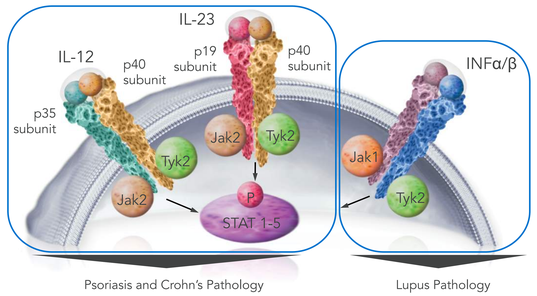
RESULTS

Here we describe the identification of NDI-031407, a small molecule catalytic inhibitor of Tyk2, that exhibits potent enzyme and cellular activity, and is highly selective against other JAK family members and against panels of receptors, transporters, ion channels, CYP enzymes and the hERG channel. This compound inhibits Tyk2 with a Ki of 0.2 nM in a biochemical assay, and is 218-, 148-, and 20-fold selective against JAK1, JAK2, and JAK3, respectively. Cell-based potency and selectivity of NDI-031407 was demonstrated in human PBMC assays by blockade of IL-12 induced phospho-STAT4, GM-CSF induced phospho-STAT5, and IL-2 induced phospho-STAT5, with IC_{50} of 0.10 μM, 4.1 μM and 0.25 μM, respectively. In addition, NDI-031407 inhibited IL-12 induced IFNα with IC_{50} of 2.7 μM and 7.0 μM in human and mouse whole blood, respectively. We investigated the *in vivo* efficacy of NDI-031407 in mouse models of psoriasis and IBD. In an IL-23-induced mouse ear inflammation model, NDI-031407 dose-dependently reduced disease with up to 74% inhibition of ear swelling and 96% inhibition of tissue levels of IL-17A at 100 mg/kg, highlighting the crucial role of Tyk2 inhibition in Th17 pathogenesis mediated by IL-23. In addition, NDI-031407 was highly efficacious in an imiquimod-induced mouse psoriasis model. NDI-031407 at 100 mg/kg achieved the same efficacy as dexamethasone in reduction of psoriasis score without body weight reduction. NDI-031407-treated mice had improved skin histology and dose-dependent reduction of spleen weight. To investigate the role of Tyk2 inhibition in Th1-driven pathogenesis, we tested NDI-031407 in a $CD4^+CD45RB^{High}$ adoptive transfer model that resembles the pathology of human Crohns' disease. NDI-031407 treatment improved disease outcomes by reduction of body weight loss and colonic weight/length ratio, and improved colon histology. 100 mg/kg of NDI-031407 treatment also reduced colon myeloperoxidase levels to those of disease-free control mice, demonstrating the remarkable anti-inflammatory efficacy of Tyk2 inhibitors in this disease model.

CONCLUSION

Utilizing unique and innovative structure-based drug design technologies, we rapidly designed highly selective and potent Tyk2 inhibitors with suitable pharmaceutical properties as potential therapeutics in inflammatory disorders. We validated the vital role of Tyk2 in disease pathogenesis of psoriasis and IBD in preclinical mouse models.

1. Tyk2: Key Mediator of Th17 and Th1 Pathogenesis



2. Potency, Selectivity, and Drug-Like Properties of Tyk2 Lead Molecule NDI-031407

			NDI-031407
Biochemical Tyk2 Kinase Assay		Tyk2 Ki (nM)	0.21
JAK Family Kinase Biochemical Selectivity (Fold over Ki)		Fold Selectivity over JAK2	148x
		Fold Selectivity over JAK1	218x
		Fold Selectivity over JAK3	20x
Plasma Protein Binding		Human PPB (%bound)	90
Human *in vitro* metabolism		Cl_{int} (mL/min/kg) Microsomes	0.62
		Cl_{int} (mL/min/kg) Hepatocytes	5.2
Mouse PK	IV 3 mg/kg	Cl_{obs} (mL/min/kg)	25
	PO 30 mg/kg	$T_{1/2}$ (h)	0.76
		C_{max} (μM)	19
		F (%)	100%
		AUC (ng/mL*hr)	28,238

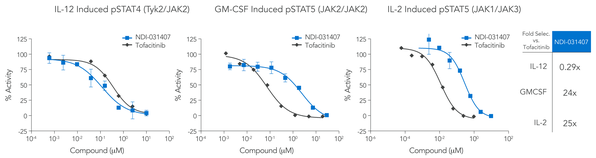
All kinase assays were performed in the radiometric format with peptide substrates. PK study was conducted in C57BL/6 mice. The vehicle for NDI-031407 was 20% HPβCD in saline for IV dosing and 0.5% methylcellulose for PO dosing.

3. NDI-031407 Is A Potent Tyk2 Inhibitor with Excellent Selectivity Against JAK Family Kinases

Cell-Based Potency

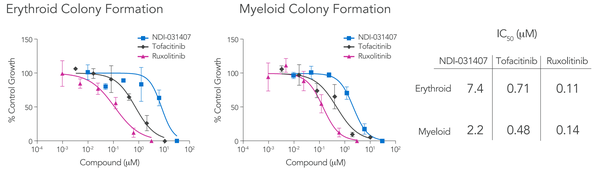
IC_{50} (nM)	PBMC_IL12/pSTAT4	PBMC_GMCSF/pSTAT5	PBMC_IL2/pSTAT5	NK92_IL12/IFNγ	hWB_IL12/IFNγ	mWB_IL12/IFNγ
NDI-031407	100	4100	250	59	2700	7000

Human PBMC Cellular Selectivity



Human PBMC were pre-incubated with the compound for one hour and stimulated with IL-12 for 30 minutes, or GM-CSF or IL-2 for 10 minutes. Cell lysates were prepared and phospho and total STAT protein was measured by MSD. The ratio of pSTAT/total STAT was used for IC_{50} calculation. NK92 assay was performed by incubating the compound with the IL-2 starved NK92 cells for one hour following by IL-12 stimulation for 24 hrs. Human and mouse whole blood assay was performed by incubating the compound with blood for one hour followed by IL-12 stimulation for 24 hours on anti-CD3 antibody coated plate. At the end of the incubation, IFNγ level in the supernatant was quantified by ELISA.

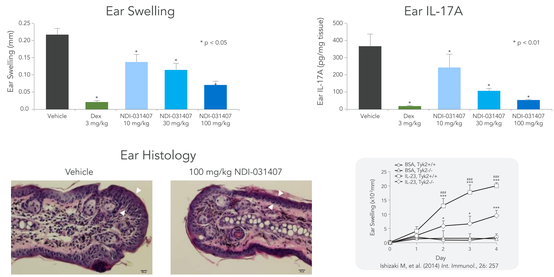
4. NDI-031407 Demonstrated Reduced Activity on Erythroid and Myeloid Progenitor Cell Proliferation Compared to Other JAK Inhibitors



IC_{50} (μM)	NDI-031407	Tofacitinib	Ruxolitinib
Erythroid	7.4	0.71	0.11
Myeloid	2.2	0.48	0.14

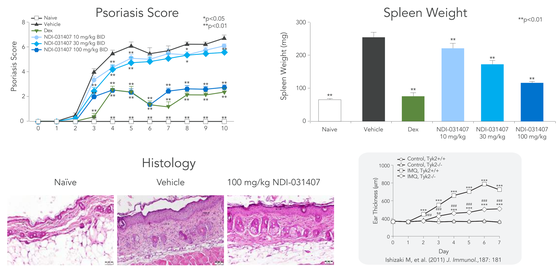
In vitro human bone marrow derived erythroid and myeloid progenitor cell colony forming assays were performed in media containing EPO, G-CSF, GM-CSF, IL-3, and SCF. Compounds were added to the culture and incubated for 14 days. Hematopoietic colonies were assessed and scored at the end of the incubation period. % control growth was calculated using DMSO-treated samples as 100%.

5. NDI-031407 Reduced Inflammation in Murine Model of IL-23-induced Psoriasis



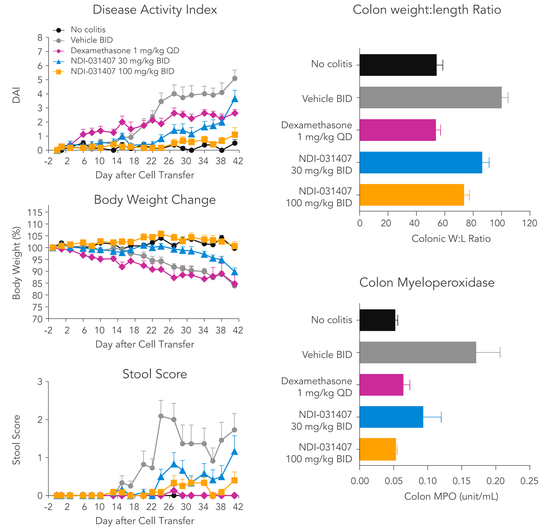
Ear swelling was induced by injecting recombinant murine IL-23 into the ears of C57BL/6 mice once daily for 4 days. Vehicle (0.5% methylcellulose) or NDI-031407 were dosed twice daily orally and dexamethasone was dosed once daily IP. Ear swelling was the difference between the ear thickness measured prior to the first IL-23 injection and one day post the last IL-23 injection. Ears were harvested and homogenized one hour post the last compound dose. IL-17A in the tissue homogenate was measured by Luminex kit. p values are student t-test vs. the vehicle. Ear skin was harvested for histology.

6. NDI-031407 Reduced Disease in Murine Model of Imiquimod-induced Psoriasis



Psoriasis was induced by topical application of Imiquimod cream once daily on the shaved back skin of Balb/c mice for 11 days. Vehicle (0.5% methylcellulose) or NDI-031407 were dosed twice daily and dexamethasone was dosed once daily orally. Skin inflammation was scored daily. Spleens were collected one hour post the last compound dose and weighed. p values are student t-test vs. the vehicle. Back skin was harvested for histology.

7. NDI-031407 Is Efficacious in $CD4^+CD45RB^{high}$ Mouse Adoptive Transfer Colitis Model



$CD4^+CD45RB^{high}$ cells were isolated from spleens of BALB/c mice and adoptively transferred into SCID mice intravenously to induce colitis. Vehicle (0.5% methylcellulose) or NDI-031407 were dosed twice daily orally and dexamethasone was dosed once daily intraperitoneally, starting on the day of T cell transfer. Body weight and stool score were collected three times per week. Daily disease activity index (DAI) was calculated based on body weight and stool scores. At the end of the study, colons were harvested to measure colon weight, length, and myeloperoxidase.

SUMMARY

- Tyk2 is a target for inflammatory diseases with pathogenic pathways involving IL-12, IL-23 and Type I Interferon.
- Nimbus has discovered potent Tyk2 inhibitors with excellent JAK family kinase selectivity.
- Lead compound NDI-031407 demonstrated potent inhibition of IL-12 signaling in human PBMC and mouse and human whole blood.
- *In vivo* studies in murine models of psoriasis and IBD demonstrated that NDI-031407 was efficacious in suppressing diseases with Th1 and Th17 pathogenic mechanisms. The preclinical safety profile of NDI-031407 is currently under evaluation.
- Nimbus compounds have excellent drug-like properties, are well tolerated, and are candidates for further development in inflammatory diseases with excessive IL-12, IL-23 and Type I Interferon signaling.

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