# Potent and Selective Tyk2 Inhibitors Block Th1- and Th17- Mediated Immune Responses and Reduce Disease Progression in Rodent Models of Delayed-Type Hypersensitivity and Psoriasis

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NIMBUS THERAPEUTICS

## BACKGROUND/PURPOSE

Tyk2 is a member of the JAK family kinases and 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 lupus, psoriasis and inflammatory bowel diseases. Supported by compelling data from human genetic association studies, Tyk2 inhibition is an attractive therapeutic strategy for these diseases.

## METHODS

One of the challenges of developing selective Tyk2 inhibitors is the high sequence homology of the active site among the members of the JAK family kinases. We utilized cutting edge proprietary structure-based drug design tools to identify highly potent and selective inhibitors of Tyk2. These inhibitors were characterized for their potency and selectivity in the enzyme and cell-based assays, and in mouse models of delayed type hypersensitivity and psoriasis.

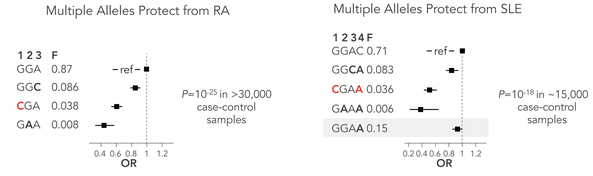
## RESULTS

We have identified Tyk2 inhibitors with up to 720-, 540-, and 210-fold selectivity against JAK1, JAK2, and JAK3 respectively, with potent cellular activity and excellent cellular selectivity against other JAK family kinases in human peripheral blood mononuclear cells. NDI-031301 is a Tyk2 inhibitor with a Ki of 0.5 nM that is 107-, 85-, and 15-fold selective against JAK1, JAK2, and JAK3 respectively. It blocks IL-12 induced phospho-STAT4 and GM-CSF induced phospho-STAT5 in human PBMCs with $IC_{50}$ of 0.1 μM and 2.6 μM, respectively. NDI-031301 has excellent selectivity against a panel of 364 kinases, showing less than 70% inhibition at 300 nM against all but 16 of the kinases tested. It also showed less than 50% inhibition up to 30 μM against human CYP enzymes and hERG channel. In addition, NDI-031301 has an attractive PK profile with good oral bioavailability in rodents and dogs. Studies with humans carrying inactive forms of Tyk2 and mice deficient in Tyk2 revealed a role in Th1 and Th17 polarization. We investigated the *in vivo* activity and mechanism of action of Nimbus Tyk2 inhibitors in a methylated-BSA induced mouse delayed type hypersensitivity model. At 100 mg/kg dose, orally administered NDI-031301 reduced paw swelling and paw weight, as well as Th1 (IFNγ) and Th17 (IL-17A and IL-22) cytokines in the inflamed paws by more than 50%. It also dramatically reduced Th1 cells in the draining lymph nodes and suppressed over 85% of in vitro antigen-induced IFNγ response in the draining lymph node cells. In an IL-23-induced mouse psoriasis model, NDI-031301 dose-dependently reduced skin inflammation with up to 76% inhibition of ear swelling at 100 mg/kg, highlighting the role of Tyk2 inhibition in Th17 pathogenesis. Finally, NDI-031301 was highly efficacious in an imiquimod-induced mouse psoriasis model, showing dose-dependent reduction of psoriasis score, spleen weight, and improved skin histology. 30 mg/kg of NDI-031301 treatment blocked disease progression and 100 mg/kg treatment reversed the disease.

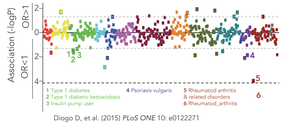
## CONCLUSION

Utilizing unique and innovative structure-based drug design technologies, we rapidly designed highly potent and selective Tyk2 inhibitors for use as potential therapeutics in inflammatory disorders involving Th1, Th17, and type I interferon pathogenesis.

## 1. Human Genetic Studies Implicate Tyk2 in RA and SLE Susceptibility




No Obvious Adverse Events in ~30K Patients




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

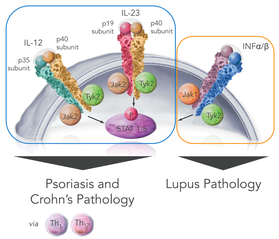
**PheWAS phenotypes (ICD9 Classification)**

- Infectious and Parasitic
- Neoplasms
- Endocrine, nutritional and metabolic; immunity disorders
- Blood and blood-forming organs
- Mental disorders
- Nervous system and sense organs
- Circulatory system
- Respiratory system
- Digestive system
- Genitourinary system
- Skin and subcutaneous tissue
- Musculoskeletal system and connective tissue

## 2. Broad Potential of Tyk2 Inhibition Across Autoimmune Indications of High Unmet Medical Need

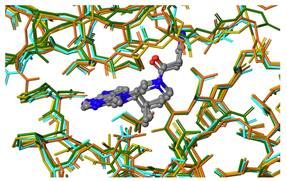
| | | | |
|---|---|---|---|
| Psoriasis | nature genetics | A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1 | OR of 1.4 |
| Crohn's Disease | nature genetics | Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci | OR of 1.12 |
| Systemic Lupus Erythematosus | PLOS GENETICS | Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus | OR of 1.2 |
| Multiple Sclerosis | The American Journal of Human Genetics | Replication analysis identifies TYK2 as a multiple sclerosis susceptibility factor [increased risk with more active Tyk2 variant] | OR of 1.32 |
| PBC | nature genetics | Dense fine-mapping study identfies new susceptibility loci for primary biliary cirrhosis | OR of 1.91 |
| Type 1 Diabetes | nature genetics | The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes | OR of 0.86 |
| Systemic Sclerosis | RHEUMATIC DISEASES | Influence of TYK2 in systemic sclerosis susceptibility: a new locus in the IL-12 pathway | OR of 0.59 |

## 3. Tyk2: Key Mediator of Th17 and Th1 Pathogenesis



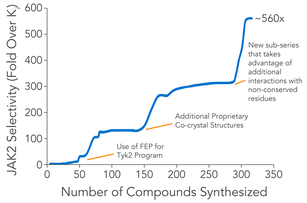

## 4. Free Energy Perturbation (FEP) Used to Develop Quantitative Selectivity Model to Drive SAR

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



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

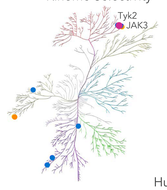
FEP-enabled Selectivity Breakthrough (Tyk2 vs JAK2)




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

## 5. NDI-031301 is a Potent and Selective Tyk2 Inhibitor

Kinome Selectivity

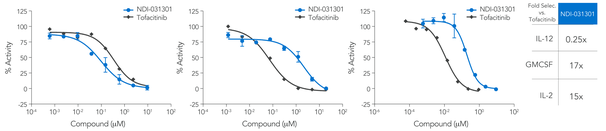



Cell-Based Potency

| $IC_{50}$ (nM) | NDI-031301 |
|---|---|
| PBMC_IL12/pSTAT4 | 97 |
| NK92_IL12/IFNγ | 56 |
| hWB_IL12/IFNγ | 460 |

*PBMC: peripheral blood mononuclear cells; hWB: human whole blood; mWB: mouse whole blood*

Human PBMC Cellular Selectivity




| Fold Selec. vs Tofacitinib | NDI-031301 |
|---|---|
| IL-12 | 0.25x |
| GMCSF | 17x |
| IL-2 | 15x |

Kinome selectivity is based on radiometric peptide kinase assays. For cell-based assays, 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 IC50 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.

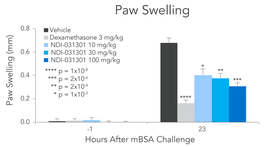
## 6. Potency, Selectivity, and Drug-Like Properties of NDI-031301

All kinase assays were performed in the radiometric format with peptide substrates. PK study was conducted in C57BL/6 mice.

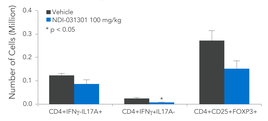
| | | | NDI-031301 |
|---|---|---|---|
| Biochemical Tyk2 Kinase Assay | | Tyk2 Ki (nM) | 0.53 |
| JAK Family Kinase Biochemical Selectivity (Fold over Ki) | | Fold Selectivity over JAK2 | 85x |
| | | Fold Selectivity over JAK1 | 107x |
| | | Fold Selectivity over JAK3 | 15x |
| Plasma Protein Binding | | Human PPB (%bound) | 75 |
| Human in vitro metabolism | | $CL_{int}$ (mL/min/Kg) Microsomes | 3.0 |
| | | Hepatocytes | 9.1 |
| Mouse PK | IV 3 mg/kg | $CL_{tot}$ (mL/min/kg) | 39 |
| | | $T_{1/2}$ (h) | 2.9 |
| | PO 30 mg/kg | $C_{max}$ (nM ) | 13 |
| | | F (%) | 100% |
| | | AUC (μM*hr) | 38 |

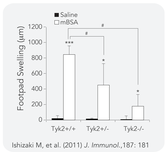
## 7. NDI-031301 Reduced Inflammation in the mBSA-induced Delayed Type Hypersensitivity Model

Paw Swelling




Draining Lymph Node T Cell Subsets

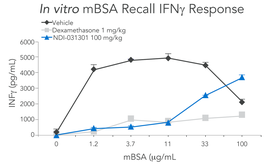



Ishizaki M, et al. (2011) *J. Immunol.*,187: 181

- Dexamethasone was dosed IP QD for 5 days
- Paw swelling is the difference between the mBSA injected vs. the saline injected paw from the same mouse
- p values are student t-test vs. the vehicle

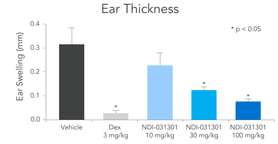
*In vitro* mBSA Recall IFNγ Response



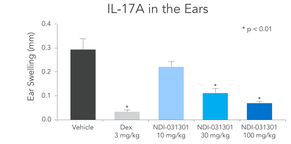

C57BL/6 mice were immunized with methylated BSA/CFA emulsion at lower back on day 0. Mice were challenged on day 5 by injecting mBSA/PBS solution into one hind paw and PBS into the other hind paw. Vehicle (20% HP-β-CD in saline) or NDI-031301 were dosed twice daily orally and Dexamethasone was dosed 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. Draining lymph node cells were harvested and T cell subsets were phenotyped by flow cytometry. The *in vitro* mBSA recall response was measured by incubating the draining lymph node cells with mBSA for 72 hours and supernatant collected for IFNγ measurement. p values are student t-test vs. the vehicle.

## 8. NDI-031301 Reduced Inflammation in the IL-23-induced Mouse Epidermal Hyperplasia Model

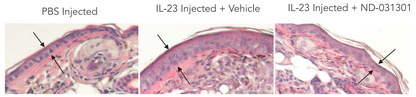
Ear Thickness

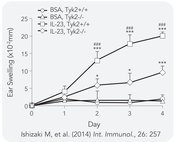



IL-17A in the Ears




Ear Histology



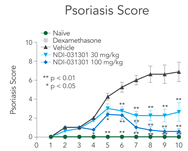




Ishizaki M, et al. (2014) *Int. Immunol.*, 26: 257

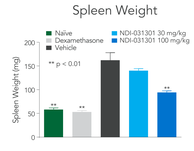
Ear swelling was induced by injecting recombinant murine IL-23 into the ears of C57BL/6 mice once daily for 4 days. Vehicle (20% HP-β-CD in saline) or NDI-031301 were dosed twice daily orally and Dexamethasone was dosed once daily IP. Ear thickness was measured prior to the first IL-23 injection and one day post the last IL-23 injection, and ear swelling was the difference of the two measurements. 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.

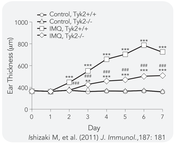
## 9. NDI-031301 Reduced Disease in Imiquimod-induced Psoriasis Model

Psoriasis Score



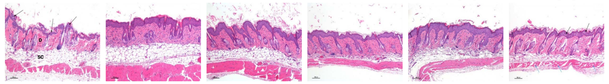

Spleen Weight




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

Histology




Psoriasis was induced by topical application of Imiquimod cream once daily on the shaved back skin of Balb/c mice for 11 days. Vehicle (20% HP-β-CD in saline) or NDI-031301 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.

## SUMMARY

- Tyk2 is a sought after target for treatment of autoimmune diseases
- Given the high degree of structural homology amongst the JAK kinase family members, designing potent and selective inhibitors has remained a 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
- *In vivo* proof of mechanism and efficacy was demonstrated in mouse models of delayed type hypersensitivity and psoriasis
- Nimbus compounds have excellent drug-like properties, are well tolerated, and are candidates for further development for inflammatory diseases with excessive IL-12, IL-23 and/or type I interferon signaling

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