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

**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 are 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 sites among the JAK family kinases. We utilized cut 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 an IC50 of 0.5 nM that is >100-, 8-, 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 IC50 of 0.1 μM and 2.6 μM, respectively. NDI-031301 has excellent selectivity against a panel of 346 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 NDI-031301 inhibitors in a methylated-BSA induced delayed type hypersensitivity model. At 100 mg/kg dose, orally administered NDI-031301 reduced paw swelling and joint density, 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 node cell populations. In an IL-23 induced mouse psoriasis model, NDI-031301 dose-dependently reduced skin inflammation with >75% 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 IL-12, IL-23, and type I interferon signaling and suppressed >85% of in vitro antigen-induced IFNγ response in >30% human PBMCs with IC50 of 0.1 μM. It dramatically reduced Th1 cells in the draining lymph nodes of NOD mice and >85% of Th17 cells in the draining lymph nodes of NOD SCID IL2−/− and NOD SCID IL2−/− IL10−/− mice. NDI-031301 also blocked IFNγ and IL-17A secretion in vitro in Th1 and Th17 cell lines. In vivo, NDI-031301 reduced disease in imiquimod-induced psoriasis model.

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

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

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

**CONCLUSION**

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

**SUMMARY**

1. Given the high degree of structural homology amongst the JAK family kinases, designing potent and selective inhibitors has remained a challenge.
2. Using a physics-based computational approach, Nimbus has uncovered previously unexploited drivers of potency and JAK family selectivity.
3. Potent inhibition of the IL-12/STAT4 phosphorylation and cytokine production was observed in human PBMCs and whole blood.
4. In vivo proof of mechanism and efficacy was demonstrated in mouse models of delayed type hypersensitivity and psoriasis.
5. 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.

**Table 1: Potency, Selectivity, and Drug-Like Properties of NDI-031301**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NDI-031301</th>
<th>JAK2</th>
<th>JAK3</th>
</tr>
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<tbody>
<tr>
<td>IC50 (μM)</td>
<td>0.1</td>
<td>0.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Fold Selectivity over JAK2</td>
<td>25</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Fold Selectivity over JAK3</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>38</td>
<td>38</td>
<td>38</td>
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<tr>
<td>M*hr)</td>
<td>25</td>
<td>25</td>
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**Figure 1: NDI-031301 Reduced Inflammation in the mBSA-induced Delayed Type Hypersensitivity Model**

- **In vivo mBSA recall IFNγ Response**
- **Ear Thickness**
- **Ear Histology**
- **Paw thickness**
- **Dietary Adherence**

**Figure 2: NDI-031301 Reduced Disease in Imiquimod-induced Psoriasis Model**

- **Psoriasis Score**
- **Spleen Weight**
- **Paw thickness**
- **Dietary Adherence**

**Figure 3: Tyk2 Program**

- **Stat 1-5**
- **Tyk2 Jak1**
- **Jak3**
- **Tyk2 Jak2**
- **Tyk2+/+ Tyk2+/- Tyk2-/-**

**Figure 4: Free Energy Perturbation (FEP) Used to Develop Quantitative Selectivity**

- **M*hr)**
- **Potency, Selectivity, and Drug-Like Properties of NDI-031301**
- **IC50 (nM)**
- **% Activity**
- **Response**

**Table 2: IC50 and Fold Selectivity of NDI-031301 vs. JAK2 and JAK3**

<table>
<thead>
<tr>
<th>Compound</th>
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**Figure 5: NDI-031301 is a Potent and Selective Tyk2 Inhibitor**

- **IC50 (nM)**
- **% Activity**
- **Response**

**Table 3: IC50 and Fold Selectivity of NDI-031301 vs. JAK2 and JAK3**

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<td>15</td>
</tr>
<tr>
<td>JAK2</td>
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<td>150</td>
</tr>
<tr>
<td>JAK3</td>
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**Figure 6: Potency, Selectivity, and Drug-Like Properties of NDI-031301**

- **IC50 (nM)**
- **% Activity**
- **Response**

**Table 4: IC50 and Fold Selectivity of NDI-031301 vs. JAK2 and JAK3**

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**Figure 7: NDI-031301 Reduced Inflammation in the mBSA-induced Delayed Type Hypersensitivity Model**

- **In vivo mBSA recall IFNγ Response**
- **Ear Thickness**
- **Ear Histology**
- **Paw thickness**
- **Dietary Adherence**

**Figure 8: NDI-031301 Reduced Inflammation in the IL-23-induced Mouse Epidermal Hyperplasia Model**

- **In vivo mBSA recall IFNγ Response**
- **Ear Thickness**
- **Ear Histology**
- **Paw thickness**
- **Dietary Adherence**