Ruxolitinib

JAK1 and JAK2 Inhibitor

Catalog code: tlrl-rux

https://www.invivogen.com/ruxolitinib

For research use only

Version 21J05-MM

PRODUCT INFORMATION

Contents

- 5 mg of Ruxolitinib

Storage and stability

- Ruxolitinib is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension in DMSO, prepare aliquots of ruxolitinib and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6 months when properly stored.

Quality Control:

- Purity: ≥97% (UHPLC)
- The biological activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

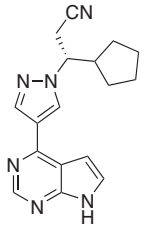
Ruxolitinib (also known as INCB018424) is a potent, reversible, and selective Janus Kinase (JAK) 1 and JAK2 inhibitor[1]. JAKs are constitutively bound to cytokine receptors, such as Type I interferons (IFNs) and interleukin-6 (IL-6). Upon binding of the ligand to the receptor, JAKs phosphorylate downstream targets such as STAT3/5, Akt, and ERK. This induces the production of cytokines and chemokines, including IFN-stimulated genes (ISGs). JAK-STAT signaling is crucial for the regulation and homeostasis of hematopoiesis and immunity[2]. Ruxolitinib was developed as a potential therapeutic for a family of blood cancers termed myeloproliferative neoplasms (MPNs), which are characterized by the aberrant activation of the JAK–STAT pathway due to a mutation (V617F) in JAK2[1,3].

Ruxolitinib competes with ATP for binding to the catalytic site in the kinase domain, and thus inhibits not only the mutated JAK2 but wild-type JAK1-2 signaling pathways. Inhibition of the JAK-STAT signaling pathway by Ruxolitinib results in a dramatic decrease in levels of inflammatory cytokines, such as IL-6 and TNF-α. It is this attenuation of the inflammatory response that the clinical efficiency of Ruxolitinib is attributed[2]. Ruxolitinib is approved for the treatment of the MPNs, myelofibrosis and polycythemia vera[3]. Pre-clinical data suggest that Ruxolitinib shows potential in the treatment of inflammatory conditions such as acute graft versus host disease[4]. Additionally, synergy has been reported between Ruxolitinib and the chemotherapy drug, dexamethasone, in the treatment of acute lymphoblastic leukemia in both *in vitro* and *in vivo* pre-clinical models[5].

1. Quintas-Cardama, A. *et al.*, 2010. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood 115, 3109-17. **2. Ajayi S. *et al.*, 2018.** Ruxolitinib. Recent Results Cancer Res 212, 119-132. **3. Mascarenhas J. & Hoffman R. 2012.** Ruxolitinib: the first FDA approved therapy for the treatment of myelofibrosis. Clin Cancer Res 18:3008-14. **4. Zeiser R. *et al.*, 2020.** Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. N Engl J Med 382:1800-10. **5. Verbeke D. *et al.*, 2019.** Ruxolitinib Synergizes With Dexamethasone for the Treatment of T-cell Acute Lymphoblastic Leukemia. Hemasphere 3:e310.

CHEMICAL PROPERTIES

Structure:



Synonym: INCB018424
Solubility: 10 mg/ml in DMSO
CAS number: 941678-49-5
Formula: $C_{17}H_{18}N_6$
Molecular weight: 306.37 g/mol

METHODS

Preparation of Ruxolitinib stock solution (20 mM)

1. Add 816 µl DMSO to 5 mg ruxolitinib and vortex until completely resuspended.
2. Prepare aliquots and store at -20°C. Prepare further dilutions using aqueous buffers. Do not store aqueous dilutions for more than 1 day.

Working concentration: 100 nM-10 µM for cell culture assays

Inhibition assay

Described below is a protocol to study the JAK-STAT pathway in the interferon (IFN) regulatory factor-inducible secreted embryonic alkaline phosphatase (SEAP) reporter B16 melanocytes, B16-Blue™ ISG cells.

1. Add 20 µl of ruxolitinib (final concentration 100 nM-10 µM) per well of a flat-bottom 96-well plate.
2. Add 160 µl (~75,000 cells) of a B16-Blue™ ISG cell suspension per well.
3. Incubate for 1 hour at 37°C in a 5% CO_2 incubator.
4. Add 20 µl of murine IFN-β (final concentration 100-1000 U/ml).
5. Incubate the plate at 37 °C in a 5% CO_2 incubator for 18-24 hours.
6. Monitor SEAP production using a SEAP detection assay, such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

Product	Description	Cat. Code
3-Methyladenine	PI3K inhibitor	tlrl-3ma
Amlexanox	TBK1/IKKε inhibitor	inh-amx
B16-Blue™ ISG Cells	Reporter melanocytes	bb-ifnabg
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
SB203580	p38 MAP kinase inhibitor	tlrl-sb20
U0126	MEK1 and MEK2 Inhibitor	tlrl-u0126

TECHNICAL SUPPORT
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