

## Abstract

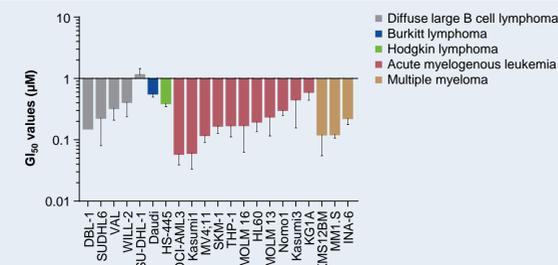
Inhibitors of the Bromodomain and Extra-Terminal (BET) family of bromodomain containing proteins regulate expression of key cell fate, cell cycle, and survival genes including *c-myc*. In preclinical models, BET inhibitors have demonstrated significant efficacy in a variety of different oncology indications, including hematological malignancies. Here we describe the preclinical profile of the novel, orally bioavailable BET inhibitor INCB057643 in preclinical models of hematologic malignancies. INCB057643 inhibited binding of BRD2/BRD3/BRD4 to an acetylated histone H4 peptide in the low nM range, and was selective against other bromodomain containing proteins. *In vitro* analyses showed that INCB057643 inhibited proliferation of human AML, DLBCL, and multiple myeloma cell lines, with a corresponding decrease in MYC protein levels. Cell cycle analyses indicated that G<sub>1</sub> arrest and a concentration-dependent increase in apoptosis were seen within 48 hours of treatment with INCB057643. BRD proteins also regulate the expression of many pro-inflammatory genes. Production of several cytokines, including IL-6, IL-10 and MIP-1 $\alpha$ , was repressed by INCB057643 in human and mouse whole blood stimulated *ex vivo* with LPS. Consistent with these effects, analyses of gene expression in cells treated with INCB057643 revealed that pathways involved in cell cycle progression, apoptosis, and IL-6 were among the most significantly altered *in vitro*. Oral administration of INCB057643 resulted in significant anti-tumor efficacy in xenograft models of AML, myeloma, and DLBCL. Additionally, combining INCB057643 with standard of care agents used for the treatment of DLBCL including rituximab and bendamustine resulted in enhanced anti-tumor efficacy relative to that achieved with single agent therapies at doses that were well tolerated. In addition, many B cell malignancies are reliant on the PI3K $\delta$  pathway for proliferation and survival, suggesting that the combination of INCB057643 with the clinical stage PI3K $\delta$  specific inhibitor INCB050465 may be a rational therapeutic strategy for DLBCL. Compared with single agent BET or PI3K $\delta$  therapy, the combination significantly potentiated tumor growth inhibition in DLBCL models representative of the ABC subtype (HBL-1), and the double hit GCB subtype (WILL-2). These data suggest that clinical exploration of INCB057643 as a monotherapy or in combination in hematologic malignancies is warranted.

## Biochemical Properties of INCB057643

Assay	Mean IC <sub>50</sub> $\pm$ SD (nM)	Number of Repeats
BRD2-BD1	81 $\pm$ 13	14
BRD2-BD2	59 $\pm$ 11	14
BRD3-BD1	18 $\pm$ 4	14
BRD3-BD2	9 $\pm$ 2	14
BRD4-BD1	39 $\pm$ 6	14
BRD4-BD2	6 $\pm$ 1	14
BRDT-BD1	106 $\pm$ 13	7
BRDT-BD2	100 $\pm$ 11	7

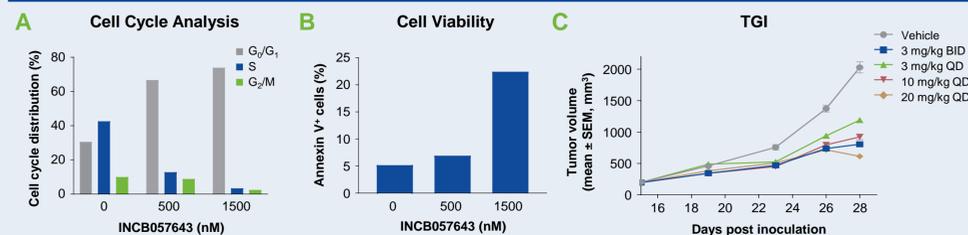
Inhibition of Bromodomain and Extra-Terminal (BET) bromodomain (BD) binding to an acetylated H4 peptide by INCB057643 measured using an AlphaScreen assay or Fluorescence Anisotropy Binding assay (BRDT-BD2).

## Sensitivity of Hematologic Cell Lines to INCB057643 *In Vitro*



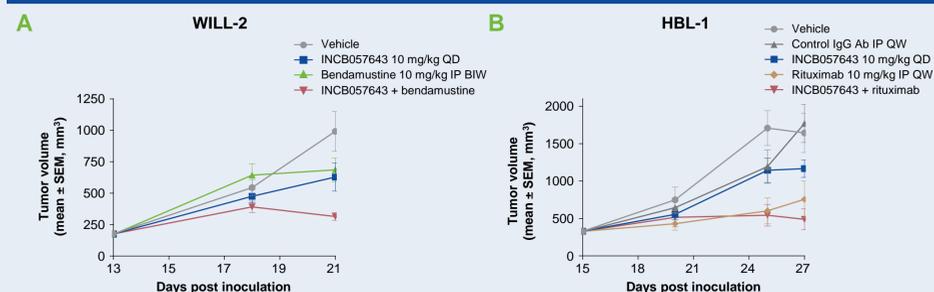
Cells were treated for 72 hours with 11 concentrations of INCB057643 (1 nM–5  $\mu$ M). Growth was measured using the ATP assay (Promega, Madison, WI), values converted to percent inhibition, and growth inhibitory (GI<sub>50</sub>) values were calculated by nonlinear regression curve fitting using 4 parameter dose-response curves (GraphPad Prism).

## Effects of INCB057643 *In Vitro* and *In Vivo* in the WILL-2 Xenograft Model of Double-Hit Diffuse Large B Cell Lymphoma



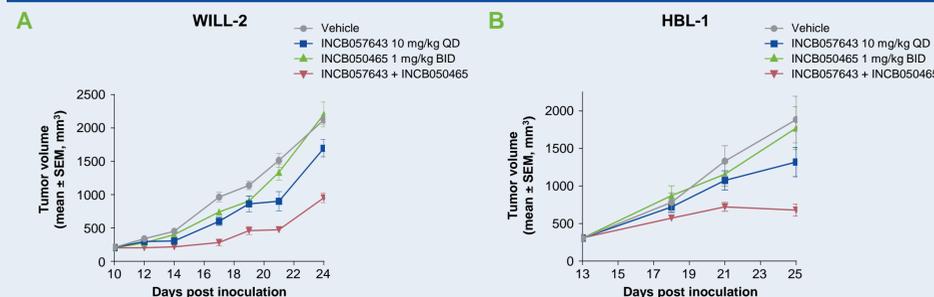
WILL-2 (double-hit) diffuse large B cell lymphoma cells were treated with INCB057643: cell cycle (A) and viability/apoptosis (B) were analyzed by propidium iodide and annexin V staining, respectively. Inhibition of tumor growth (C) was determined in female SCID mice inoculated with  $1.5 \times 10^7$  WILL-2 cells in matrigel. Mice were dosed orally with INCB057643 at 3, 10, or 20 mg/kg QD, or INCB057643 at 3 mg/kg BID or vehicle BID. All INCB057643 treatment groups showed statistically significant decreases ( $P < 0.05$ ) in % tumor growth inhibition (TGI) by study termination compared with the vehicle control group. Each INCB057643 dose was well tolerated.

## Efficacy of INCB057643 Combined With Standard of Care Agents in Diffuse Large B Cell Lymphoma Xenograft Models



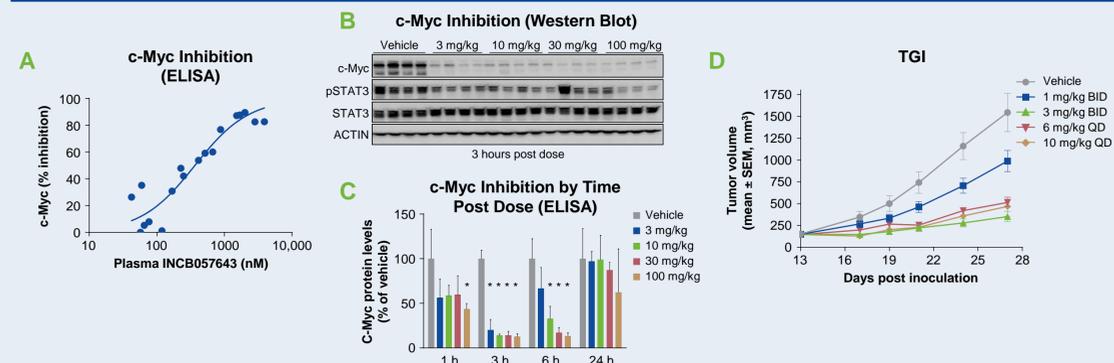
A. Female SCID mice were subcutaneously inoculated with  $1.5 \times 10^7$  WILL-2 cells in matrigel and dosed orally with vehicle, or INCB057643 and bendamustine alone or in combination ( $n = 10$ /group). The combination of INCB057643 and bendamustine resulted in significantly greater %TGI versus each agent alone ( $P < 0.02$  for each group). All doses were well tolerated.  
B. Female SCID mice were subcutaneously inoculated with HBL-1 tumor fragments. Mice were dosed orally with vehicle, INCB057643, or dosed IP with control rat IgG antibody, or rituximab, or with INCB057643 plus rituximab ( $n = 9$ /group). %TGI was significantly greater with INCB057643 plus rituximab versus either vehicle or IgG controls ( $P = 0.0013$ ;  $P = 0.0004$ ); importantly, the combination resulted in significantly greater %TGI relative to INCB057643 alone ( $P = 0.0033$ ). All dosing regimens were well tolerated.

## Efficacy of INCB057643 Combined With the PI3K $\delta$ Inhibitor INCB050465 in Diffuse Large B Cell Lymphoma Xenograft Models



Female SCID mice were subcutaneously inoculated with (A)  $1.5 \times 10^7$  WILL-2 cells in matrigel or (B) with HBL-1 tumor fragments ( $n = 9$ /group). In each study, despite the lack of efficacy from either single agent alone, combining INCB050465 with a minimally active dose of INCB057643 resulted in a significant enhancement in efficacy; %TGI was significantly greater with the combination versus each single agent (WILL-2:  $P < 0.0012$  for each; HBL-1:  $P < 0.007$  for each). All dosing regimens were well tolerated.

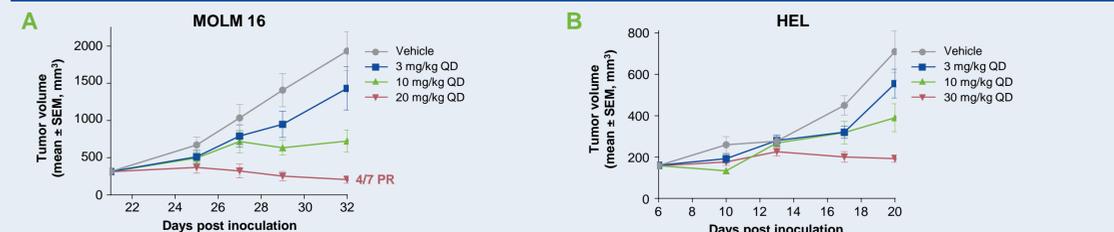
## Efficacy and Pharmacodynamic Response to INCB057643 in a Xenograft Model of Multiple Myeloma



Female SCID mice were inoculated with  $1 \times 10^7$  MM1.S cells in matrigel and were dosed once orally with INCB057643 at 0.1–100 mg/kg or vehicle. At 3 hours post dose, tumors were harvested for protein analyses by c-Myc ELISA and Western blots; blood was drawn for plasma PK analysis.

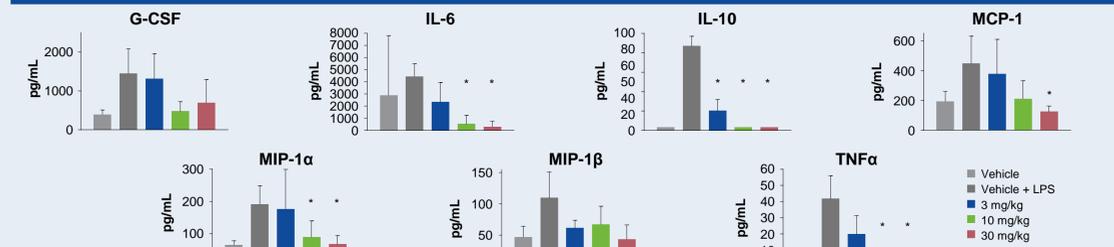
A. The *in vivo* IC<sub>50</sub> of INCB057643 was 367 nM as assessed by c-Myc ELISA.  
B. Western blotting verified the reduction in c-Myc protein levels at doses greater than 1 mg/kg.  
C. At 1, 3, 6, and 24 hours post dosing, tumors were harvested for protein analyses by c-Myc ELISA ( $n = 3$ /group); asterisks indicate statistically significant deviations versus vehicle controls ( $P < 0.05$ ).  
D. Compared with vehicle control, %TGI was significantly greater for all INCB057643 doses ( $P < 0.05$  for each;  $n = 10$ /group). All doses were well tolerated.

## Efficacy of INCB057643 in Xenograft Models of Acute Myelogenous Leukemia



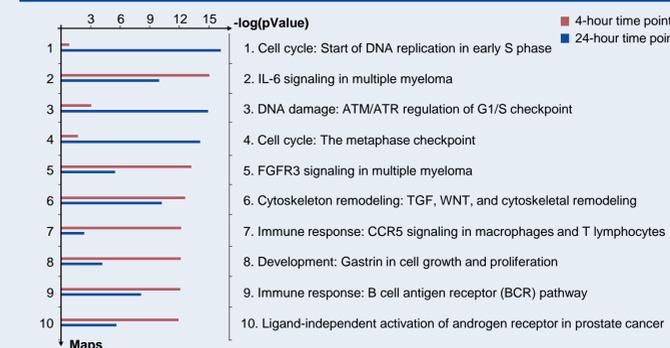
A. Female SCID mice were inoculated with  $1 \times 10^7$  MOLM16 cells in matrigel and dosed orally with INCB057643 at 3, 10, or 20 mg/kg QD, or vehicle control ( $n = 8$ /group). Dose-dependent antitumor activity was observed; %TGI with 10- and 20-mg/kg QD doses differed significantly from vehicle controls ( $P < 0.05$  for each group). In the 20-mg/kg group, 4 of 7 tumor-bearing mice achieved partial tumor regressions, where tumor volumes diminished to less than 50% of the pretreatment volume. All doses were well tolerated.  
B. Female SCID mice were inoculated with  $1 \times 10^7$  HEL cells in matrigel and dosed orally with INCB057643 at 3, 10, or 30 mg/kg QD or vehicle control ( $n = 10$ /group). The %TGI with all dose groups except the 3-mg/kg QD group differed significantly from the vehicle control group ( $P < 0.02$  for each group). All doses were well tolerated.

## INCB057643 Alters Levels of Inflammatory Cytokines *Ex Vivo*



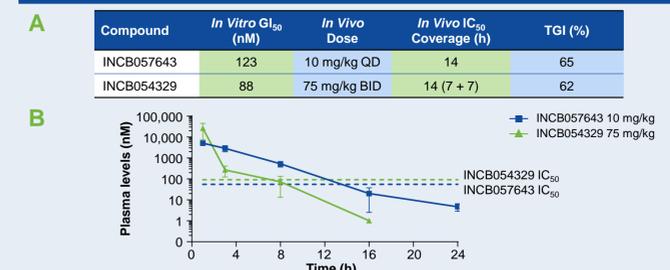
An *ex vivo* LPS assay was performed on blood drawn from mice treated once orally with INCB057643 at the indicated doses to evaluate its effects on cytokine profiles using a Luminex cytokine panel. Profiles 3 hours post-dose are shown ( $n = 3-4$ /group); asterisks indicate statistically significant differences versus the LPS treatment group.

## Transcriptional Analyses of INCB057643-Treated MM1.S Multiple Myeloma Cells



Affymetrix microarray analyses of MM1.S cells treated with INCB057643 for 4 or 24 hours. Differentially expressed genes were determined for both time points relative to vehicle controls ( $> 1.5$ -fold change and false discovery rate  $< 0.05$ ). The 10 most significantly enriched pathways are depicted with bars showing  $-\log(pValue)$ .

## Comparison of INCB057643 to INCB054329



A. Using the MM1.S cell line as a standard, INCB057643 and INCB054329 have comparable GI<sub>50</sub> values in a 72-hour *in vitro* proliferation assay.  
B. *In vivo* xenograft modeling demonstrates that covering an IC<sub>50</sub> MYC protein level repression for similar amounts of time results in similar %TGI despite the differing half-lives of these compounds.

## Conclusions

- INCB057643 potently inhibits each BRD isoform in biochemical assays and inhibits the viability of a wide range of hematologic cancer cell lines *in vitro*
- Both *in vitro* and *in vivo*, INCB057643 regulates markers of proliferation, survival, and inflammation and oncogenic transcription factors such as c-Myc
- INCB057643 exhibits significant single agent activity in models of multiple myeloma, acute myelogenous leukemia, and diffuse large B cell lymphoma, and its activity is enhanced in combination with bendamustine, rituximab, or the PI3K $\delta$  specific inhibitor, INCB050465
- A phase 1 clinical trial of INCB057643 in patients with advanced malignancies is ongoing (NCT02711137)

## Author Disclosures

All Authors: Incyte Corporation; Employment and Stock Ownership.

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