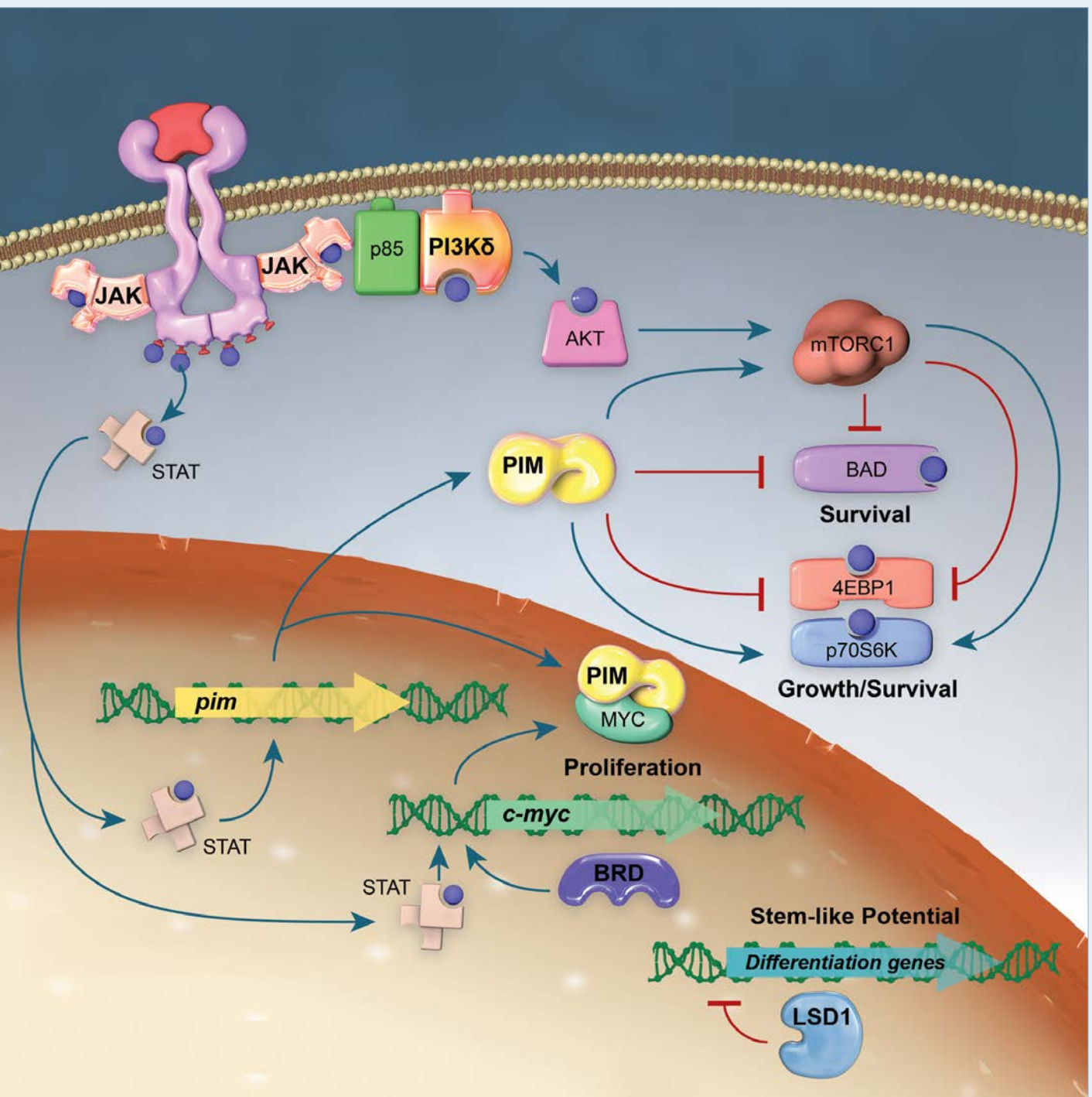




Abstract

Combinatorial therapeutic strategies have achieved improved response rates and durability of responses in several malignancies either by selectively targeting distinct and non-overlapping oncogenic signaling pathways (e.g. PARP and phosphoinositide 3-kinase (PI3K) inhibition in subsets of breast and ovarian cancers), or alternatively, inhibiting distinct nodal points of regulation downstream in common oncogenic signaling pathways (e.g. BRAf and MEK inhibition in subsets of melanoma). Recent data suggest that deregulated epigenetic modifications may be just as significant as genetic mutations in driving cancer development and growth by inhibition of tumor suppressor activity and activation of oncogenic pathways. We therefore hypothesized that an epigenetic regulator could potentiate the efficacy of a protein kinase inhibitor to result in robust tumor growth inhibition. We previously reported that the potent and selective LSD1 inhibitor INCB059872 potently inhibited tumor growth in multiple tumor xenograft models of AML and SCLC as a single agent and in a combination with standard of care of agents. In this study, we explored the anti-tumor effect of combining INCB059872 and various signal transduction pathway inhibitors, including the PIM kinase inhibitor INCB053914, the JAK1/2 inhibitor ruxolitinib, or the PI3K δ -selective inhibitor INCB050465 in models of human hematologic malignancies. Each of these therapeutic combinations significantly inhibited tumor growth in the Molm-16 human AML xenograft model. Mechanistic studies suggested that MYC expression levels were downregulated by these combinations both *in vitro* and *in vivo*. Treatment with INCB059872 alone or in combination with signal transduction kinase inhibitors significantly downregulated cytokines levels, particularly IL-10, sCD40L, and MCP-1 in Molm-16 tumors. These data suggest that the combination of an LSD1 inhibitor and signal transduction inhibitors can co-regulate key tumor intrinsic and extrinsic pathways involved in paracrine or autocrine signaling in AML. In addition to the improved efficacy observed in AML models, the combination of INCB059872 with the PI3K δ inhibitor INCB050465 enhanced tumor growth inhibition in the WILL-2 xenograft model (GCB subtype, double hit lymphoma), whereas the activity of these single agents were modest in this particular subtype of lymphoma. Additional mechanistic studies are ongoing to further understand the molecular bases of these observations. Taken together, these data suggest that targeting distinct epigenetic and oncogenic signaling pathways may potentiate anti-tumor efficacy and overcome intrinsic resistance mechanisms in specific hematologic malignancies.

Critical Pathways in Hematologic Malignancies

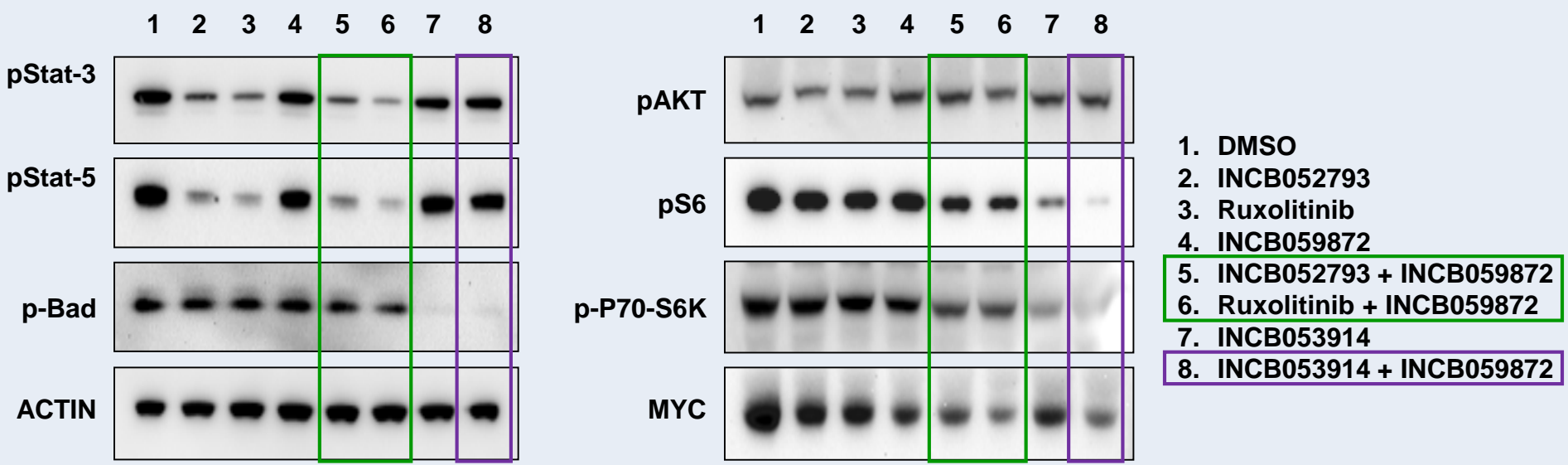


Biochemical Potencies of INCB059872, INCB050465, INCB053914, INCB052793, and Ruxolitinib

Inhibitory Activity of INCB059872		Inhibitory Activity of INCB050465	
Enzyme	INCB059872 IC ₅₀ (nM)	Enzyme	INCB050465 IC ₅₀ (nM)
LSD1	18	PI3K α	>20,000
LSD2	>5000	PI3K β	>20,000
MAO-A	>20,000	PI3K γ	19,000
MAO-B	>20,000	PI3K δ	1

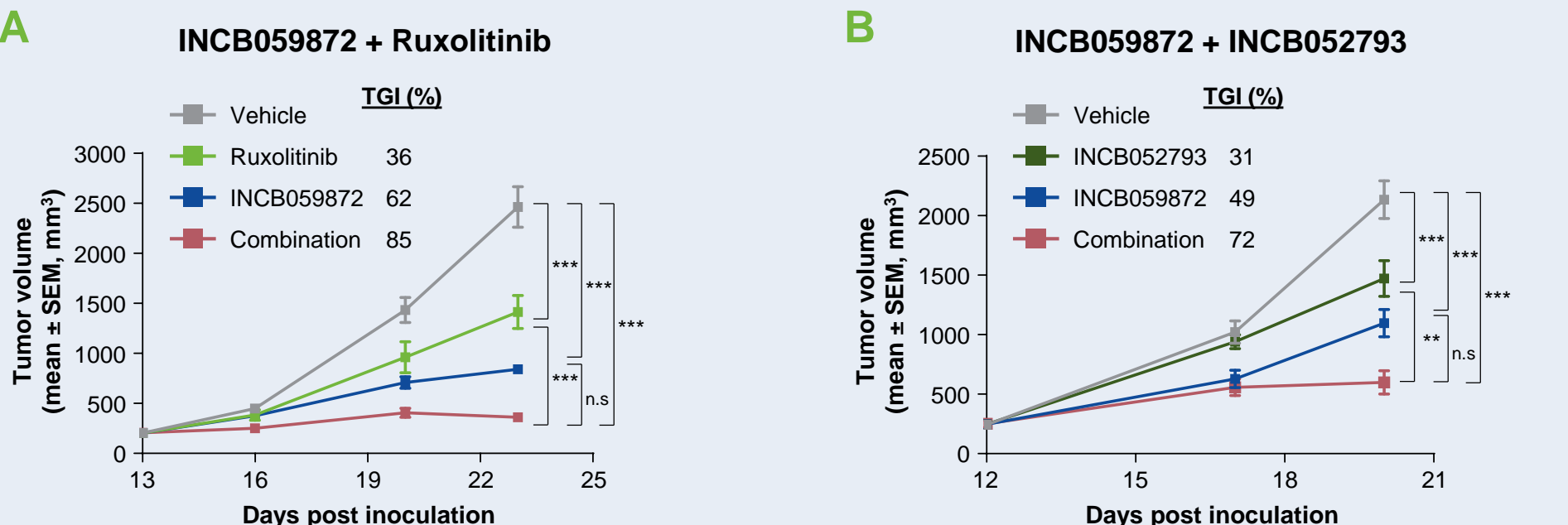
Inhibitory Activity of INCB053914		Inhibitory Activity of INCB052793 and Ruxolitinib	
Enzyme	INCB053914 IC ₅₀ (nM)	Enzyme	INCB052793 IC ₅₀ (nM) Ruxolitinib IC ₅₀ (nM)
PIM1	0.24	JAK1	1.8 3.3
PIM2	30	JAK2	277 2.8
PIM3	0.12	JAK3	1548 428

Combination of LSD1 Inhibition With JAK Inhibition or PIM Kinase Inhibition Decreases MYC Expression and pS6 Signaling in Molm-16 AML Cells



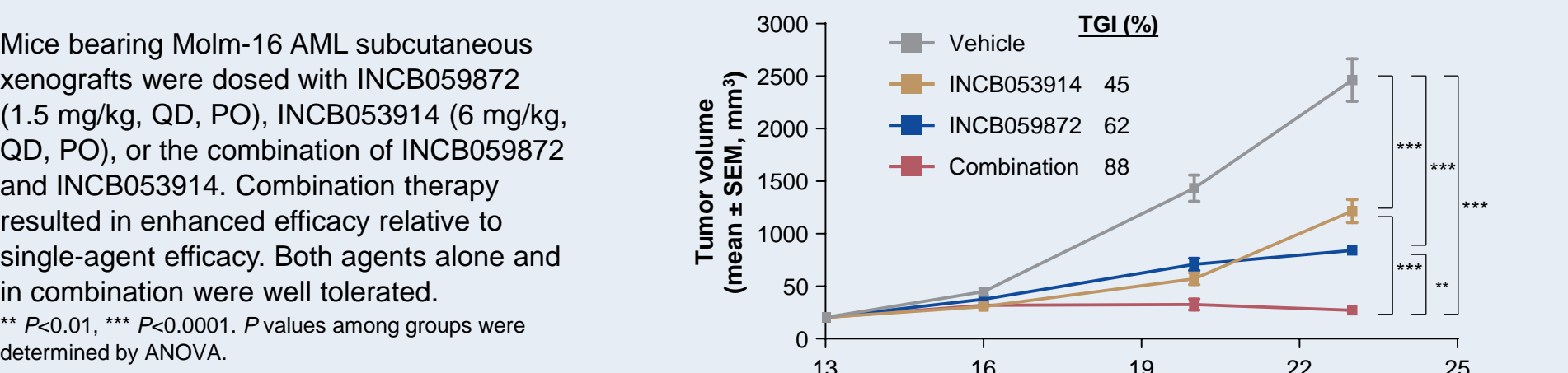
Molm-16 human acute myeloid leukemia (AML) cells were incubated with inhibitors (100 nM each) as indicated. Western blot data suggested that the combination of INCB059872 and protein kinase-associated signal transduction pathway inhibitors decreased MYC expression and inhibited the pS6 signal transduction pathway. These data suggest that either paracrine or autocrine kinase-associated signaling pathways may be co-regulated by LSD1.

Combination of LSD1 Inhibition and JAK Inhibition Significantly Enhances TGI in Molm-16 AML Xenografts

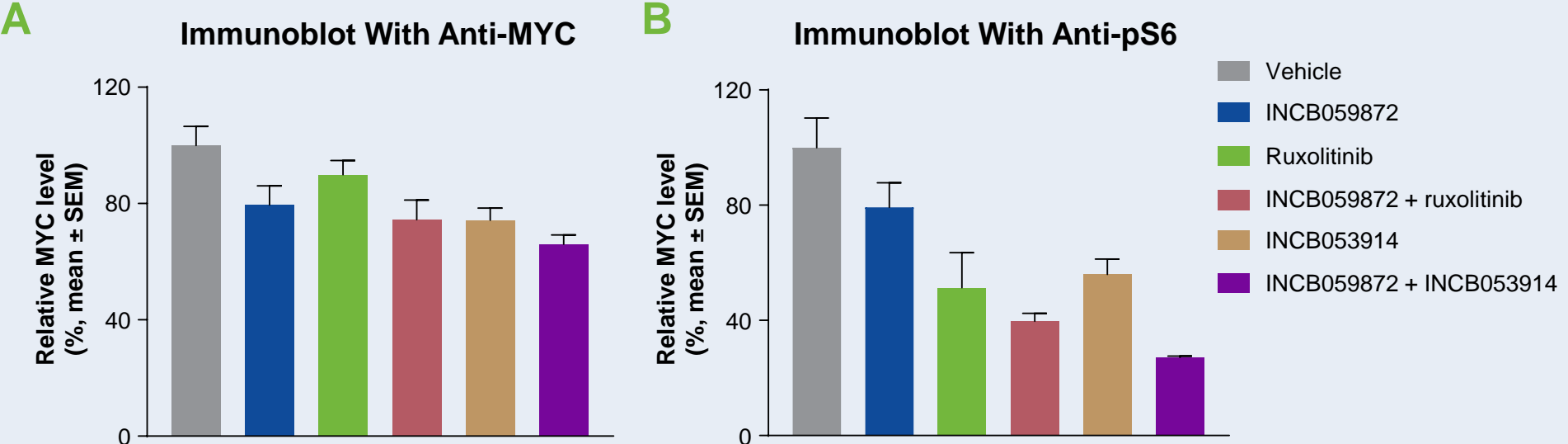


Mice bearing Molm-16 AML subcutaneous xenografts were dosed with INCB059872 (1.5 mg/kg, QD, PO), ruxolitinib (30 mg/kg, BID, PO), or the combination of INCB059872 and ruxolitinib (A), or INCB052793 (3 mg/kg, BID, PO), INCB059872 (1.5 mg/kg, QD, PO), and the combination of INCB059872 and INCB052793 (B). Combination therapies resulted in enhanced efficacy relative to single-agent efficacy. Both agents alone and in combination were well tolerated. ** *P* < 0.01, *** *P* < 0.0001. *P* values among treated groups were determined by ANOVA. n.s., non-significant; TGI, tumor growth inhibition.

Combination of LSD1 Inhibition and PIM Inhibition Enhances TGI in the Molm-16 Xenograft Model

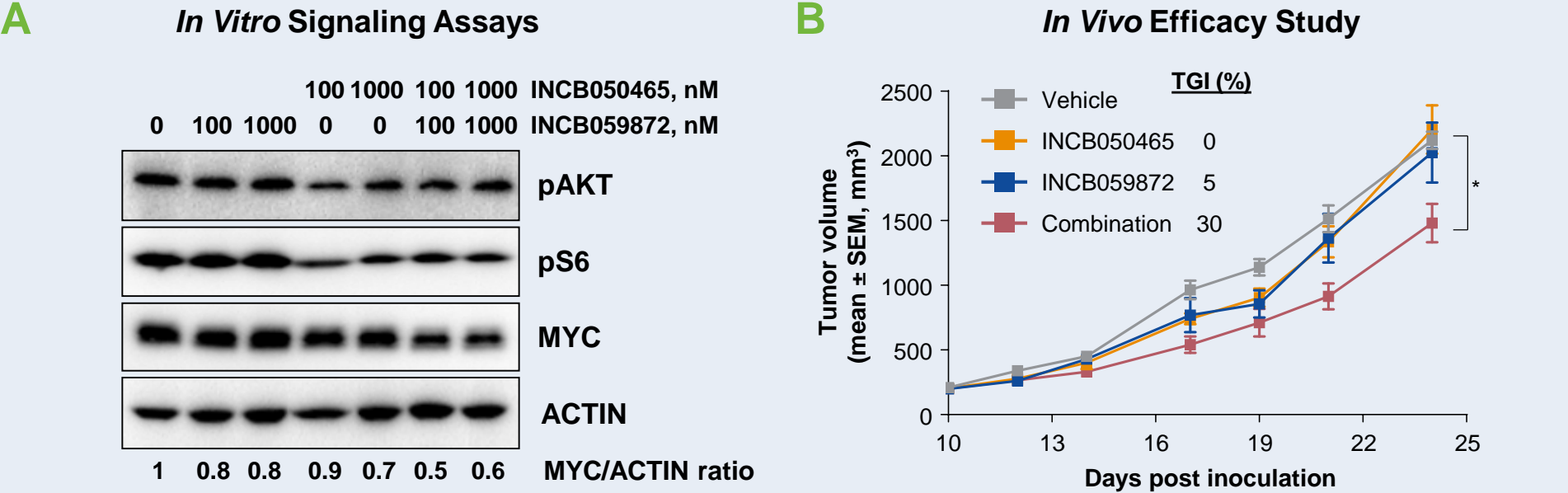


Effects of the Combination of LSD1 Inhibition With JAK1/2 Inhibition, JAK1 Inhibition or PIM Kinase Inhibition on Signal Transduction Pathways in Molm-16 AML Xenografts



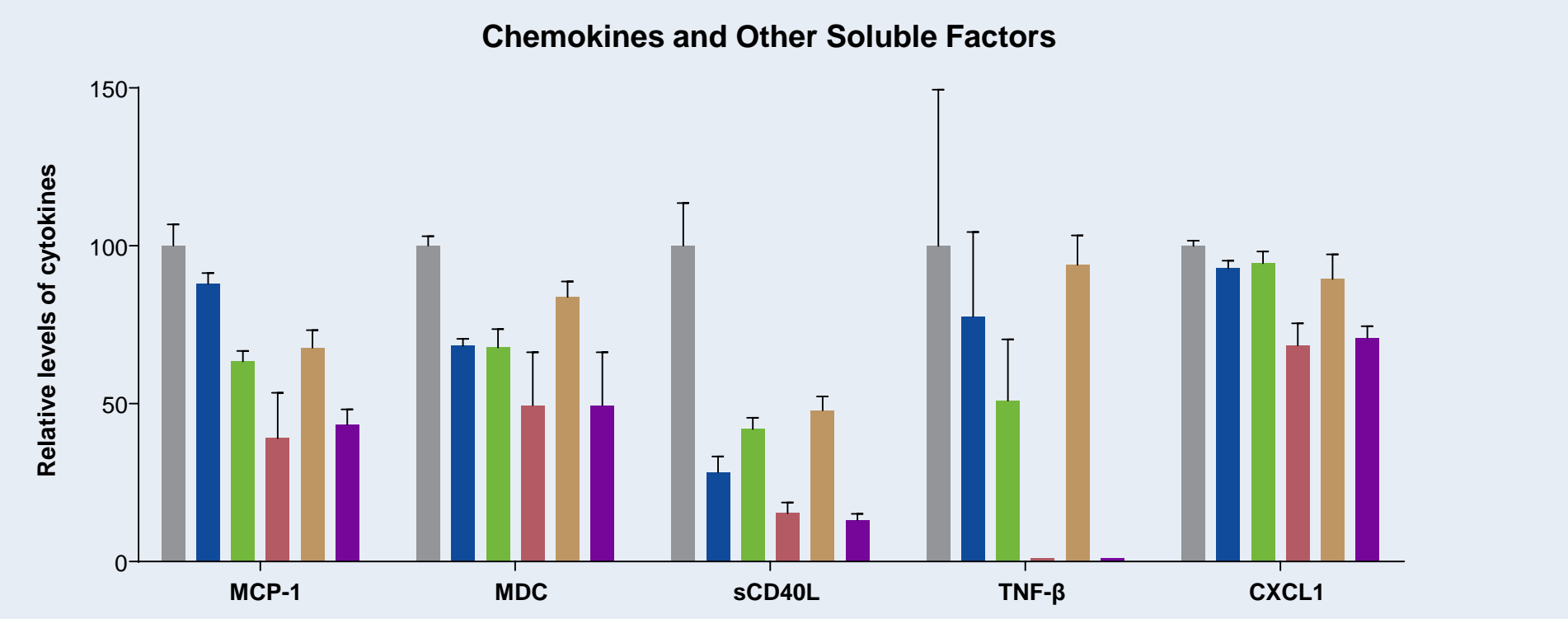
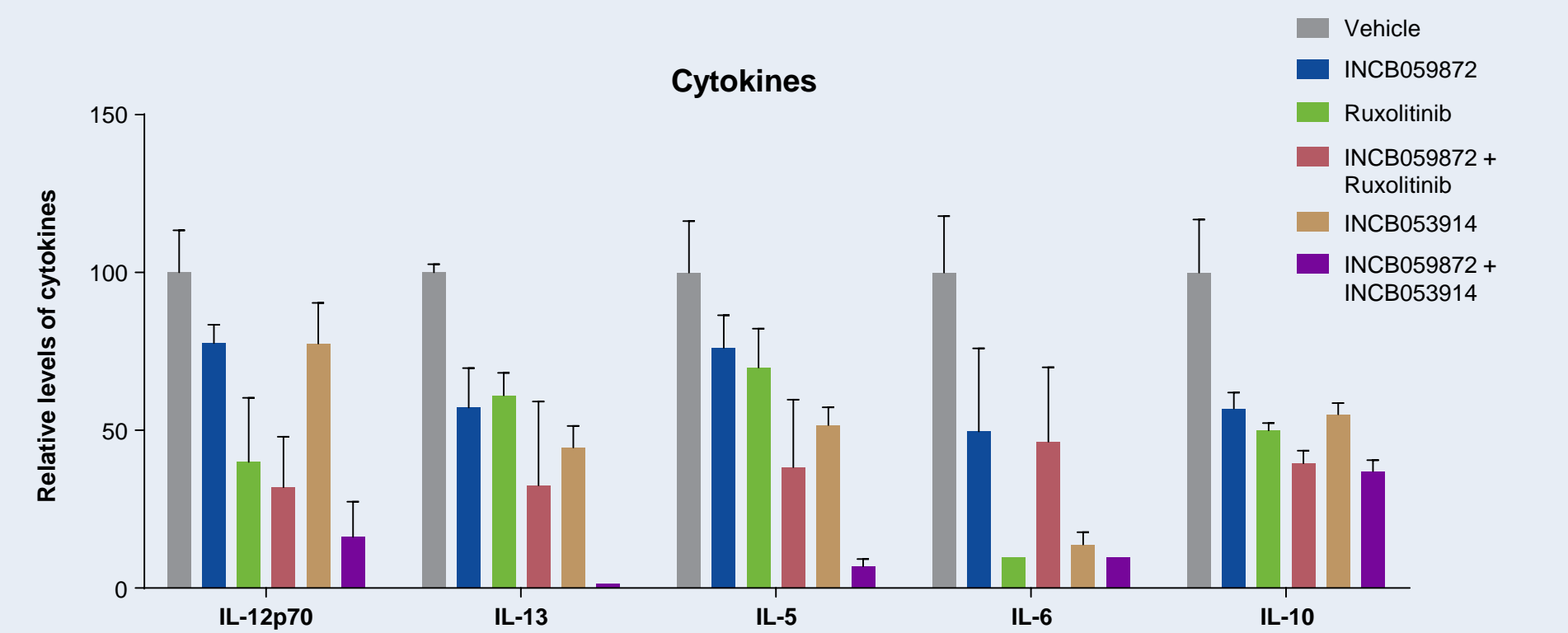
Mice bearing Molm-16 AML subcutaneous xenografts were dosed with INCB059872 (1.5 mg/kg, QD, PO), ruxolitinib (30 mg/kg, BID, PO), INCB053914 (6 mg/kg, QD, PO), the combination of INCB059872 and ruxolitinib, or the combination of INCB059872 and INCB053914. Four hours after the fifth dose, tumor tissues were harvested and total cellular lysates were prepared for immunoblot analyses with anti-MYC (A) and anti-pS6 antibody (B). The relative level of proteins was determined by the AlphaView Software (Alpha Innotech, Protein Simple, San Jose, CA). INCB059872 enhanced downregulation of MYC expression and suppressed the pS6 signaling pathway.

Combination of LSD1 Inhibition and PI3K δ Inhibition Enhances Downregulation of MYC Expression in the WILL-2 Double-Hit DLBCL Model



WILL-2 cells (germinal center B cell [GCB] subtype, double-hit lymphoma) were treated with INCB059872 (100 nM and 1000 nM), INCB050465 (100 nM and 1000 nM), and the combination of both agents as shown. Four hours after treatment, cells were harvested and total cellular lysates were prepared for immunoblot analyses with anti-MYC, anti-pAKT Ser473, and anti-pS6 Ser240/244 antibodies. The relative level MYC expression was determined by the AlphaView Software (Alpha Innotech, Protein Simple, San Jose, CA). Combination treatment resulted in suppressed MYC expression relative to single-agent efficacy. Mice bearing WILL-2 subcutaneous xenografts were dosed with INCB059872 (1.5 mg/kg, QD, PO), INCB050465 (1 mg/kg, BID, PO), or the combination of INCB059872 and INCB050465. Combination treatment resulted in enhanced efficacy relative to single-agent efficacy. Both agents alone and in combination were well tolerated. * *P* < 0.05, *P* values versus vehicle group determined by ANOVA.

LSD1 Inhibition Enhances Downregulation of Cytokines When Combined With JAK or PIM Kinase Inhibition



Mice bearing Molm-16 AML subcutaneous xenografts were dosed with INCB059872 (1.5 mg/kg, QD, PO), ruxolitinib (30 mg/kg, BID, PO), INCB053914 (6 mg/kg, QD, PO), the combination of INCB059872 and ruxolitinib, or the combination of INCB059872 and INCB053914 for 4 days. Tumor lysates were prepared at 4 hours post final dose. Tumor lysates were subjected to Luminex analyses for evaluation of cytokine levels.

Conclusions

- The combination of an LSD1 inhibitor (INCB059872) with a PIM inhibitor (INCB053914) or JAK inhibitors (ruxolitinib and INCB052793) significantly inhibited tumor growth in the Molm-16 human AML xenograft model
- MYC expression and pS6 levels were downregulated by the combination of INCB059872 and protein kinase inhibitors both *in vitro* and *in vivo*
- Treatment with INCB059872 enhanced downregulation of cytokines that were regulated by the JAK/STAT/PIM pathways by autocrine- and/or paracrine-dependent signaling in Molm-16 tumors
- These data suggest that autocrine and/or paracrine cytokine signaling cascades can be targeted by novel combination therapies of an LSD1 inhibitor and signal transduction pathway inhibitors in AML
- The combination of INCB059872 with a PI3K δ inhibitor (INCB050465) enhanced tumor growth inhibition in the WILL-2 GCB subtype, double-hit lymphoma xenograft model
- Mechanistic studies are ongoing to further understand the molecular bases of these observations

