

Preclinical Studies on Potential Therapeutic Combination Partners for the Potent and Selective PI3K δ Inhibitor INCB050465 in Various Subtypes of DLBCL

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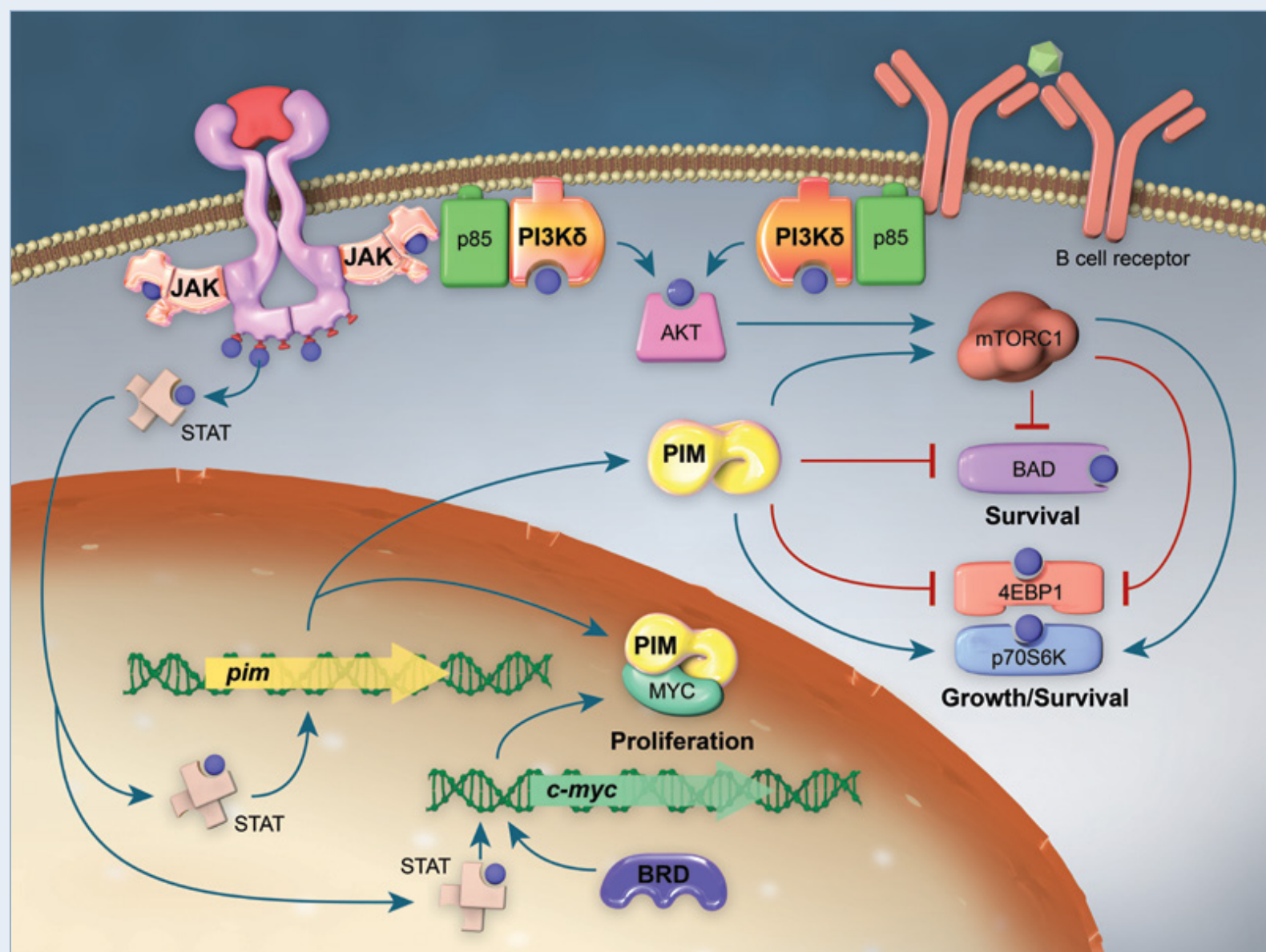
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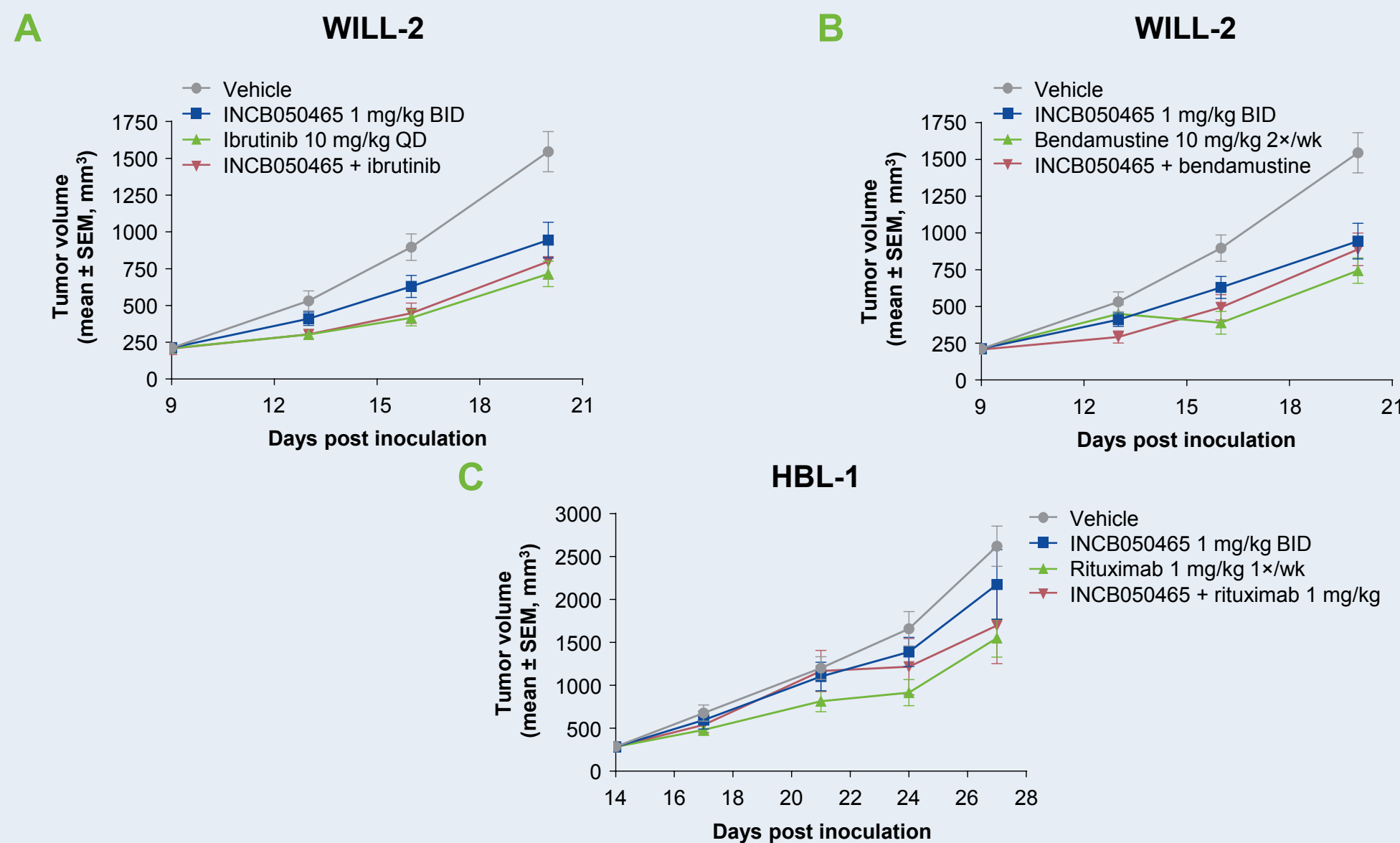
Abstract

The delta isoform of PI3K (PI3K δ) plays an essential role in B-cell development and function by mediating the signaling of key receptors on B cells. Increased malignant B cell proliferation and survival has also been associated with aberrant activation of PI3K δ , making selective inhibition of this isoform an attractive therapeutic approach for the treatment of B cell malignancies. INCB050465 is a potent inhibitor of PI3K δ , with a >20,000 fold selectivity over other PI3K isoforms. Emerging clinical data indicate that INCB050465 monotherapy is well tolerated and results in promising clinical responses in patients with various lymphoma histologies, including those with DLBCL. We therefore sought to explore rational combination strategies for INCB050465 using mouse xenograft models of ABC-subtype (HBL-1), GCB-subtype (Pfeiffer), and GCB/double-hit (WILL-2) human DLBCL, evaluating standard of care agents such as bendamustine and rituximab, as well as with targeted agents. PIM inhibition is a logical addition to PI3K δ inhibition as a therapeutic approach as both kinases play a critical role in the AKT signaling pathway, having overlapping substrates. Likewise BET inhibition is a rational addition to PI3K δ inhibition in "double-hit" DLBCL due to de-regulation of MYC transcriptional activity. *In vivo* studies performed in the Pfeiffer xenograft model demonstrate that INCB050465 combined with the pan-PIM inhibitor INCB053914 yielded complete tumor regressions. This profound decrease in tumor cell survival was due in part to the significant reduction in pBAD levels resulting from dual PIM and PI3K δ inhibition. Despite modest single agent activity *in vivo*, the combination of INCB050465 with BET inhibitors, INCB054329 or INCB057643, resulted in significant anti-tumor efficacy in all of the DLBCL models studied, and caused a marked repression in tumor MYC expression. To study the transcriptional effects of combining PI3K δ and BET inhibitors in this lymphoma model, WILL-2 xenograft tumors from mice treated with single dose INCB050465, INCB054329, the combination, or vehicle control were analyzed by RNAseq. INCB050465 enhanced the ability of INCB054329 to repress a MYC-driven transcriptional program, and the combination also regulated multiple developmental and inflammatory pathways. Together, these data support the clinical evaluation of the PI3K δ inhibitor INCB050465 as part of a combination regimen with PIM or BET inhibitors for the treatment of DLBCL.

PI3K δ Pathway Interactions

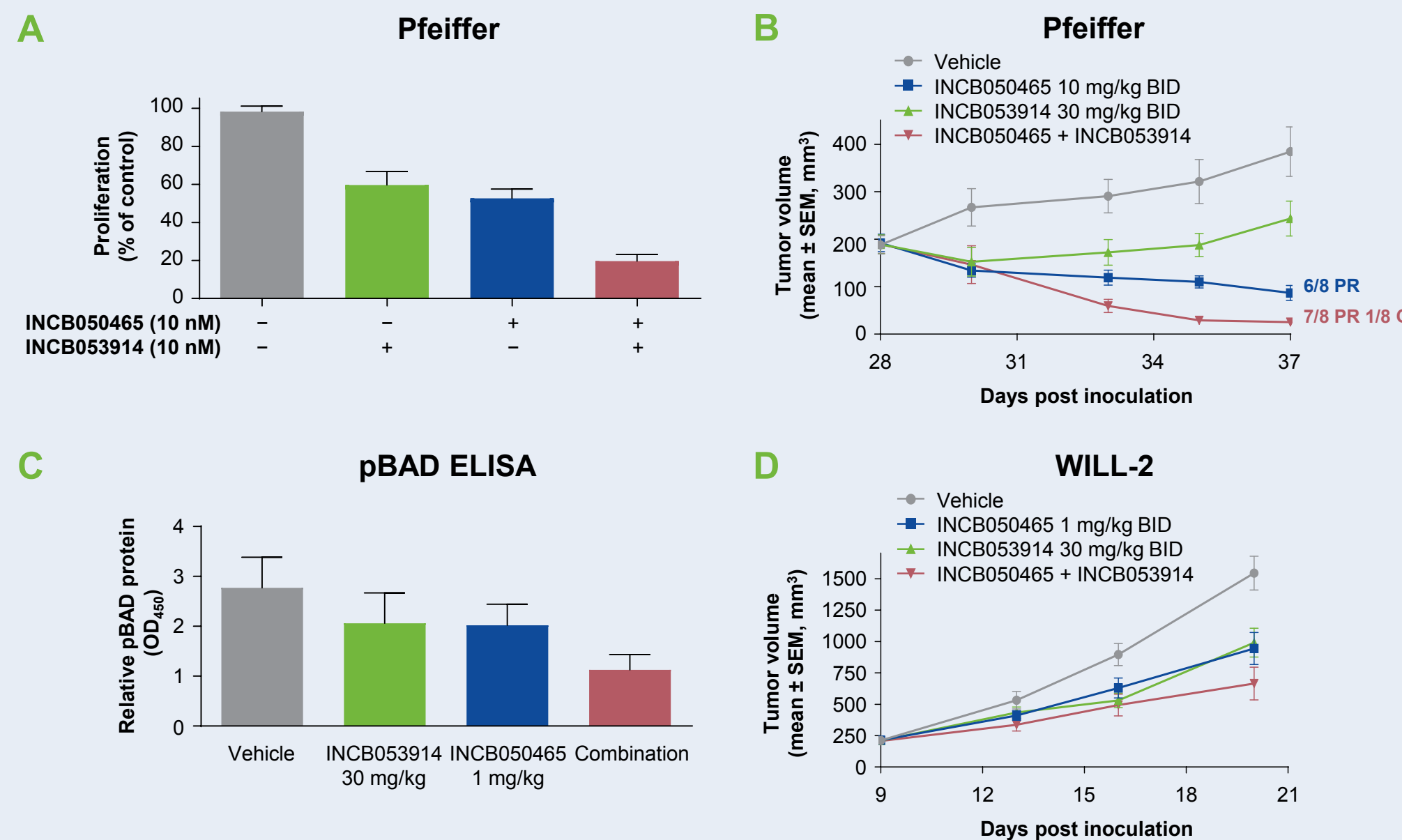


Efficacy of Combinations of INCB050465 and Current Therapeutic Agents for DLBCL



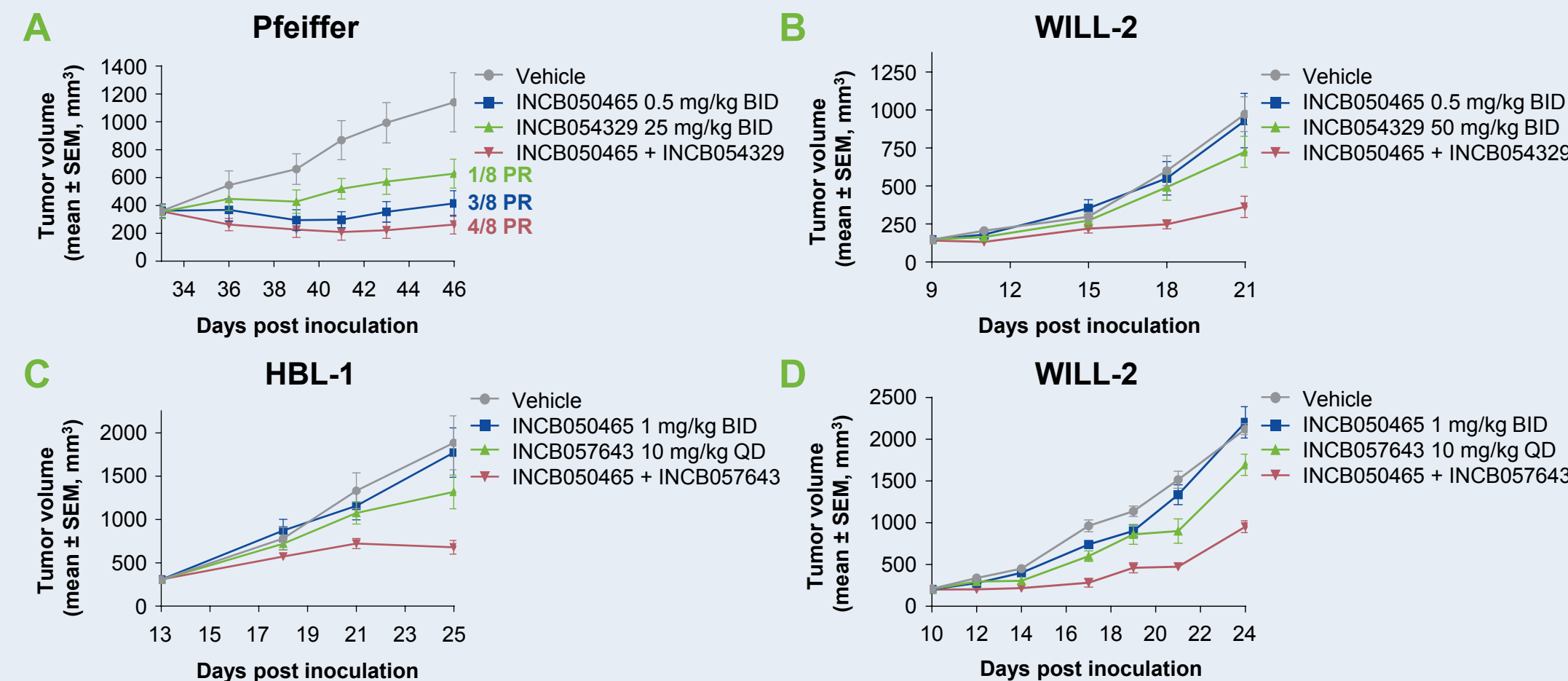
Combinations of INCB050465 with either ibrutinib (A) or bendamustine (B) in the WILL-2 xenograft model did not show any benefit over single agents alone. Combining rituximab and INCB050465 in the HBL-1 xenograft model did not enhance the efficacy of either INCB050465 or rituximab (C). All dosing combinations were well tolerated. The HBL-1 model expresses CD20, the target of rituximab, while the WILL-2 model does not express CD20.

Efficacy and Pharmacodynamics of Combination of INCB050465 and the Pan-PIM Inhibitor INCB053914

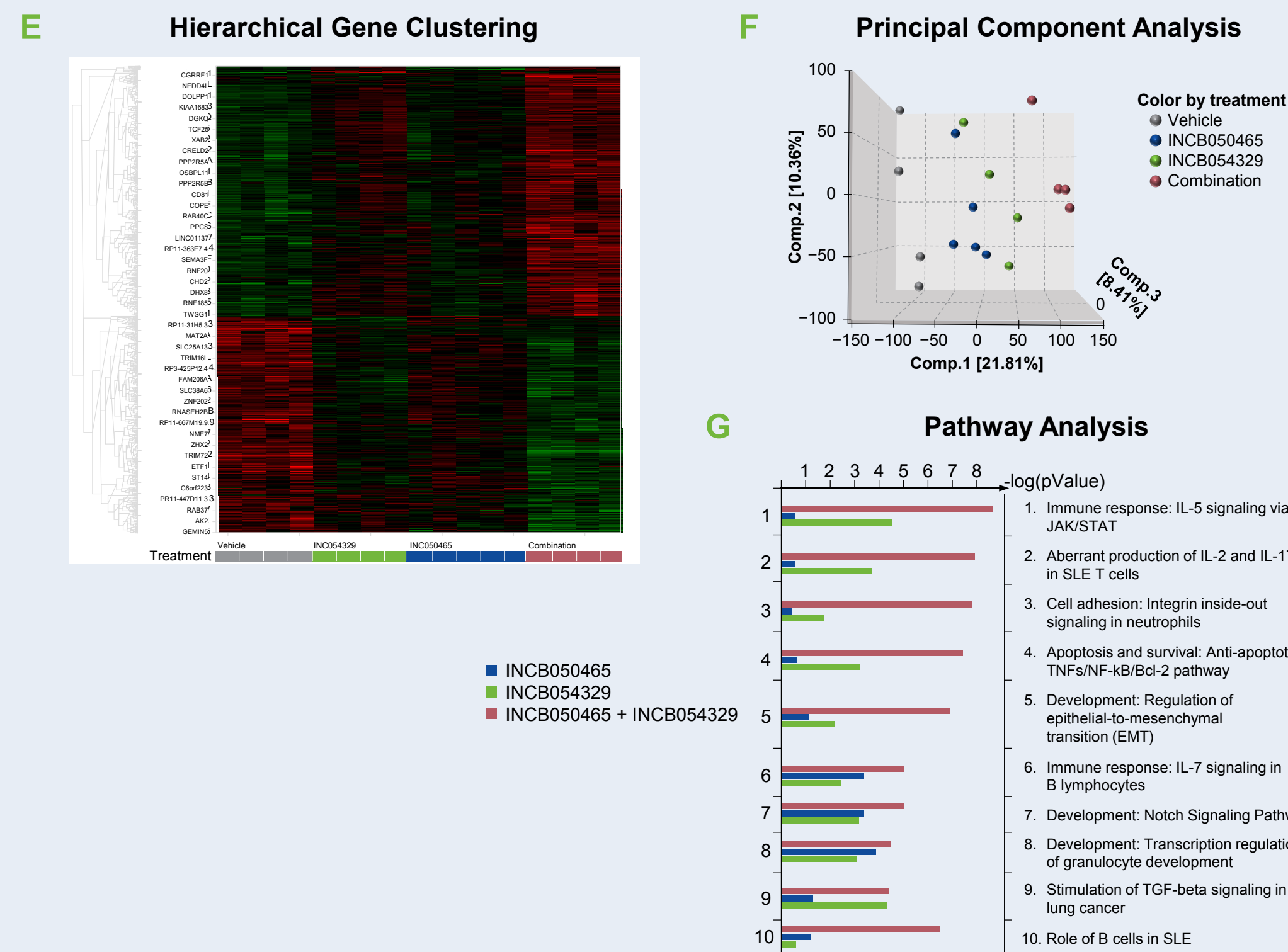


- A. INCB050465 and pan-PIM inhibitor INCB053914 cooperate to slow growth of Pfeiffer cells *in vitro*.
B. Addition of INCB053914 to INCB050465 enhances efficacy in the Pfeiffer xenograft model (INCB050465 vs combination, $P < 0.002$, t test).
C. Pfeiffer xenograft tumors show enhanced inhibition of pBAD levels when treated with both INCB050465 and INCB053914.
D. Combination of INCB050465 with INCB053914 in the WILL-2 xenograft model yields increased efficacy over either single agent alone.

Efficacy of Combinations of INCB050465 and the BET Inhibitors INCB054329 and INCB057643

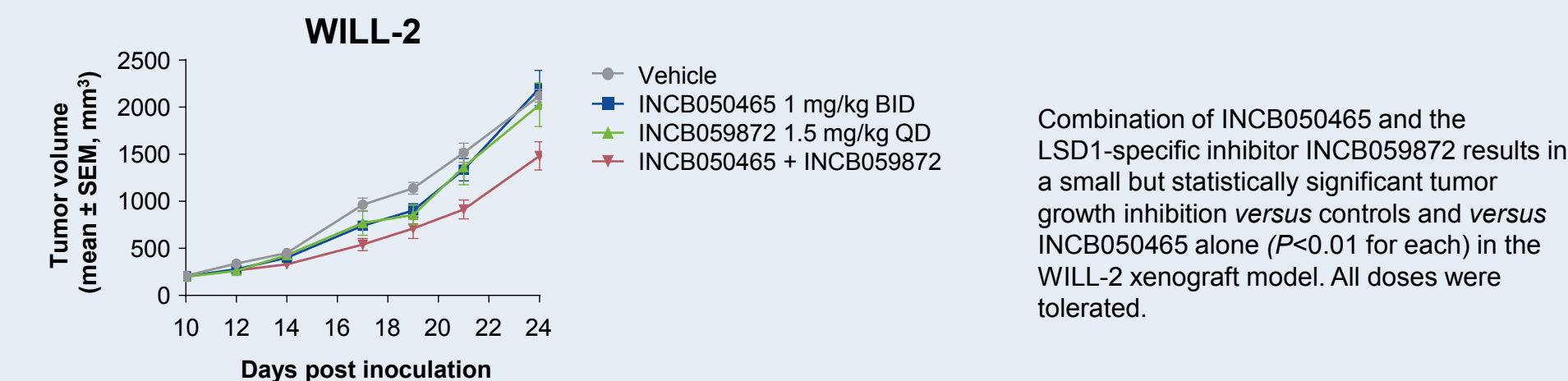


Combination of INCB050465 with the BET inhibitor INCB054329 in the Pfeiffer (A) or WILL-2 (B) xenograft models, or with the BET inhibitor INCB057643 in the HBL-1 (C) or WILL-2 (D) xenograft models. In the HBL-1 and WILL-2 tumor models, tumor growth inhibition from the combination groups significantly differed from that of each single agent ($P < 0.02$, t test for all).



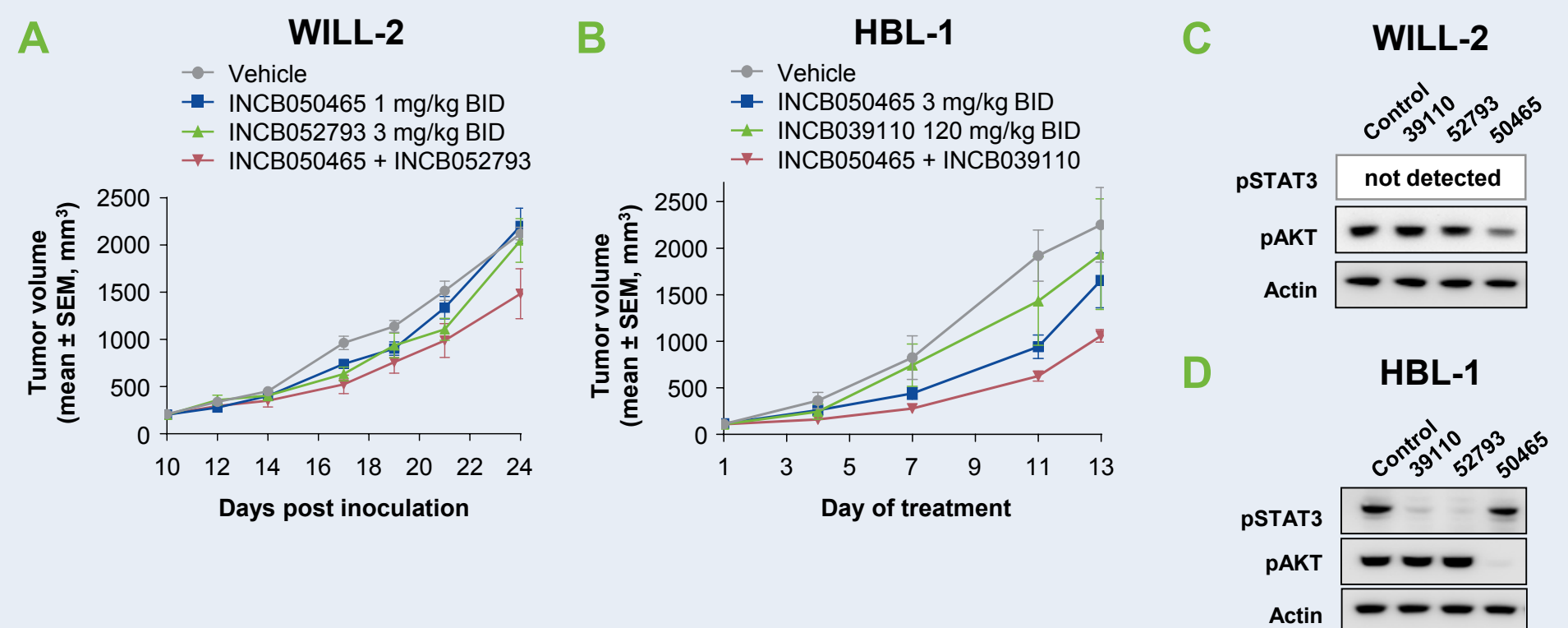
- WILL-2 xenograft tumors were harvested 4 hours after 1 dose of INCB050465 (1 mg/kg), INCB054329 (50 mg/kg), their combination, or vehicle control. RNA was generated from tumors for sequencing (RNAseq).
E. General linear model and hierarchical clustering of 5654 differentially expressed genes showing distinct expression patterns of each treatment group.
F. Principal component analysis was performed with the treatment groups being denoted by colored circles.
G. Pathway analysis of differentially expressed genes with bars showing $-\log(pValue)$ for the treatment groups relative to vehicle controls.

Efficacy of Combination of INCB050465 and the LSD1 Inhibitor INCB059872



Combination of INCB050465 and the LSD1-specific inhibitor INCB059872 results in a small but statistically significant tumor growth inhibition *versus* controls and *versus* INCB050465 alone ($P < 0.01$ for each) in the WILL-2 xenograft model. All doses were tolerated.

Efficacy of Combination of INCB050465 and the JAK1-Specific Inhibitors INCB052793 and INCB039110



- A. Combination of INCB050465 and the JAK1 specific inhibitor INCB052793 results in a small but statistically significant tumor growth inhibition *versus* controls and *versus* INCB050465 alone ($P < 0.05$ for each) in the WILL-2 xenograft model. All doses were tolerated.
B. Combination of INCB050465 and the JAK1 specific inhibitor INCB039110 (itacitinib) in the HBL-1 model also results in an increase in efficacy over INCB050465 alone, with the tumor growth inhibition from the combination group giving the only significant change from vehicle ($P < 0.02$, t test). All doses were tolerated.
C & D. Western blots from *in vitro* testing of the JAK1 inhibitors (1 μ M) or INCB050465 (10 nM) on the WILL-2 (C) and HBL-1 (D) cell lines. In the WILL-2 line, pSTAT3 was undetectable.

Conclusions

- INCB050465 can enhance efficacy of JAK1 and pan-PIM selective kinase inhibitors as well as inhibitors of epigenetic regulators (BET, LSD1) to varying degrees *in vivo*
- Combinatorial effects from INCB050465 on tumor growth may be context dependent
- Combination of INCB050465 with a pan-PIM inhibitor strongly inhibits tumor cell growth in 2 DLBCL models, due in part to suppressing pBAD levels
- Combining INCB050465 with BET inhibitors gives a synergistic effect brought on by stronger repression of BET regulated genes as well as novel regulation of several key lymphoma pathways
- Levels of pSTAT3 may indicate whether combination of INCB050465 with JAK1 inhibitors will give a robust effect on tumor growth
- Further studies are required to determine the context for combining INCB050465 with current therapeutic options for DLBCL

Author Disclosures

All Authors: Incyte Corporation: Employment and Stock Ownership.

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