Identification of Highly Potent and Selective Tyk2 Inhibitors for the Treatment of Autoimmune Diseases Through Structure-based Drug Design

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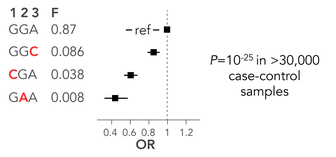
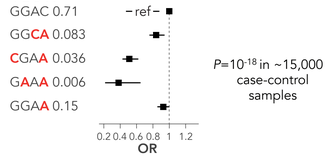
ABSTRACT

The JAK family kinase Tyk2 is essential for IL-12 and IL-23 signaling, which are associated with Th1 and Th17 cell differentiation and activation. The Th1 and Th17 pathways have been implicated in the pathogenesis of psoriasis and inflammatory bowel diseases (IBD) thereby making Tyk2 a highly attractive target for the treatment of these disorders. However, given the high degree of sequence homology between the JAK family kinases, designing potent and selective Tyk2 inhibitors remains a challenge. Using an innovative structure-based approach, we have designed, synthesized and characterized small molecule inhibitors optimized for JAK family selectivity using computational free energy perturbation (FEP) methods and medicinal chemistry SAR. We have identified selective Tyk2 inhibitors with pM activity against Tyk2 (K_i=140-520 pM) and >100 fold selectivity over JAK2 and JAK1 with more moderate selectivity over JAK3. These analogs are orally bioavailable (85%F) with suitable drug-like properties. NDI-031232 was determined to be highly selective across 359 kinases, and is a potent inhibitor of IL-12 induced pSTAT4 in hPBMCs (IC_{50}=17 nM) and IL-12 induced IFNγ in human whole blood (IC_{50}=520 nM). NDI-031232 also demonstrated robust efficacy in blocking IL-12-mediated IFNγ production in an *ex vivo* mouse model. Therefore, selective inhibitors of Tyk2 retain the anti-inflammatory activity while reducing potential for dose-limiting side effects observed with non-selective JAK inhibitors.

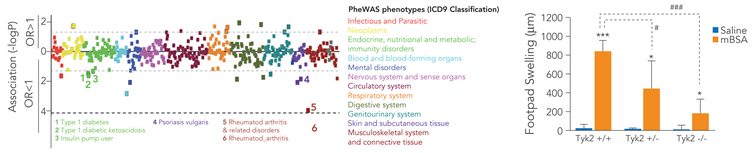
Multiple Alleles Protect from RA



Multiple Alleles Protect from SLE



No Obvious Adverse Events in ~30K Patients

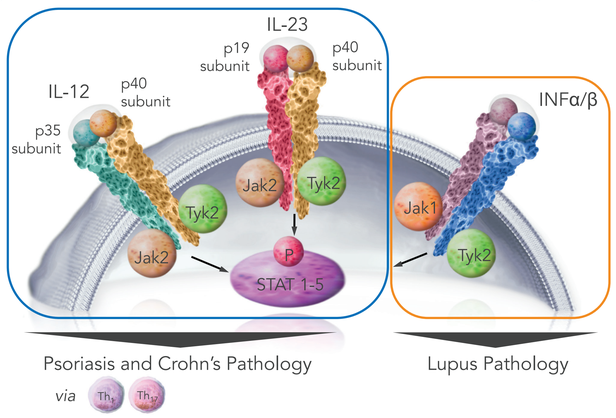


Diogo D, et al. (2015) PLoS ONE 10: e0122271

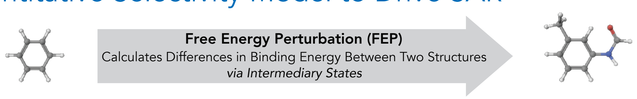
mBSA-induced DTH Model

Ishizaki M, et al. (2011) J. Immunol.,187: 181

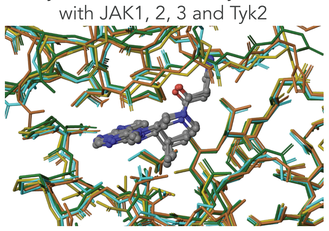
1. Tyk2: Key Mediator of Th17 and Th1 Pathogenesis



2. Free Energy Perturbation (FEP) Used to Develop Quantitative Selectivity Model to Drive SAR

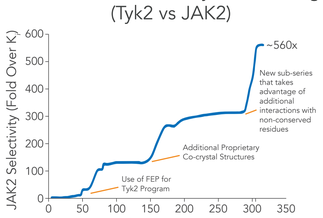


Similarity of Tofacitinib Co-Crystal Structure with JAK1, 2, 3 and Tyk2



- Active sites virtually identical
- Gatekeeper residue (methionine) conserved across JAK family

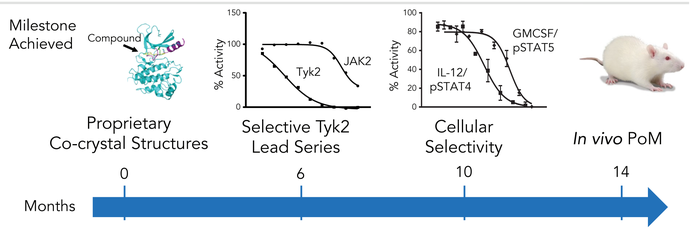
FEP-enabled Selectivity Breakthrough (Tyk2 vs JAK2)



See details in Wang, L., et al. (2015) J. Am. Chem. Soc.,137: 2695

3. Highly Selective Lead Series Identified in ~10 Months

Ideas	6MM	3,000	12,400	31,979
Compounds Synthesized		90	260	400
Tyk2 Potency		2.5 nM	0.5 nM	0.3 nM
Selectivity over JAK2	0x	>120x	>250x	>500x



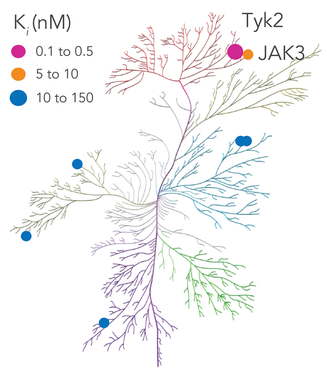
4. Potency, Selectivity, and Drug-Like Properties of Tyk2 Lead Candidates

			NDI-031232	NDI-031301
Biochemical Tyk2 Kinase Assay		Tyk2 Ki (nM)	0.14	0.53
JAK Family Kinase Biochemical Selectivity (Fold over Ki)		Fold Selectivity over JAK2	210x	85x
		Fold Selectivity over JAK1	93x	107x
		Fold Selectivity over JAK3	24x	15x
Plasma Protein Binding		Human PPB (%bound)	83	74
Human *in vitro* metabolism		Cl_{int} (mL/min/kg) Microsomes	5	3
		Cl_{int} (mL/min/kg) Hepatocytes	2	9
Mouse PK	IV 3 mg/kg	Cl_{obs} (mL/min/kg)	35	39
	PO 30 mg/kg	$T_{1/2}$ (h)	1.6	4.8
		C_{max} (μM)	15	13
		F (%)	85	100
		AUC (μM*hr)	29	38
Physical Properties		MW (Da); solubility (μM)	400-450; 10	400-450; 302

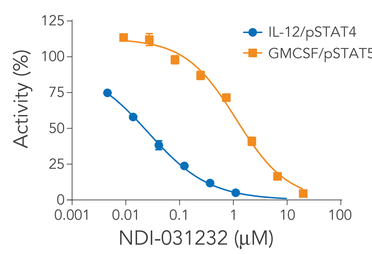
All kinase assays were performed with radiolabeled peptides. PK study was conducted in C57BL/6 mice. The vehicle for NDI-031232 was 20% DMSO/60% PEG400 in saline, and the vehicle for NDI-031302 was 20% HPβCD in saline.

5. NDI-031232 Is A Potent and Selective Tyk2 Inhibitor

Kinome Selectivity

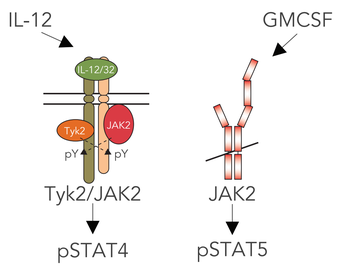


Cellular Selectivity

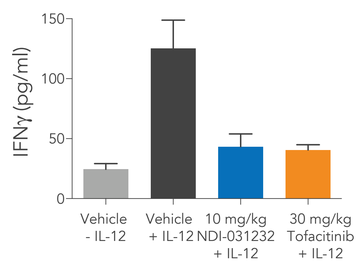


- IL-12 induced pSTAT4 IC_{50} = 17 nM
- GMCSF induced pSTAT5 IC_{50} ~ 1.2 μM
- Selectivity Tyk2/JAK2 = 70x

Cell-Based Assay



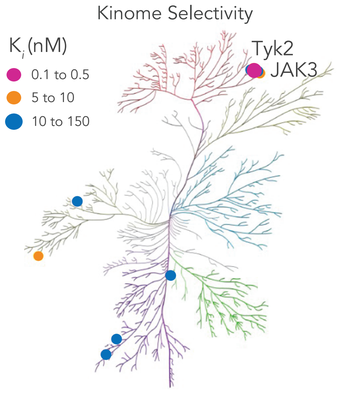
Blocks *ex vivo* IL-12-induced INFα in the Whole Blood of NDI-031232 Dosed Mice



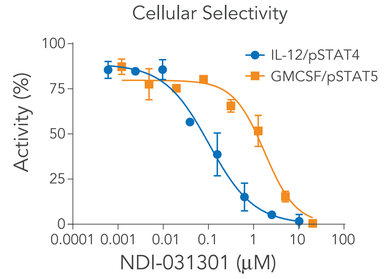
Kinome selectivity is based on radio-peptide kinase assays. For cell-based assays, human PBMC were pre-incubated with NDI-031232 for one hour and stimulated with 1.67 ng/ml of IL-12 for 30 minutes or 10 ng/ml of GMCSF for 10 minutes. Cell lysates were prepared and phospho and total STAT protein was measured by MSD. The ratio of pSTAT/total STAT was used for IC_{50} calculation. For the *ex vivo* study, C57BL/6 mice were dosed orally with vehicle (20% DMSO/60% PEG400 in saline), NDI-031232, or Tofacitinib. Whole blood was collected 30 minutes post dose and stimulated with 2 ng/ml IL-12 on anti-CD3 antibody coated plate for 24 hours. At the end of the incubation, IFNγ level in the supernatant was quantified by MSD.

6. NDI-031301 Is a Potent and Selective Tyk2 Inhibitor

Kinome Selectivity

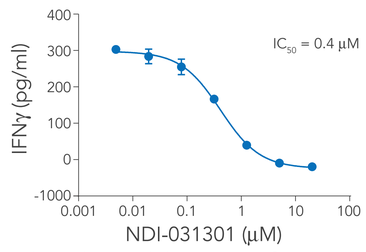


Cellular Selectivity



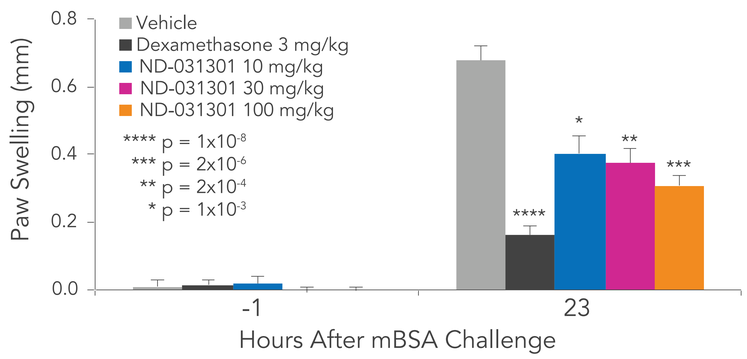
- IL-12 induced pSTAT4 IC_{50} = 74 nM
- GMCSF induced pSTAT5 IC_{50} ~ 4.5 μM
- Selectivity Tyk2/JAK2 = 61x

Human Whole Blood Activity



Kinome selectivity is based on radio-peptide kinase assays. Cell selectivity assays were performed with human PBMC as above. Human whole blood assay was performed by incubating NDI-031301 with human whole blood for one hour followed by 0.5 ng/ml of 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.

7. NDI-031301 Reduces Inflammation in the mBSA-induced Delayed Type Hypersensitivity Model



C57BL/6 mice were immunized with 0.11 mg of methylated BSA/CFA emulsion at lower back on day 0. Mice were challenged on day 5 by injecting 0.1 mg mBSA/PBS solution into one hind paw and PBS into the other hind paw. Vehicle (20% HPβCD in saline) or NDI-031301 were dosed twice daily orally and Dexamethasone was dosed once daily IP on day 0 through day 5. Paw thickness was measured one day post the challenge (day 6) and paw swelling was calculated by subtracting the thickness of the PBS paw from the mBSA paw of the same mouse. Mice received a final dose on day 6 and plasma was collected one hour post the dose and analyzed for NDI-031301 by LC/M/MS. p values are student t-test vs. the vehicle.

CONCLUSIONS

- Tyk2 is a sought after target for the treatment of autoimmune diseases with compelling human genetic data from GWAS/PheWAS studies
- Given the high degree of structural homology amongst the JAK kinase family members, designing potent and selective inhibitors has remained a considerable challenge
- Using a physics-based computational approach, Nimbus has uncovered previously unexploited drivers of potency and JAK family selectivity
- Potent inhibition of IL-12-induced STAT4 phosphorylation and cytokine production was observed in human PBMC and whole blood and the Nimbus compounds maintain high levels of functional selectivity over JAK2
- *In vivo* proof of mechanism was demonstrated in a mouse delayed type hypersensitivity model
- Nimbus compounds have good drug-like properties and are candidates for further development for inflammatory diseases

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