



# The Pan-PIM Inhibitor INCB053914 Displays Potent Synergy at Low Doses in Combination With Ruxolitinib in Pre-clinical Models of MPNs



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## ABSTRACT

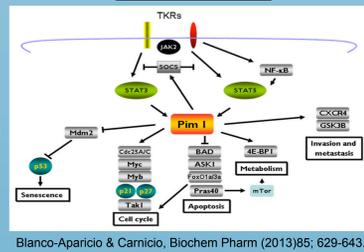
Classic Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are hematopoietic stem cell disorders that affect about 300,000 people in the U.S. and can progress to bone marrow failure and acute myeloid leukemia. MPNs are driven by aberrant activation of the JAK2 tyrosine kinase, which leads to activation of downstream signaling molecules including STAT5, ERK, and AKT, among others. While the JAK1/2 inhibitor ruxolitinib is approved for use with patients with intermediate and high risk myelofibrosis and patients with polycythemia vera who are resistant or intolerant to hydroxyurea, molecular responses to ruxolitinib therapy occur only rarely. In order to identify new MPN regimens that may enhance patient benefits, we investigated the effect of combining the JAK1/JAK2 inhibitor ruxolitinib with a new inhibitor (INCB053914) of the PIM family of Ser/Thr kinases, which are downstream targets of JAK2/STAT signaling. Members of the PIM family of kinases are proto-oncogenes that can cooperate with cMyc to induce lymphomagenesis in mice. PIM kinase activity regulates a variety of cell processes, including cell proliferation and apoptosis. For example, PIM kinases phosphorylate and inactivate the pro-apoptotic protein BAD. In addition, PIM activity can regulate translation initiation via activation of the mTORC1 complex, leading to inactivation of the translational initiation repressor 4EBP1. Triple PIM1/2/3 knockout mice demonstrate no major deleterious abnormalities suggesting specific targeting of PIM kinase activity may have limited adverse effects. However, the elimination of PIM1 in mice has suggested a role of PIM1 in the function of hematopoietic stem cells. Thus, deregulated PIM activity could affect hematopoietic stem cell disorders such as MPNs. We have demonstrated that PIM family members are elevated in MPN cells that persistently grow in the presence of ruxolitinib and that exogenous expression of PIM1 can induce ruxolitinib resistance. Taken together, PIMs appear to be strong candidates as therapeutic targets in MPNs.

INCB053914 (PIMi) exhibits biochemical IC50s against PIM1/2/3 of 0.24 nM/30 nM/0.12 nM (at 1 mM ATP). In this study, we demonstrate that PIMi exhibits potent combination effects with ruxolitinib in pre-clinical models of MPNs. PIMi displayed synergistic effects in combination with ruxolitinib in MPN model cell lines, including BaF3/EpoR-JAK2-V617F, UKE1, and SET2. These effects include growth inhibition and, importantly, synergistic induction of apoptosis. The phosphorylation of biomarkers for PIM activity, including BAD, p70S6K, S6, and 4EBP1 was significantly diminished with combination of low dose ruxolitinib (100 nM) and PIMi (100 nM). Cells that were generated to persistently grow in ruxolitinib remained sensitive to PIMi. Importantly, neoplastic growth of erythroid colonies from JAK2-V617F-positive MPN patients was sensitive to low dose PIMi (5 nM), with colony formation from some patient samples displaying sensitivity to as low as 1 nM of PIMi. In combination with ruxolitinib (25-50 nM), low dose PIMi displayed impressive synergy in inhibiting neoplastic colony formation of primary patient cells. Finally, in an in vivo model of JAK2-V617F-driven MPN cell growth, the combined treatment of ruxolitinib and PIMi suppressed tumor formation in CB17-SCID mice at doses that had little anti-tumor effect as single agents. The combination therapy was well tolerated in mice.

Together, these data suggest that the pan-PIM inhibitor INCB053914 may provide a highly effective novel anti-MPN therapeutic, particularly in combination with ruxolitinib. INCB053914 is currently under investigation in a Phase 1/2 study in patients with advanced hematological malignancies (ClinicalTrials.gov: NCT02587598). Our findings support a clinical study investigating the combination of INCB053914 and ruxolitinib in MPN patients.

- Identified as the site of proviral integration of moloney virus in retroviral-induced lymphoma in mice
- Three family members – PIM1, PIM2, and PIM3
- Cooperate with Myc to induce lymphomas in mice
- Encode similar serine-threonine kinases
- Proteins have a very short half-life (e.g. PIM1 half life is <5 min)
- Kinase activity is constitutive due to nature of the kinase domain activation loop
- Because they are always active, their activity is regulated by protein expression
- Known direct transcriptional targets of JAK/STAT signaling
- PIM1 is required for normal hematopoietic stem cell function

## PIM Substrates

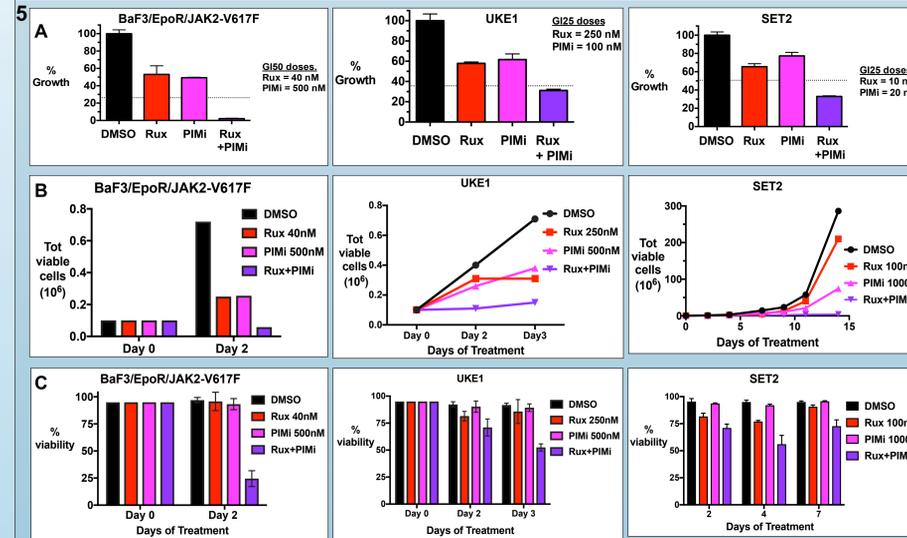
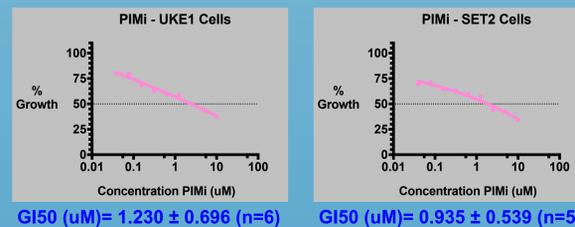


## Panel 3: Biochemical IC50 of PIMi (INCB053914) on PIM kinase activity

	IC50 (nM)		
	PIM1	PIM2	PIM3
	0.24	30	0.12

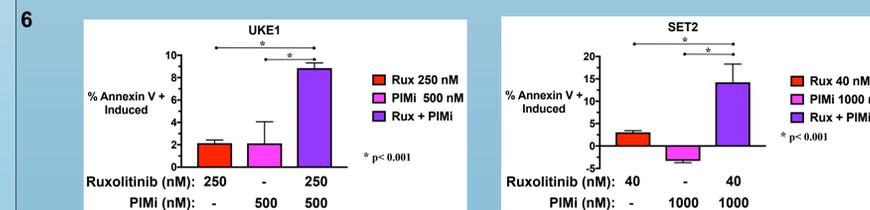
## Panel 4: Dose Response of the JAK2-driven MPN model cells UKE1 and SET2 to PIMi.

Relative viable cells for GI50 studies for PIMi were assessed at 72 hr with CellTiter-Glo. GI50 values were determined by Prism (GraphPad Software, Inc.), +/- s.d.



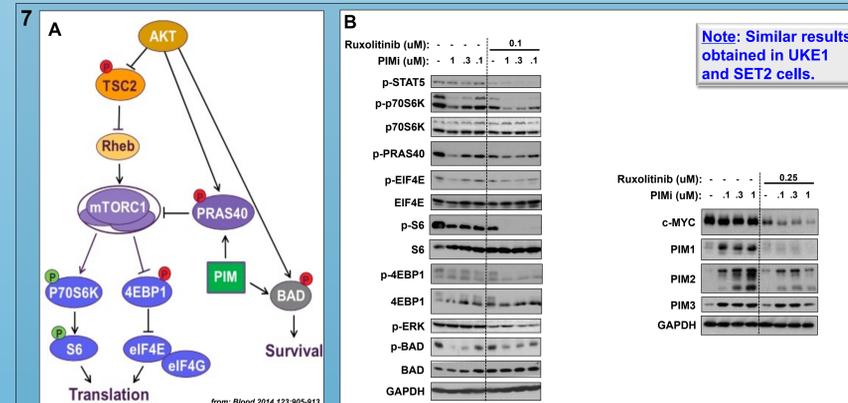
## Panel 5: PIMi Synergizes with Ruxolitinib to Inhibit MPN Model Cell Growth.

BaF3/EpoR/JAK2-V617F, UKE1, SET2 cells were either left untreated (DMSO-vehicle only, black), or treated with Rux (red), PIMi (pink), and these two together (purple). A) Synergy studies were performed by CellTiter-Glo at 72hr. Expected % Growth of combinations using the Bliss independence model is indicated by the dotted line: the lower measured % growth suggests synergy. B) Viable cells and C) % viability were determined by trypan blue exclusion over time.



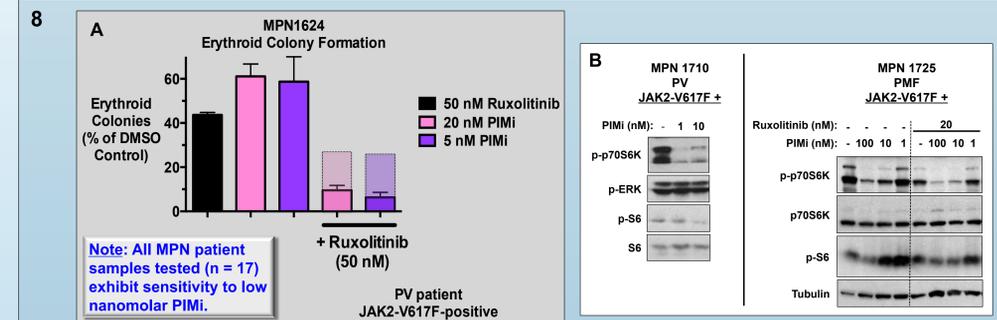
## Panel 6: PIMi and Ruxolitinib Synergistically Induce Apoptosis.

UKE1 and SET2 cells were treated with either DMSO, ruxolitinib (red), PIMi (pink), or these two together (purple). Cells were stained using FITC Annexin V and PI and analyzed by flow cytometry. Error bars indicate s.d. The p value was calculated by one-way ANOVA using Prism (GraphPad Software, Inc.).



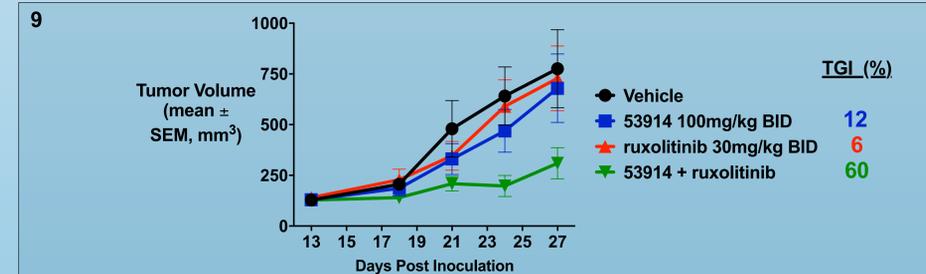
## Panel 7: PIMi and Ruxolitinib Combination Leads to Inhibition of mTOR Signaling.

**BAD Phosphorylation, c-MYC Expression.** UKE1 cells were left untreated (DMSO only), or treated with Rux, PIMi, or these two drugs together for 4hr. Cell lysates were analyzed by immunoblotting for the indicated phosphorylated and total proteins. PIM stabilization further demonstrates on-target effect of PIMi.



## Panel 8: Inhibition of Neoplastic Erythroid Colony Formation of JAK2-V617F(+) MPN Hematopoietic Progenitors by PIMi and Synergistic Inhibition in Combination With Ruxolitinib. Low Nanomolar PIMi Inhibits mTOR Signaling in JAK2-V617F(+) MPN Granulocytes.

A) Peripheral blood mononuclear cells from an MPN patient (PV, JAK2-V617F+) were plated in methylcellulose containing cytokines without erythropoietin (Epo), and in the presence of either DMSO-vehicle, 50nM Rux, 20nM or 5nM PIMi or the two drugs in combination. Epo-independent erythroid colonies were scored after 12 days. Expected % Growth of combinations using the Bliss independence model is indicated by the transparent bars with dotted lines. The lower measured % growth indicates synergy. B) Immunoblot analysis of MPN patient granulocytes left untreated (DMSO only) or treated with the indicated concentrations of PIMi or ruxolitinib.



No single agent activity of INCB053914 or ruxolitinib was observed at the doses utilized in this model.

A statistically significant effect of INCB053914 & ruxolitinib in combination, relative to vehicle & single agent treatments (both p<0.05 by 2-way ANOVA), was observed.

All doses alone and in combination were well tolerated.

## Panel 9: Efficacy of the Combination of PIMi INCB053914 and Ruxolitinib in vivo.

CB17 SCID mice were injected with SET2 cells. Once tumors formed, animals were treated with vehicle, (black line), PIMi (100mg/kg BID, blue line), ruxolitinib (30 mg/kg BID, red line), and the two drugs in combination (green line). Data represents tumor volumes over time. TGI = tumor growth inhibition.

## 10 SUMMARY

- PIMi (INCB053914) synergizes with ruxolitinib to inhibit JAK2-V617F-driven cell growth.
- PIMi and ruxolitinib synergize to induce apoptosis.
- PIMi and ruxolitinib synergistically inhibit mTORC1 signaling pathway and decrease p-BAD.
- As a single agent, PIMi inhibits Epo-independent erythroid colony formation of primary MPN patient cells at low nanomolar concentrations and inhibits mTORC1 signaling pathway in MPN patient granulocytes.
- Epo-independent erythroid colony formation of primary MPN patient cells is synergistically suppressed when PIMi is combined with ruxolitinib.
- PIMi and ruxolitinib synergistically suppress SET2 cell tumor growth in vivo.
- These data suggest that the pan-PIM inhibitor INCB053914 may provide a highly effective novel anti-MPN therapeutic, particularly in combination with ruxolitinib, supporting clinical testing of this combination in MPNs.