Ruxolitinib (INCB018424) Datasheet

**Biological Activity**

<table>
<thead>
<tr>
<th>Description</th>
<th>INCB018424 (Ruxolitinib) is a JAK family inhibitor for JAK1, JAK2 and JAK3 with IC50 of 2.7 nM, 4.5 nM and 322 nM, respectively.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Targets</td>
<td>JAK1  \nJAK2  \nJAK3</td>
</tr>
<tr>
<td>IC50</td>
<td>2.7 nM  \n4.5 nM  \n322 nM(^{[1]})</td>
</tr>
</tbody>
</table>

**In vitro**

INCB018424 demonstrates modest selectivity against Tyk2 (6-fold) and marked selectivity (130-fold) against JAK3. INCB018424 potently inhibits JAK1 and JAK2 in the blood. INCB018424 potently and selectively inhibits JAK2\(\text{V}617\text{F}\)-mediated signaling and proliferation. Growth of HEL cells is also affected by INCB018424 with EC50 of 186 nM. Unlike the BaF3 cells, cell proliferation is not completely inhibited by INCB018424, even though pSTAT3 and pSTAT5 are completely absent at concentrations more than 100\(\text{nM}\). Treatment with INCB018424 markedly increases apoptosis compared with DMSO, with a 4.3-, 7.2-, and 13.2-fold increase at concentrations of 150 nM, 400 nM, and 1000 nM, respectively. Treatment with INCB018424 (64 \(\text{nM}\)) results in a doubling of cells with depolarized mitochondria, an effect that is dose-dependent. INCB018424 inhibits hematopoietic progenitor cell proliferation in primary MPN patient samples.\(^{[2]}\)

**In vivo**

INCB018424 treatment improves viability and splenomegaly in a JAK2\(\text{V}617\text{F}\)-driven model of malignant disease. Spleens from INCB018424-treated mice demonstrates significantly fewer of these neoplastic features and retained normal lymphoid components. The mass of white pulp in INCB018424-treated mice is similar to that of naive and vehicle-treated mice. In agreement with the biochemical selectivity of INCB018424 against JAK3, whereas cyclophosphamide significantly decreases the absolute lymphocyte counts compared with vehicle-treated animals after 2 weeks of treatment, daily administration of INCB018424 for a period of 4 weeks has no significant effect on circulating lymphocyte numbers. Similarly, INCB018424 has minimal effects on thymus weights.\(^{[2]}\)

**Clinical Trials**

INCB018424 has entered in a Phase II clinical trial for the treatment of breast cancer.

**Features**

- INCB018424 has been approved for marketing in Japan and is undergoing Phase III trials for the treatment of myelofibrosis.
- It is also being investigated for the treatment of other diseases such as psoriasis and rheumatoid arthritis.

**Protocal** (Only for Reference)

**Kinase Assay:**\(^{[2]}\)

- **Biochemical assays.** The kinase domains of human JAK1 (837-1142), JAK2 (828-1132), JAK3 (781-1124), and Tyk2 (873-1187) are cloned by PCR with N-terminal epitope tags. Recombinant proteins are expressed using 921 cells and baculovirus vectors and purified with affinity chromatography. JAK kinase assays used a homogenous time-resolved fluorescence assay with the peptide substrate \(-\text{EQEDEPEGDYFEWLE}\). Each enzyme reaction is carried out with INCB018424 or control, JAK enzyme, 500 nM peptide, adenosine triphosphate (ATP; 1 mM), and 2.0% dimethyl sulfoxide (DMSO) for 1 hour. The 50% inhibitory concentration (IC50) is calculated as the compound concentration required for inhibition of 50% of the fluorescent signal. Biochemical assays for CHK2 and c-MET enzymes are performed using standard conditions (Michaelis constant [K\text{m}] ATP) with recombinantly expressed catalytic domains from each protein and synthetic peptide substrates. An additional panel of kinase assays (Abl; Akt1, AurA, AurB, CDC2, CDK2, CDK4, CHK2, c-ki, c-Met, GFR, EphB4, ERK1, ERK2, FLT-1, HER2, IGF1R, IGF2R, IGF3, JAK2, JNK1, Lck, MEK1, p38, p70S6K, PKA, PKCα, Src, and ZAP70) is performed using standard conditions including 200 nM INCB018424. Significant inhibition is defined as more than or equal to 30% (average of duplicate assays) compared with control values.

- **Cell Assay:**\(^{[2]}\)
  - **Cell Lines.** Ba/F3-EpoR-JAK2\(\text{V}617\text{F}\) or HEL cells
  - **Concentrations.** 0-4 nM
  - **Incubation Time.** 48 hours
  - **Methods.** Ba/F3-EpoR-JAK2\(\text{V}617\text{F}\) or HEL cells are seeded at 2000/well of white bottom 96-well plates, treated with INCB018424 from DMSO stocks (0.2% final DMSO concentration), and incubated for 48 hours at 37°C with 5% CO\text{2}. Viability is measured by cellular ATP determination using the Cell-Titer Glo luciferase reagent or viable cell counting. Values are transformed to percent inhibition relative to vehicle control, and IC50 curves are fitted according to nonlinear regression analysis of the data.

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**Animal Study**

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>6- to 8-week-old female BALB/c mice with Ba/F3-JAK2V617F tumors</th>
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</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>5% dimethyl acetamide, 0.5% methocellulose</td>
</tr>
<tr>
<td>Doses</td>
<td>180 mg/kg</td>
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<tr>
<td>Administration</td>
<td>Oral gavage</td>
</tr>
</tbody>
</table>

**References**


**PLEASE KEEP THE PRODUCT UNDER -20°C FOR LONG-TERM STORAGE.**

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