Preclinical Evaluation of INCB160058 – A Novel and Potentially Disease Modifying Therapy for JAK2V617F Mutant Myeloproliferative Neoplasms

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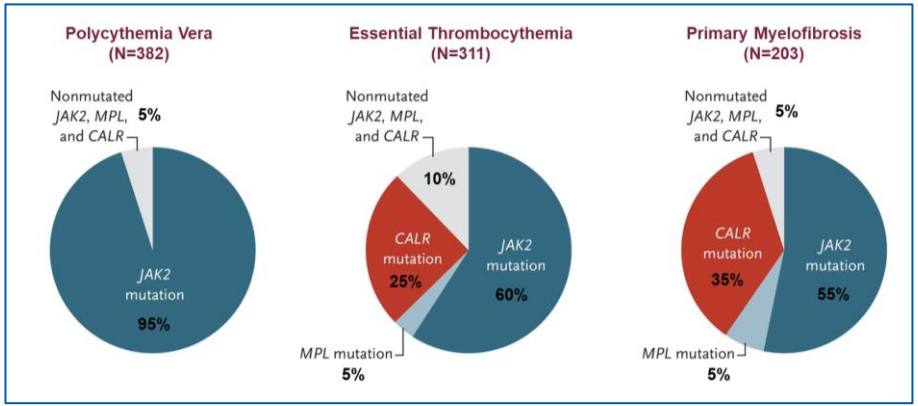
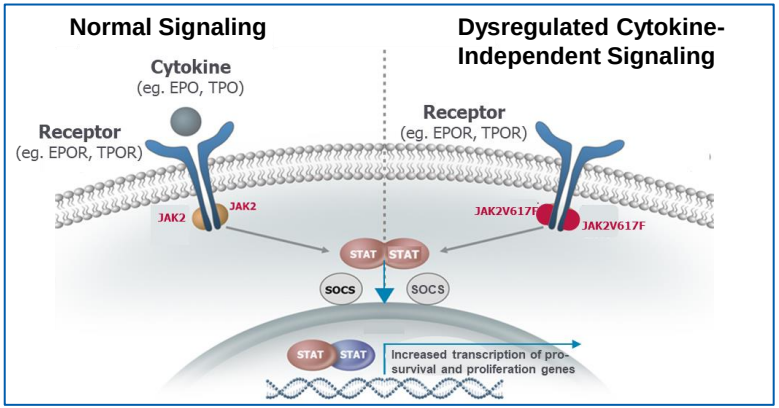
JAK2V617F Is the Most Prevalent Driver Mutation in MPN



From *The New England Journal of Medicine*, Klampfl T, et al. Somatic Mutations of Calreticulin in Myeloproliferative Neoplasms,369, 2379-90. Copyright © (2013). Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.



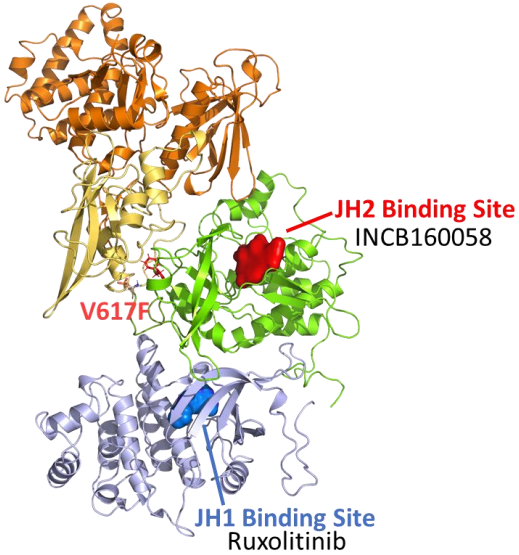
Potential therapeutic option: selectively targeting *JAK2V617F*

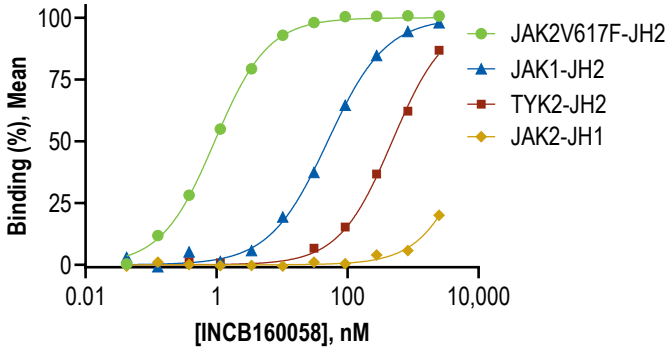
Therapeutic goals of a JAK2V617F-selective agent:

- Molecular remission
- Improved hematologic tolerability

CALR, calreticulin; EPO, erythropoietin; EPOR, erythropoietin receptor; JAK, Janus kinase; MPL, myeloproliferative leukemia virus oncogene; MPN, myeloproliferative neoplasm; SOCS, suppressors of cytokine signaling; STAT, signal transducers and activators of transcription; TPO, thrombopoietin; TPOR, thrombopoietin receptor.

INCB160058 Is a Selective Pseudokinase (JH2)-Binding Inhibitor of JAK2V617F





	Selectivity for JAK2V617F-JH2
JAK2-JH1	>2500×
JAK1-JH2	50×
TYK2-JH2	500×

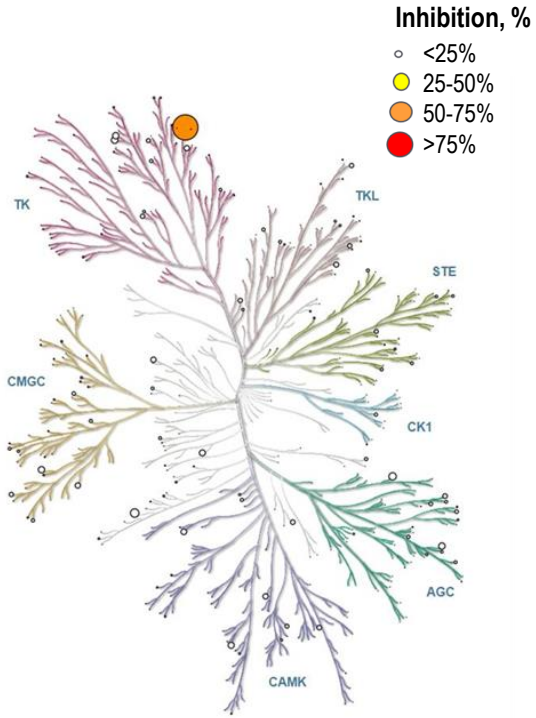
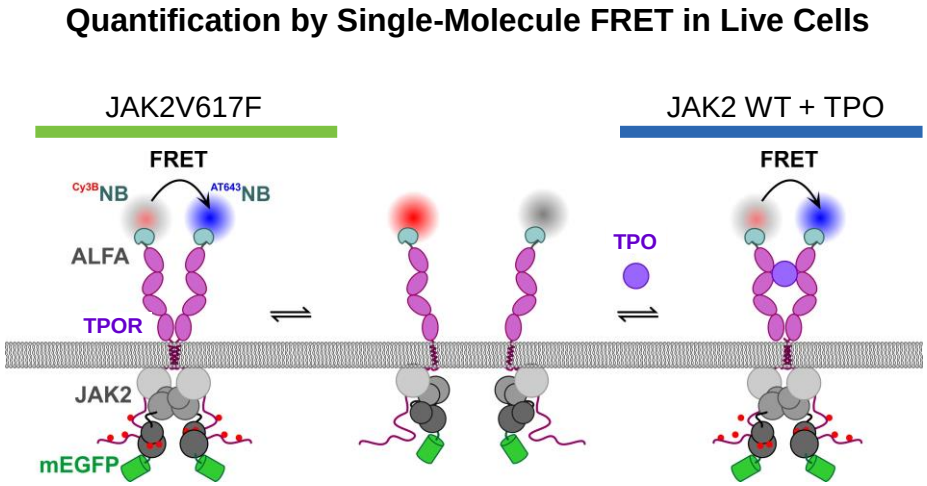


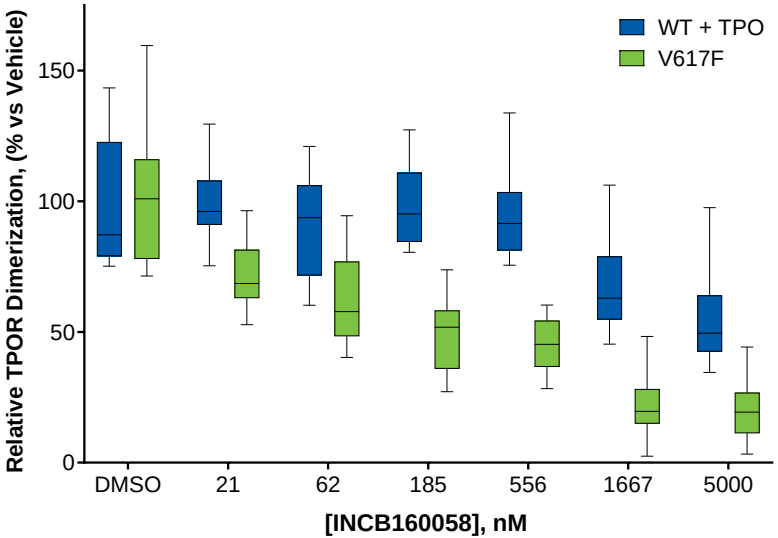
Image reproduced courtesy of Cell Signaling Technology, Inc (www.cellsignal.com).

JAK, Janus kinase; JH, JAK homology; TYK, tyrosine kinase.

Binding of INCB160058 to the Pseudokinase Domain Results in Inhibition of JAK2V617F-Induced TPOR Dimerization



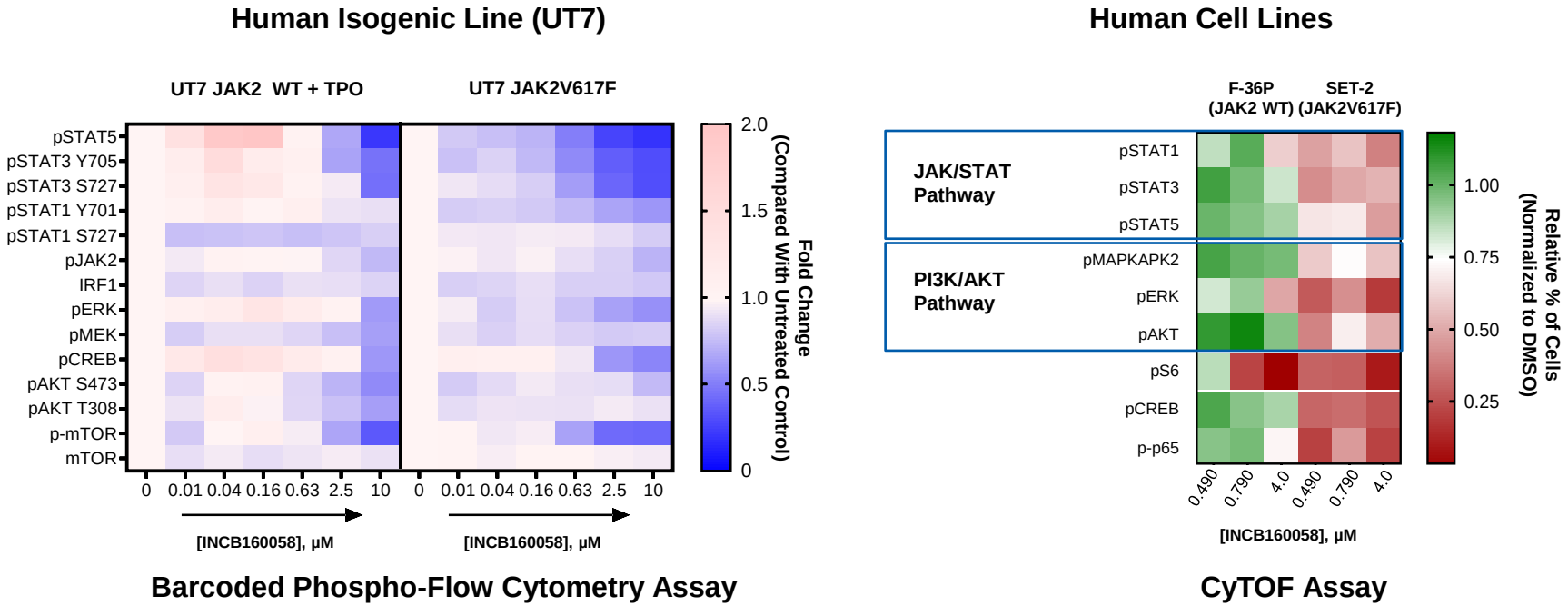
From Wilmes S, et al. *Science*. 2020;367:643-52. Mechanism of homodimeric cytokine receptor activation and dysregulation by oncogenic mutations, Reprinted with permission from AAAS.



Data from B. Pathmalolan, C. Pollmann, and J. Piehler (Osnabrück University).

ALFA, ALFA-tag; DMSO, dimethyl sulfoxide; FRET, fluorescence resonance energy transfer; JAK, Janus kinase; mEGFP, monomeric enhanced green fluorescent protein; NB, anti-ALFA nanobody; TPO, thrombopoietin; TPOR, thrombopoietin receptor; WT, wild-type.

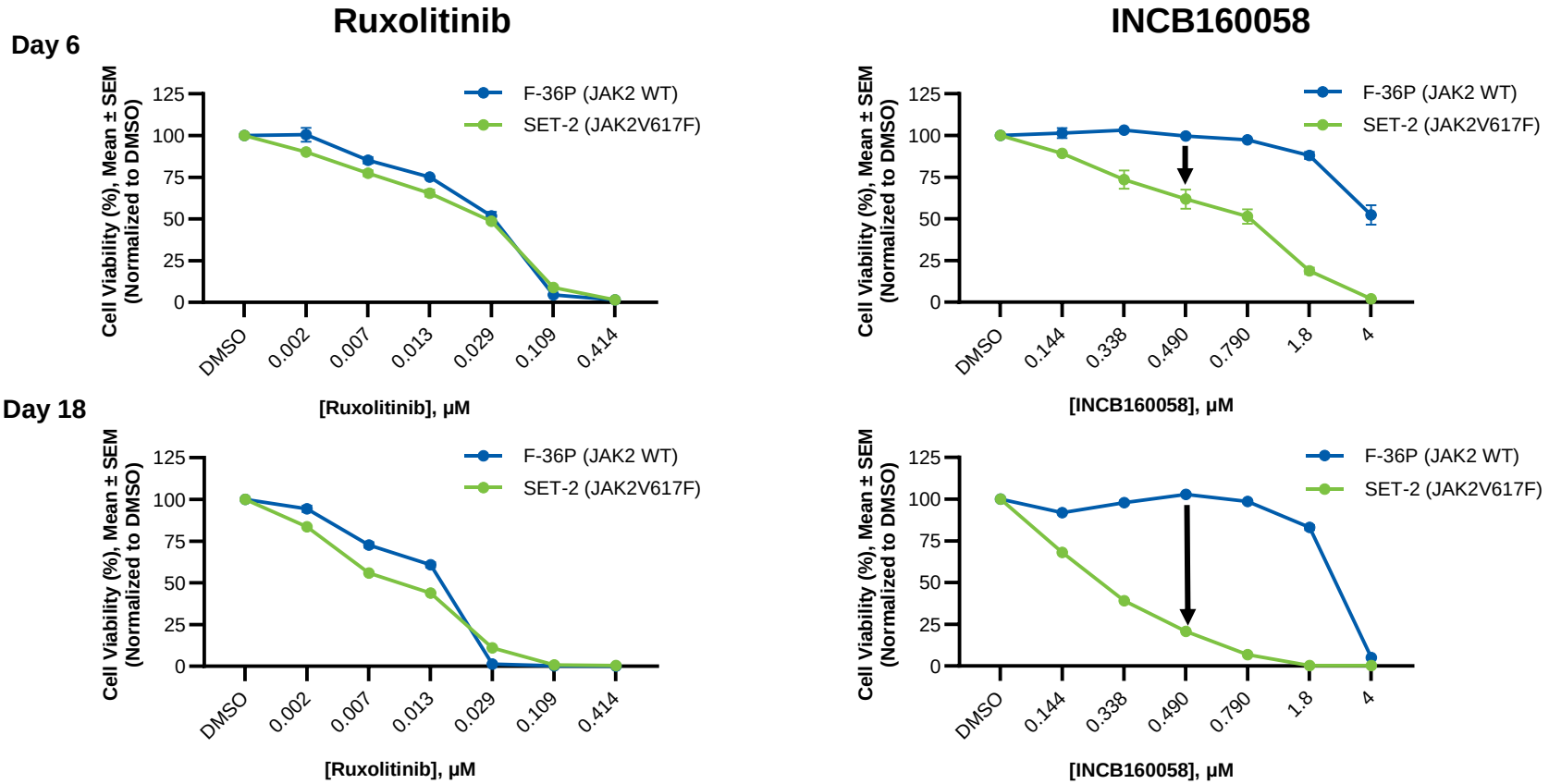
INCB160058 Selectively Inhibits JAK2V617F Signaling



Data from B. Ferreira and I. Hitchcock (University of York).

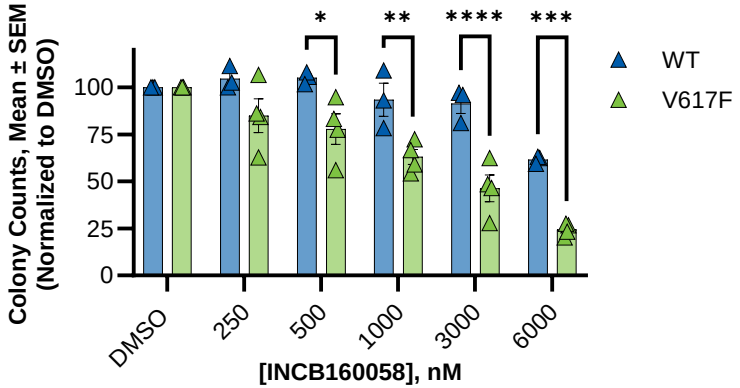
AKT, protein kinase B; CREB, cyclic adenosine monophosphate response element binding protein; CyTOF, cytometry by time-of-flight; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; IRF, interferon regulatory factor; JAK, Janus kinase; MAPKAPK, mitogen-activated protein kinase-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3 kinase; STAT, signal transducers and activators of transcription; TPO, thrombopoietin; WT, wild-type.

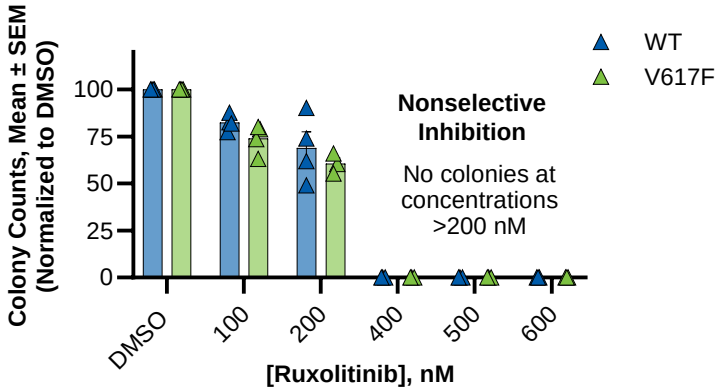
INCB160058 Selectively Inhibits Growth of JAK2V617F-Expressing Cells



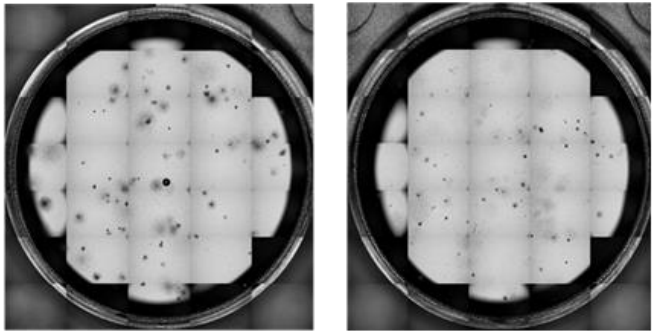
DMSO, dimethyl sulfoxide; JAK, Janus kinase; SEM, standard error of the mean.

INCB160058 Selectively Inhibits Expansion of JAK2V617F-Expressing Primary Human CD34⁺ Cells

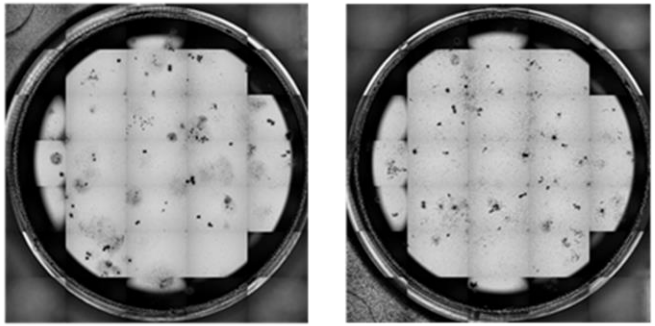




JAK2V617F Patient CD34⁺ Cells



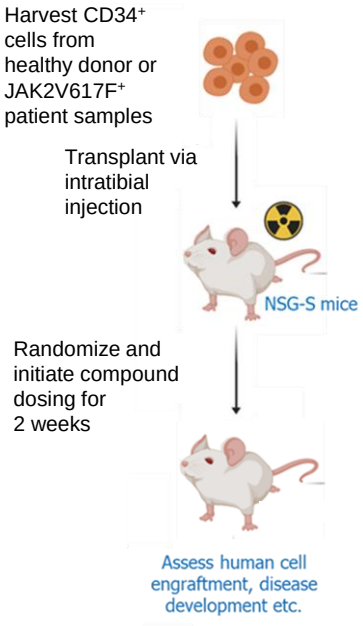
Healthy Donor CD34⁺ Cells



DMSO | **INCB160058 1 µM**

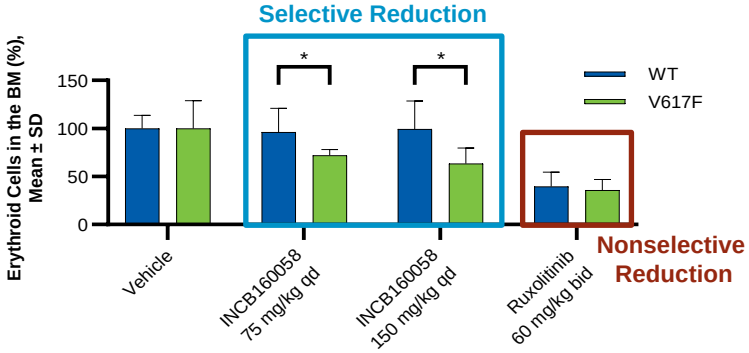
*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.
DMSO, dimethyl sulfoxide; JAK, Janus kinase; SEM, standard error of the mean; WT, wild-type.

INCB160058 Selectively Reduces Human JAK2V617F^{+} Cell Engraftment in the MPN PDX Model

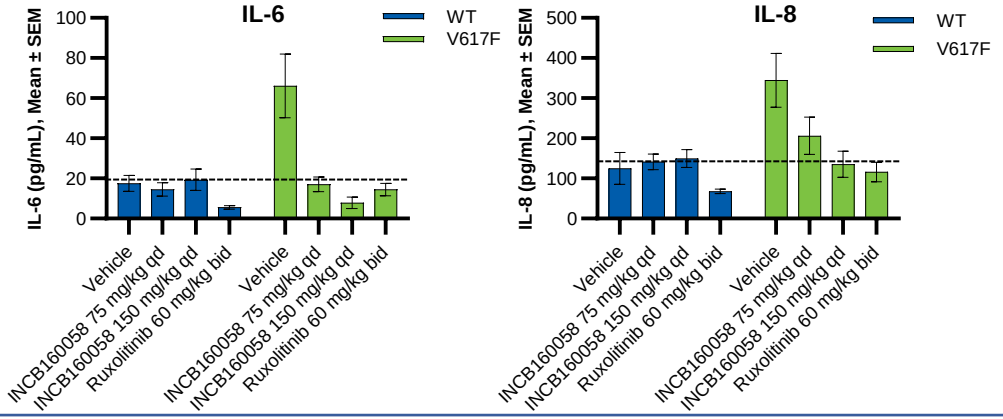


Selective Efficacy

Human Erythroid Cell Engraftment (hTer119^{-} hCD71^{+} hCD235a^{+})



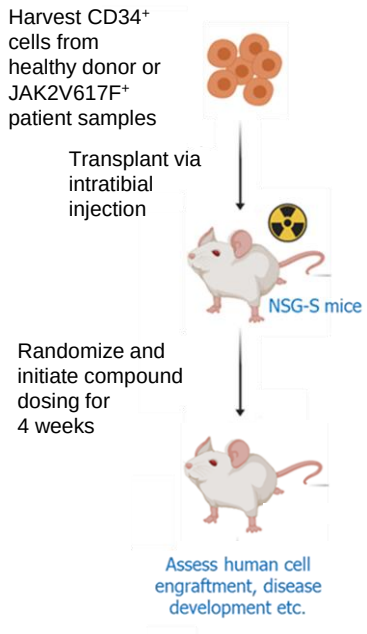
Normalization of Pathogenic Cytokine Levels



*P<0.05.

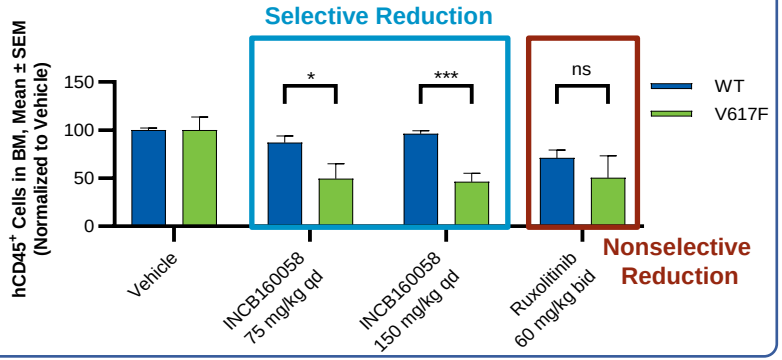
bid, twice daily; BM, bone marrow; IL, interleukin; JAK, Janus kinase; MPN, myeloproliferative neoplasm; qd, once daily; SD, standard deviation; SEM, standard error of the mean; WT, wild-type.

INCB160058 Selectively Reduces Human JAK2V617F^{+} Cell Engraftment and HSPCs in the MPN PDX Model



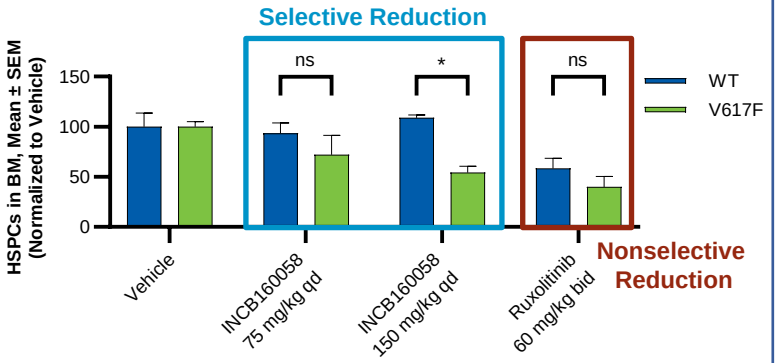
Selective Efficacy

Human Cell Engraftment in BM



Selective Efficacy

HSPCs
(hCD45^{+} Lin^{-} CD34^{+} CD38^{-})



*P<0.05; ***P<0.001.
bid, twice daily; BM, bone marrow; HSPC, hematopoietic stem and progenitor cell; JAK, Janus kinase; MPN, myeloproliferative neoplasm; ns, not significant; qd, once daily; SEM, standard error of the mean; WT, wild-type.

Summary

- INCB160058 is a potent and selective JAK2 pseudokinase domain binder
- Pseudokinase binding offers a new mechanism of action for selective inhibition of JAK2V617F, with potential to eradicate mutant clones
- INCB160058 inhibits cytokine-independent activity of *JAK2V617F* while sparing WT JAK2
  - In vitro, INCB160058 selectively targets *JAK2V617F*-harboring cell lines as well as MPN patient-derived $CD34^+$ cells, repressing pSTAT5 levels and slowing cell growth
  - In vivo, INCB160058 maintains its selective nature, reducing human JAK2V617F-derived cell engraftment; erythropoietic cells and proinflammatory cytokines were selectively reduced
- Initiation of clinical trials of INCB160058 is expected in 2024

JAK, Janus kinase; MPN, myeloproliferative neoplasm; pSTAT, phosphorylated signal transducer and activator of transcription; WT, wild-type.

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