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OR-101: A Next Generation JAK3 Selective Compound

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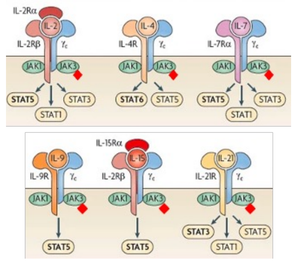
Abstract

The Janus Kinase (JAK) family is a family of cytoplasmic non-receptor tyrosine kinases that includes three JAKS (JAK1, JAK2, JAK3) and tyrosine kinase 2. Upon activation, JAKs additionally activate the signal transducer and activator of transcription (STAT) pathway to regulate the downstream signaling of inflammatory cytokines and growth factors. Targeting the JAK family kinases with small-molecule inhibitors has proved to be effective in the treatment of different types of diseases and the identification of more selective pharmacologic JAK inhibitors has been an ongoing research and development goal. Here we describe the preclinical characterization of a next generation JAK inhibitor, OR-101, with selective inhibition of JAK3-mediated inflammation. JAK3 is involved in signal transduction by receptors that employ the common gamma chain (γc) of the type I cytokine receptor family (e.g. IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R) and therefore has applicability in a number of inflammatory conditions. The selectivity profile of OR-101 was delineated using an enzymatic assay (NanoBRET), phenotypic kinome screens, and cytokine release assays. Ritlecitinib, a potent JAK3/TEC inhibitor in Phase 3 trials for inflammatory diseases, was used as a comparator in all assays. Enzymatically, OR-101 exhibited an improved selectivity profile compared with ritlecitinb. The IC50 measurement for JAK3 was 269 nM for OR-101 and 78 nM for ritlecitinib. Additionally, the IC50 measurements for ITK, BTK, and TEC were 13nM, 287nM, and >10,000nM, respectively for OR-101 as compared to 54nM, 41nM, and 10nM, respectively, for ritlecitinib. Differences in kinase activity were confirmed using the KINOMEscan assay. Specifically, both OR-101 and ritlecitinib exhibited potent JAK3 activity, 8.4nM and 7.5nM, respectively. Further comparison determined that OR-101 was 2 to 165-fold more selective than ritlecitinib with additional kinases (BLK, BTK, EGFR, ITK, JAK1, JAK2, TYK2), suggesting an improved selectivity profile. Finally, both compounds were evaluated in cytokine release assays using peripheral blood mononuclear cells (PBMC) from healthy human volunteers. PBMC were stimulated with anti-CD3 to activate T cells in the presence/absence of OR-101 or ritlecitinib for 24 hours. While OR-101 and ritlecitinib had similar inhibitory activity against, IL-2, IL-6, and IFN-g; OR-101 exhibited greater inhibitory activity against IL-4 and IL-15. These studies confirm the JAK3 selective profile of OR-101 and suggest the potential for OR-101 in inflammatory diseases characterized by JAK3 driven inflammation.

Developing a Next-GEN JAKinib

- Aberrant cytokine signaling has been shown to drive immune-mediated disease through type I and type II cytokine receptors. These receptors rely on Janus family of kinases (JAK) for signal transduction.
- The mammalian JAK family contains three JAKs (JAK1–3) and tyrosine kinase 2 (TYK2), which selectively bind different receptor chains.
- Pharmacological targeting of the JAKs has proven to be efficacious in treating immune and inflammatory diseases; however, the lack of selectivity has been associated with significant safety concerns[1]
- Selective small-molecule inhibition of both JAK3 and ITK is challenging due to the highly conserved ATP binding pocket within the Janus kinase family (JAK1, JAK2, JAK3, TYK2) and the TEC kinase family (ITK, RLK, BMX, BTK, TEC)
- OR-101 is a selective dual inhibitor of Tyr (Y) kinases, JAK3 and ITK, interfering with the JAK-STAT6 and T Cell Receptor signaling pathways

Figure 1. JAK3 Mediated Signaling through the common γc[2]



Selectivity Profile Characterization of OR-101

Nano-BRET Target Engagement Assay

- NanoBRET assays are compound competition assays that relies on bioluminescence resonance energy transfer (BRET) between a luciferase-tagged kinase and a fluorescent tracer. Quantification and specificity are key attributes of the NanoBRET system.[3]
- HEK293 cells transiently transfected with kinase/NanoLuc fusion plasmids and seeded into 384-well plates.
- NanoBRET allows for interrogation of full-length kinases in their native intracellular setting and the direct interaction of the compounds binding to target kinases in cells
- Data is expressed as the Normalized BRET response

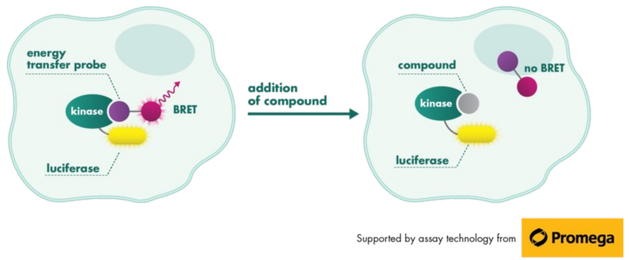
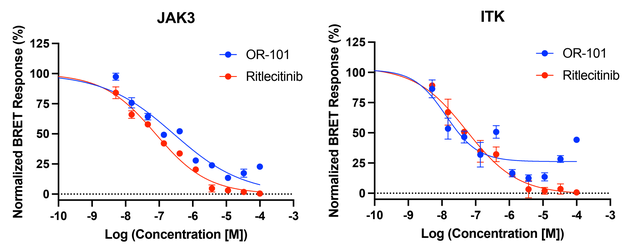


Table 1. IC50 Values for Kinase Engagement

	OR-101 (IC50 nM)	Ritlecitinib (IC50 nM)
JAK3	269	78
ITK	13	54
BTK	287	41
TEC	>10,000	10

HEK293 cells were transfected with 1 µg JAK3, ITK, BTK, or TEC vector and 9 µg transfection carrier DNA. Transfected cells were treated for 1 hour with OR-101 and Ritlecitinib starting at 100 µM, 10-dose with 3-fold dilution.

Figure 2. Target Engagement Curves for JAK3 and ITK



KdSELECT ASSAY

- KINOMEscan® screening platform employs a novel and proprietary active site-directed competition binding assay to quantitatively measure interactions between test compounds and more than 489 kinase assays and disease relevant mutant variants.[4]
- KdELECT quantifies compound binding affinity against any kinase assay.
- Inhibitor binding constants (Kd values) are calculated from duplicate 11-point dose-response curves. Measurements are made under optimized conditions that generate true thermodynamic Kd values which facilitate direct comparison of inhibitor affinity across kinases.

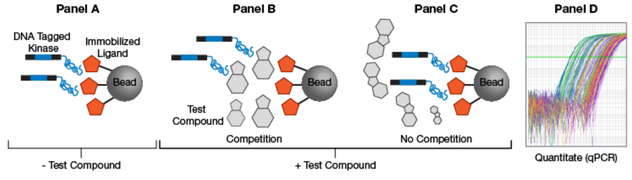
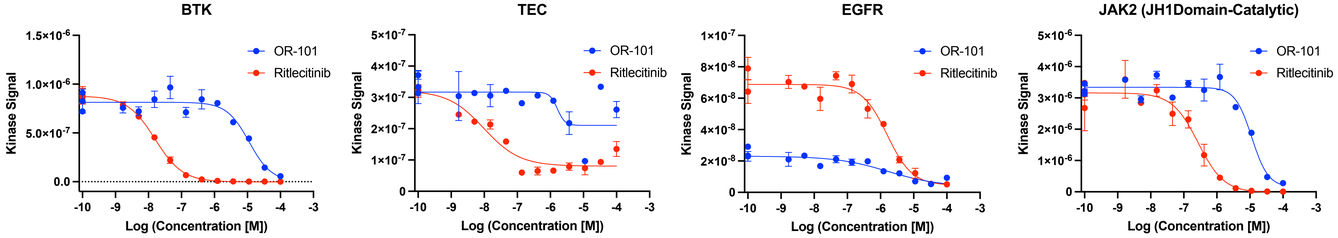


Table 2. Thermodynamic Kd Values

Kinase	OR-101 (Kd nM)	Ritlecitinib (Kd nM)
BLK	1008	45
BMX	130	40
BTK	11270	16
EGFR	4300	1590
ERBB2	26570	2173
ERBB4	5732	232
ITK	1052	37
JAK1 (JH1 Domain-Catalytic)	11390	1677
JAK2 (JH1 Domain-Catalytic)	11340	318
JAK3 (JH1 Domain-Catalytic)	8.4	7.5
TEC	1624	9.8
TYK2 (JH1 Domain-Catalytic)	101000	1337

Figure 3. Thermodynamic Kd Values for Kinase Engagement



Measurement of true thermodynamic Kd values using an 11-point dose response curve with OR-101 and Ritlecitinib starting at 100 µM, 10-dose with 3-fold dilution. The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis).

Cytokine Release Assay

Peripheral blood mononuclear cells (PBMC) from healthy donors were stimulated with anti-CD3 (or isotype control) in the presence/absence of either OR-101 or Ritlecitinib for 24 hours

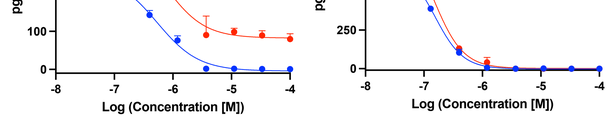
Cell supernatants were further analyzed by Luminex for the levels of IL-2, IL-4, IL-6, IL-7, IL-15, IFN-γ, and TPO

IC50s were calculated using 4 parameter non-linear regression analysis

Table 3. IC50 Values for Cytokine Release Assay

Cytokine	OR-101 (IC50 mM)	Ritlecitinib (IC50 mM)
IL-2	0.56	0.78
IL-4	0.29	Indeterminate
IL-6	0.37	0.33
IL-7	Below LOD	Below LOD
IL-15	0.35	Indeterminate
IFN-g	0.15	0.16
TPO	Below LOD	Below LOD

Figure 4: Cytokine Inhibition Curves



Conclusions

- OR-101 is JAK3 specific, with no activity towards the other Janus Kinases, JAK1, JAK2 and TYK2
- OR-101 appears to be more selective than Ritlecitinib for JAK3, based on the IC_{50}, and has added ITK inhibition
- Cellular profiling indicates that OR-101 has no impact on EPO and BTK, and does not impact IL-6 and IFN-gamma production, which are all usual culprits for AEs

References

1. Howell et al. 2019. Targeting the Janus Kinase Family in Autoimmune Skin Diseases. *Frontiers in Immunology*. 10:2342.
2. Rochman et al. 2009. New insights into the regulation of T cells by γc family cytokines. *Nature Immunology.* 9: 480–490.
3. https://www.reactionbiology.com/services/kinase-assays/nanobret-intracellular-kinase-assay
4. https://www.eurofinsdiscovery.com/solution/kdelect

Disclosures

M. Howell, P. Walker, W. Ahmad, and F. Hasan are shareholders in Ornovi