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Presented at the

American Association for Cancer Research

Orlando, FL, USA • April 14-19, 2023

Preclinical Characterization of the BET Inhibitor, INCB057643, in Combination With Ruxolitinib for Treatment of Myeloproliferative Neoplasms

Michelle Pusey,[1*] Gaurang Trivedi,[1*] Robert Collins,[1] Nina Zolotarjova,[1] Patricia Feldman,[1] Monica Bomber,[2] Jacob Ellis,[2] Kristy Stengel,[3] Cynthia Timmers,[1] Hamza Celik,[1] Matthew Stubbs,[1] Jeffrey Jackson,[1] Scott Hiebert,[2] Susan Wee,[1] Sunkyu Kim[1]

[1]Incyte Research Institute, Wilmington, DE; [2]Vanderbilt University, Nashville, TN; [3]Albert Einstein College of Medicine, Bronx, NY

*Authors contributed equally to this study

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Abstract

Patients with myeloproliferative neoplasms (MPNs) share common characteristics including overproduction of myeloid cells and chronic inflammation. In MPNs, bromodomain and extra-terminal (BET) proteins can bind to acetylated chromatin to regulate the activity of transcription factors such as NF-kB, leading to overproduction of inflammatory cytokines. Dysregulation of transcriptional gene expression can drive disease-defining characteristics; therefore, targeting BET proteins could be an attractive therapeutic approach. In preclinical models, BET inhibitors (BETi) decreased inflammatory cytokine production and restored hematopoietic cell differentiation. Combination of BETi with the JAK1/2 inhibitor, ruxolitinib, resulted in further reduction in cytokine production and bone marrow fibrosis.[1] Here, we investigate a novel, orally bioavailable BETi, INCB057643, for the treatment of MPN in combination with ruxolitinib. INCB057643 is a potent and selective inhibitor of the BET family. In binding assays, INCB057643 selectively inhibited both BRD4 bromodomains, BD1 and BD2, with half maximal inhibitory concentration values of 39 nM and 6 nM, respectively. INCB057643 comparably inhibited both NF-kB reporter activity and MYC protein levels. Nascent transcript analysis on the JAK2 V617F–mutant cell line SET2 showed decreased transcription of genes linked to inflammation, alone and in combination with ruxolitinib. These data suggest that INCB057643 and ruxolitinib can be used in combination to effectively suppress the pathogenic gene program driving chronic inflammation. In a SET2 xenograft model, the combination of INCB057643 and ruxolitinib potently inhibited tumor growth. Similarly, in the MPLW515L-driven MPN mouse model, INCB057643 in combination with ruxolitinib resulted in a greater reduction in spleen volume, compared with that achieved with single-agent treatment. Meso Scale Discovery assays on MPN patient–derived CD34⁺ cells and whole blood using INCB057643 showed inhibition of NF-kB–mediated production of cytokines such as interleukin-8 (IL-8). In addition, flow cytometry–based analysis on MPN patient–derived CD34⁺ cells showed that INCB057643 in combination with ruxolitinib decreased pathogenic megakaryopoiesis. Our data demonstrate that the combination of the BETi, INCB057643, with ruxolitinib can restore normal hematopoietic differentiation, reduce inflammatory gene expression, and inhibit pathogenic cell differentiation in preclinical models of MPN. INCB057643 is currently being evaluated in clinical studies as monotherapy and in combination with ruxolitinib in patients with MPN.

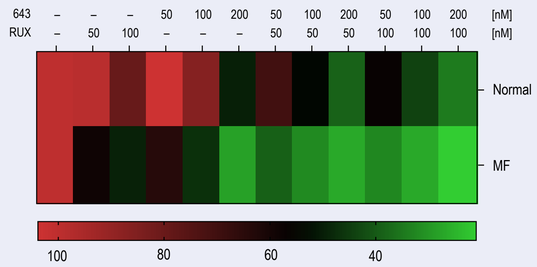
Biochemical Analysis of INCB057643

Assay	Mean IC_{50} ± SD, nM
BRD2-BD1	81 ± 13
BRD2-BD2	59 ± 11
BRD3-BD1	18 ± 4
BRD3-BD2	9 ± 2
BRD4-BD1	39 ± 6
BRD4-BD2	6 ± 1
BRDT-BD1	106 ± 13
BRDT-BD2	100 ± 11

IC_{50}, half maximal inhibitory concentration; SD, standard deviation.

- Inhibition of BET bromodomain (BD) binding to an acetylated H4 peptide by INCB057643 measured using an AlphaScreen assay or Fluorescence Anisotropy Binding assay (BRDT-BD2)

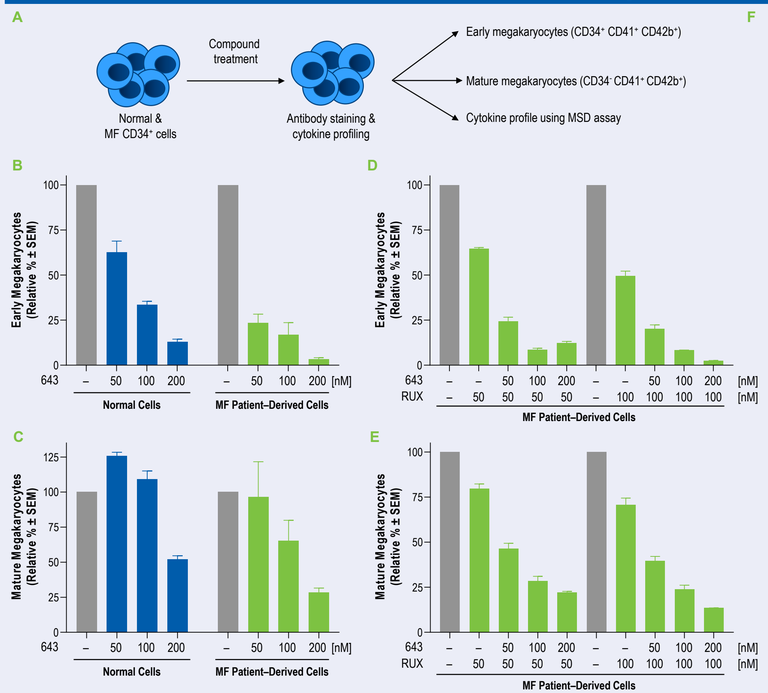
Effect of Ruxolitinib and BET Inhibitor Combination on Primary Cells



643, INCB057643; MF, myelofibrosis; RUX, ruxolitinib.

- Normal and myelofibrosis (MF) patient–derived CD34⁺ cells were treated with either ruxolitinib, INCB057643, or in combination at indicated concentrations. Percentage viability compared with dimethyl sulfoxide (DMSO) control is plotted as a heat map

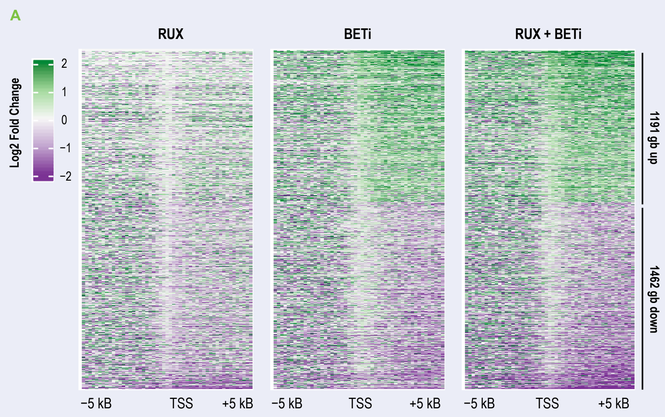
Efficacy of INCB057643, in Combination With Ruxolitinib, on Megakaryocytes Differentiation and Cytokine Production



643, INCB057643; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MIP-1b, macrophage inflammatory protein-1b; MF, myelofibrosis; MSD, Meso Scale Discovery; RUX, ruxolitinib; SEM, standard error of the mean; VEGF, vascular endothelial growth factor.

(A) Schematic diagram of the CD34⁺ cell culture experimental design. Normal and MF patient–derived CD34⁺ cells were treated with INCB057643 at 50, 100, and 200 nM concentrations. Relative percentages, normalized to DMSO control (grey bars), of (B) early megakaryocytes and (C) mature megakaryocytes are shown. MF patient–derived CD34⁺ cells were treated with INCB057643 at 50, 100, and 200 nM concentrations in combination with either 50 and 100 nM of ruxolitinib. Relative percentages of (D) early megakaryocytes and (E) mature megakaryocytes are shown. Both early and mature megakaryocyte populations were further decreased by combinatorial treatment. (F) MF cell culture supernatants were used for cytokine profiling. The heat map shows the levels of IL-8, MIP-1b, MCP-1, and VEGF cytokines. Levels of these cytokines were further decreased with combinatorial treatment

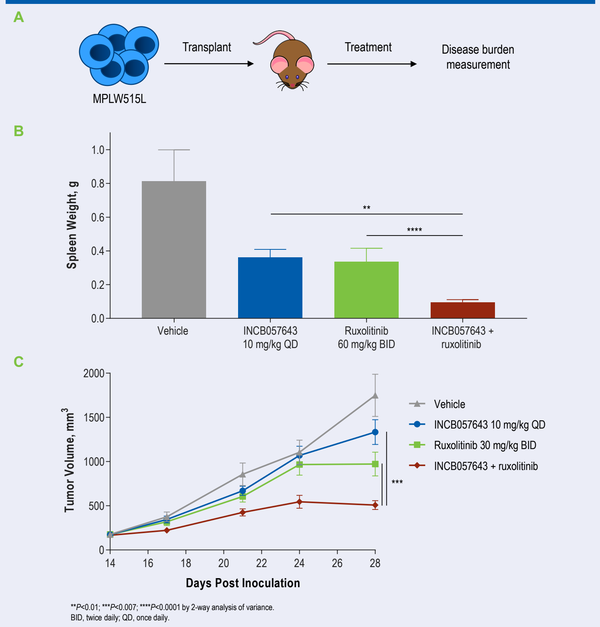
Effects of Combinatorial Treatment of INCB057643 and Ruxolitinib on Gene Expression Changes in JAK2 V617F Mutant Cells



BETi, bromodomain and extra-terminal inhibitor; FDR, false discovery rate; NES, normalized enrichment score; RUX, ruxolitinib; TSS, transcription start site.

(A) JAK2 V617F mutant SET2 cells were treated with INCB057643 and ruxolitinib, alone and in combination. Heat maps displaying log2-transformed fold change of read counts from PRO-seq in 200-bp bins ± 5 kb around the transcription start sites of the genes that were significantly up- or downregulated in the gene body by combination drug treatment for 2 hours. Genes were ranked based on log2-transformed fold change of RNA polymerase in the gene body region. (B) Gene expression profiles from gene set enrichment analysis at combinatorial drug treatment indicating a negative enrichment for the KEGG JAK/STAT and KEGG cytokine-cytokine receptor interaction gene lists

In Vivo Efficacy of Combination of INCB057643 and Ruxolitinib on Mouse Models of MPN



**P<0.01; ***P<0.007; ****P<0.0001 by 2-way analysis of variance.
BID, twice daily; QD, once daily.

(A) Schematic diagram of the MPLW515L bone marrow transplant mouse model. (B) Female BALB/c mice were transplanted with MPLW515L-transduced marrow and post-engraftment, treated with either INCB057643 10 mg/kg once daily (QD; n=10), ruxolitinib 60 mg/kg twice daily (BID; n=10), both INCB057643 and ruxolitinib (n=10), or vehicle control (n=8). Mice were dosed for 14 days, after which spleens were harvested and weighed as a surrogate for disease burden. (C) Female SCID mice were inoculated with SET2 cells in Matrigel® and dosed orally with either INCB057643 at 10 mg/kg QD (n=8), ruxolitinib 30 mg/kg BID (n=8), both INCB057643 and ruxolitinib (n=7), or vehicle control (n=8). Combination treatment resulted in a significant reduction in tumor growth compared with single agents

Conclusions

- **INCB057643, an orally bioavailable BET inhibitor, potently inhibits each bromodomain-containing protein (BRD) isoform in biochemical assays**
- **In vitro studies with INCB057643 in combination with ruxolitinib, a JAK1/JAK2 inhibitor, using MF-derived CD34⁺ cells show enhanced reduction in early and mature megakaryocytes and inflammatory cytokines**
- **Combination of INCB057643 and ruxolitinib exhibit enhanced down regulation of JAK/STAT and cytokine receptor signaling**
- **In vivo studies with MPN mouse models show that the combination of ruxolitinib and BET inhibitors leads to a greater reduction in disease burden**

Disclosures

Pusey, Trivedi, Collins, Zolotarjova, Feldman, Timmers, Celik, Stubbs, Jackson, Kim: Employment and stock ownership – *Incyte Corporation*. **Wee:** Former employee and stock ownership – *Incyte Corporation*. **Bomber, Ellis, Stengel, Hiebert:** Nothing to disclose.

Acknowledgments

Editorial and graphics support was provided by Envision Pharma Group (Philadelphia, PA), and funded by Incyte Corporation.

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2. Stubbs MC, et al. *Cancer Res*. 2017;77(13 Suppl):5071 (Abstract).

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