

The LSD1 Specific Inhibitor INCB059872 Enhances the Activity of Immune Checkpoint Blockade by Reshaping the Myeloid Compartment in the Syngeneic 4T1 Mouse Mammary Tumor Model

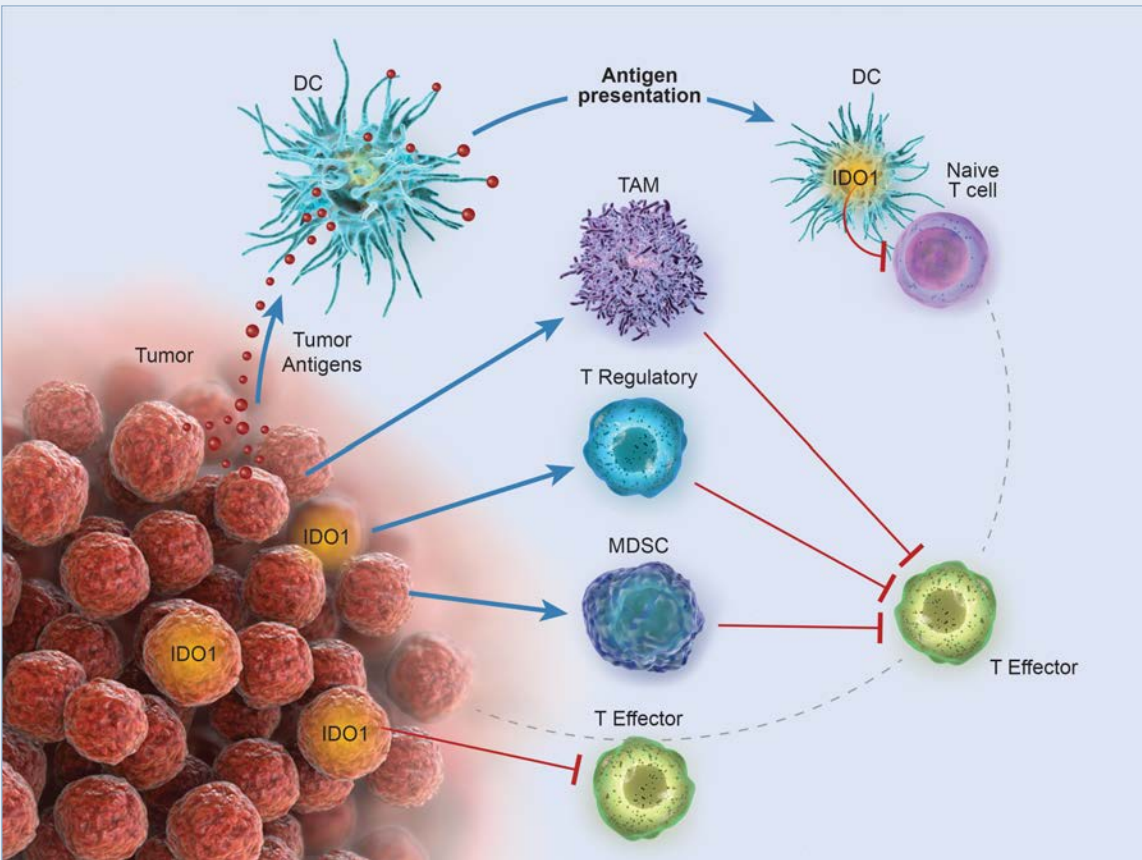
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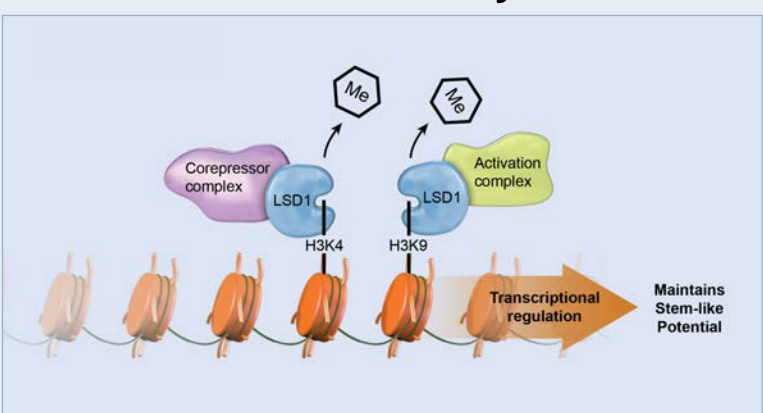
Abstract

Immune checkpoint blockade has shown considerable therapeutic promise in the clinic. However, single agent activity is compromised by the presence of suppressive myeloid cells, including myeloid derived suppressor cells (MDSC), tumor associated macrophages (TAM) and polymorphonuclear (PMN) cells, in the tumor microenvironment. Epigenetic alterations can significantly contribute to the development of the immunosuppressive tumor microenvironment and recent data have suggested that combining epigenetic-based therapies with immunotherapeutic agents can lead to improved efficacy in preclinical models. Since Lysine Specific Demethylase 1 (LSD1) has been shown to play a critical role in hematopoiesis, we hypothesized that inhibition of LSD1 could have a direct effect on myeloid cell differentiation and potentially restore normal myelopoiesis in cancer patients. To test this hypothesis, we evaluated INCB059872, a potent, selective and orally available FAD-directed covalent inhibitor of LSD1 in several experimental models. In an *in vitro* differentiation assay, the majority of CD34⁺ progenitor cells were driven to a monocytic phenotype in the presence of INCB059872, while control treated cells differentiated toward granulocytic PMN cells. Similar results were observed *in vivo*. Using the orthotopic 4T1 mammary cancer model, the myeloid compartment was characterized in tumor tissues following treatment with INCB059872. Notably, the population of PMN-MDSC was significantly decreased in tumor tissues following oral administration of INCB059872, whereas the macrophage population was increased. These data suggest that INCB059872 can redirect myeloid differentiation toward monocyte/macrophages and inhibit the differentiation of PMN-MDSC in this syngeneic tumor microenvironment. Consistently, intratumoral T lymphocyte infiltration was increased following INCB059872 treatment. The combination of INCB059872 and α -PD-L1 antibody enhanced anti-tumor efficacy in the 4T1 orthotopic tumor model. Collectively, these data suggest that inhibition of LSD1 with INCB059872 can directly affect myeloid differentiation to reduce the accumulation of myeloid suppressive cells, restoring the tumor microenvironment to be more responsive to PD-1/PD-L1 axis blockade. This study supports the therapeutic potential for the combination of an LSD1 inhibitor with immuno-therapeutic agents to improve overall clinical response in cancer patients.



INCB059872, a Potent and Selective LSD1 Inhibitor

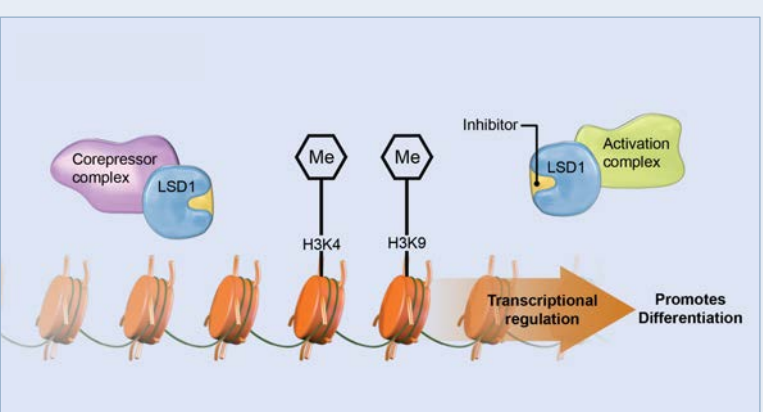
A LSD1 Activity



C Biochemical Potencies of INCB059872

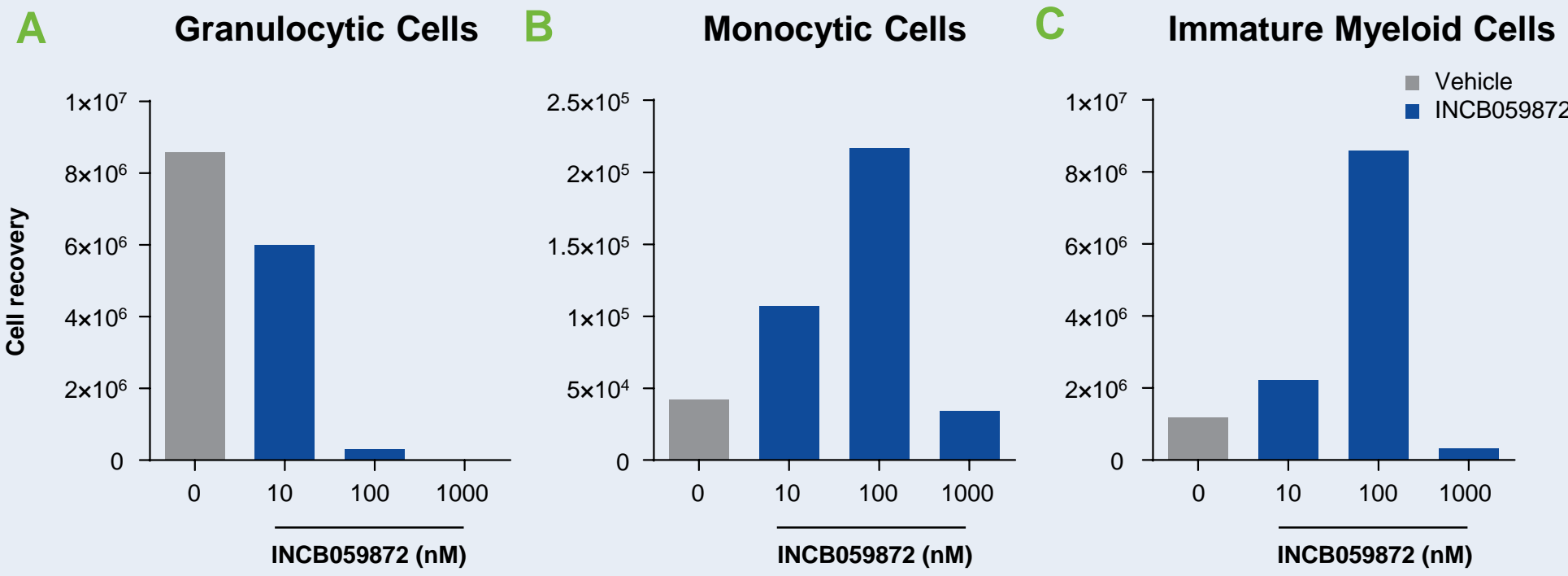
Enzyme	INCB059872 IC ₅₀ Mean \pm SD (nM)
LSD1	18 \pm 3
LSD2	>5000
MAO-A	>20,000
MAO-B	>20,000

B LSD1 Inhibition



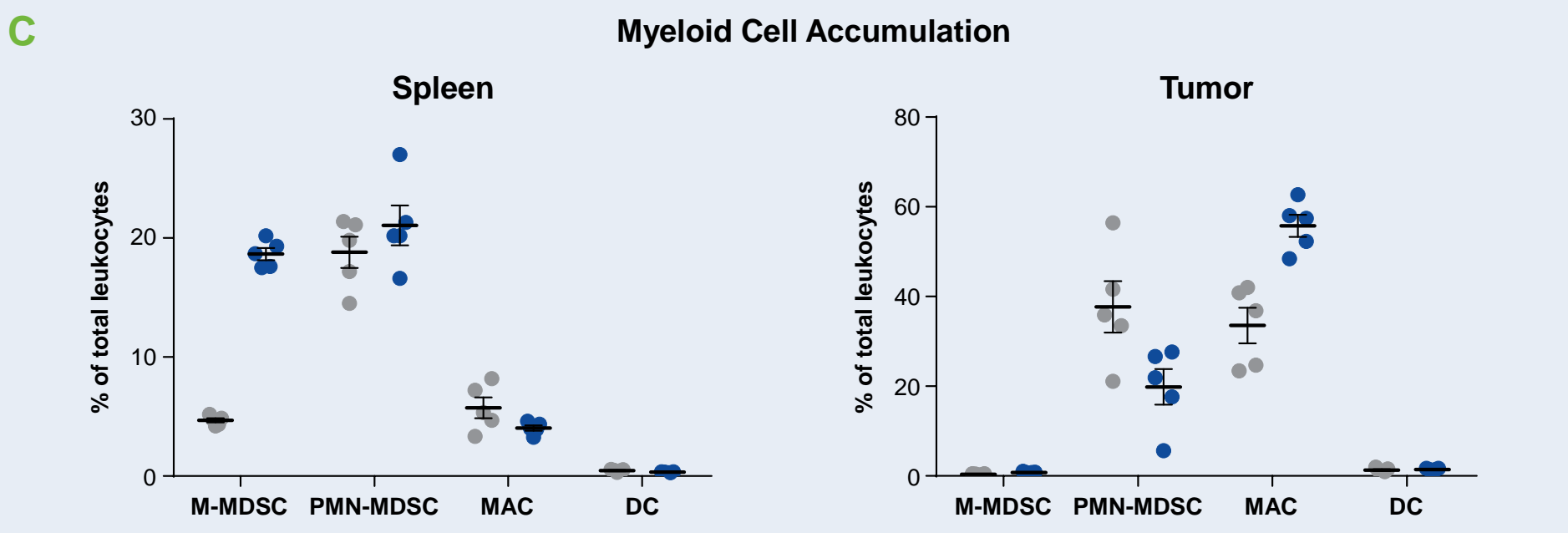
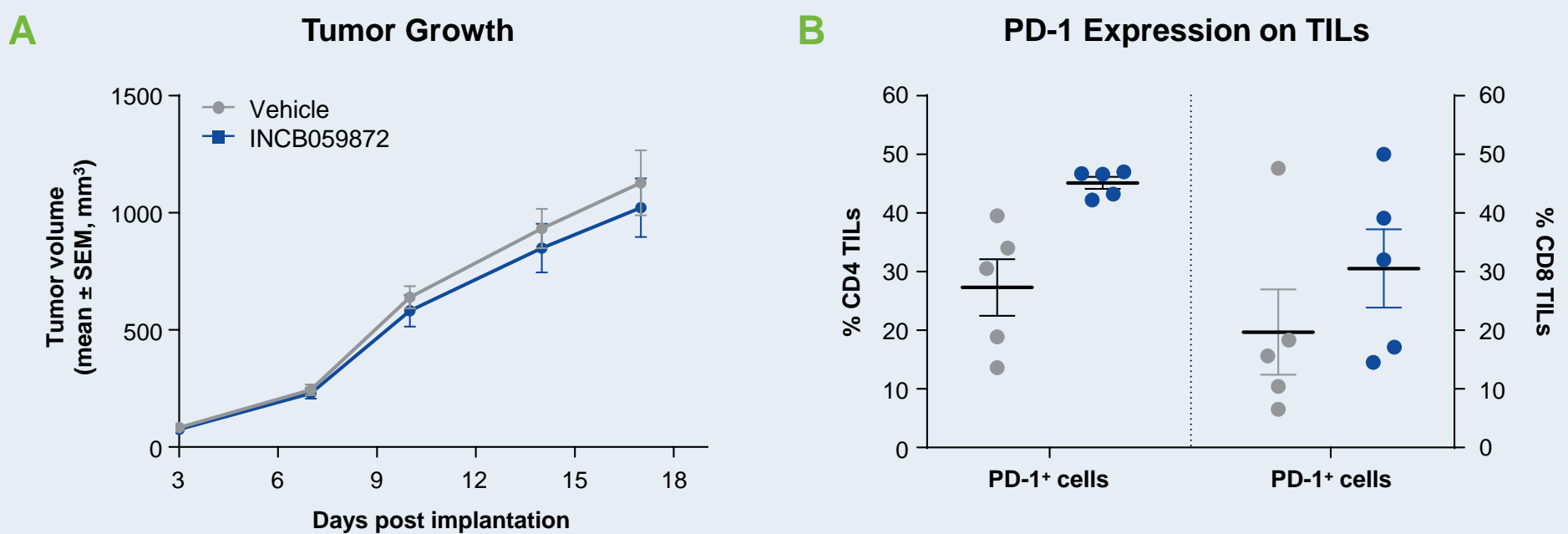
- Schematic representation of LSD1 activity.
- Schematic representation of LSD1 inhibition.
- Enzyme assays were performed with LANCE Ultra LSD1 histone H3K4 demethylase assay format (Perkin-Elmer, Waltham, MA). LSD2 and MAO-A/B were used as counterscreens.

LSD1 Inhibition Promotes Monocytic Differentiation *In Vitro*



INCB059872 redirects myeloid differentiation toward monocytic cells. CD34⁺ hematopoietic progenitor cells were cultured for 10 days in the presence of a cocktail of cytokines (GM-CSF, G-CSF, TPO, and SCF) to promote myeloid differentiation. INCB059872 (10, 100, or 1000 nM) was added and refreshed every 3 days. After 10 days of differentiation, the number of granulocytic cells (CD33⁺/CD14⁺/CD15⁺ cells – **A**), monocytic cells (CD33⁺/CD14⁺/CD15[–] cells – **B**), and immature myeloid cells (CD33⁺/CD14⁺/CD15[–] – **C**) were assessed. One representative experiment of 3 is depicted above.

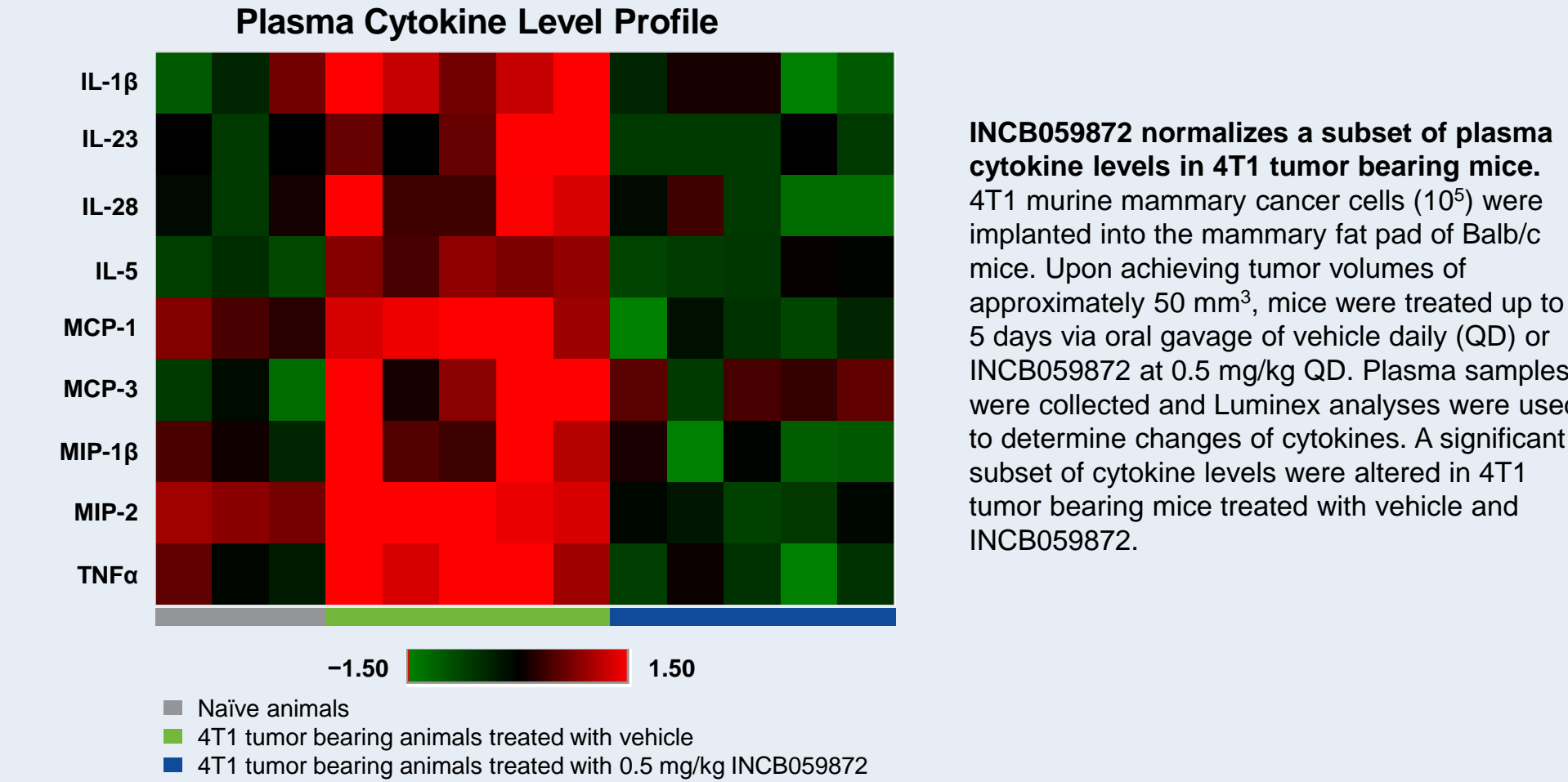
LSD1 Inhibition Reshapes the Tumor Microenvironment



INCB059872 reshapes the tumor microenvironment in 4T1 mammary carcinoma-bearing mice. 4T1 murine mammary cancer cells (10⁵) were implanted into the mammary fat pad of Balb/c mice. Upon achieving tumor volumes of approximately 50 mm³, mice were treated for up to 14 days by oral gavage with vehicle daily or INCB059872 at 1.5 mg/kg QD.

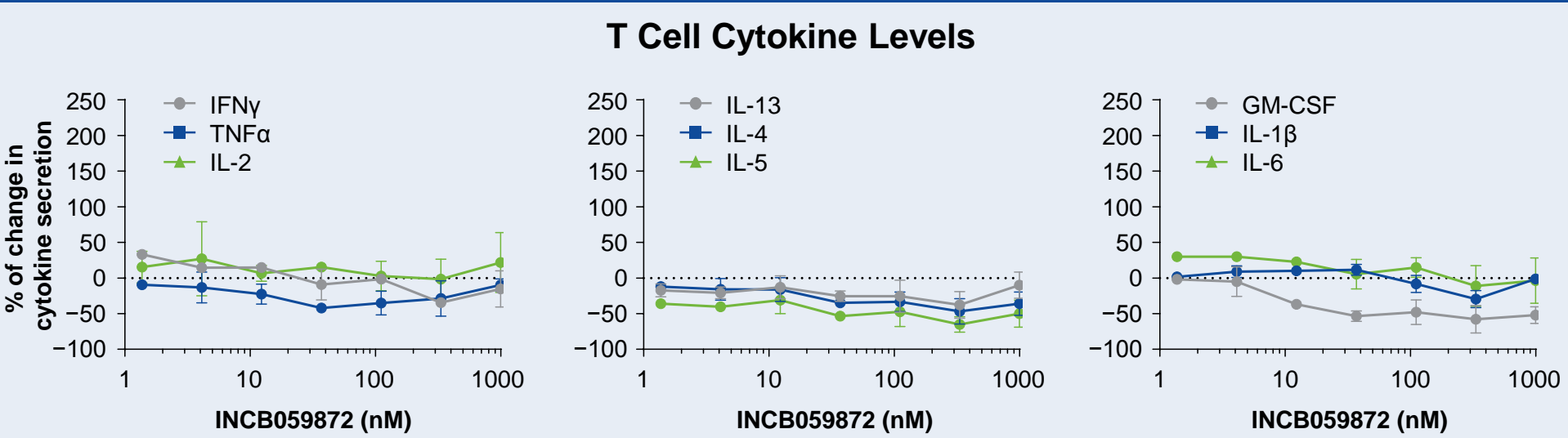
- Tumor growth was assessed over time in mice treated with INCB059872. The tumor growth was not affected by the monotherapy treatment with INCB059872.
- The percentage of PD-1⁺ CD4 and CD8 tumor-infiltrating lymphocytes (TILs) within the tumor microenvironment was assessed by flow cytometry in both vehicle and INCB059872-treated groups (n = 5 mice per group). The proportion of PD-1⁺ TILs (both CD4 and CD8 subsets) increased following treatment with INCB059872.
- Myeloid cell accumulation in the spleen (left panel) and in the tumor tissue (right panel) were also assessed by flow cytometry (n = 5 mice per group). INCB059872 drove the accumulation of monocyte/macrophages while decreasing the intratumoral proportion of PMN-MDSC (MAC, macrophages; DC, dendritic cells).

LSD1 Inhibition Decreases the Levels of Several Plasma Cytokines



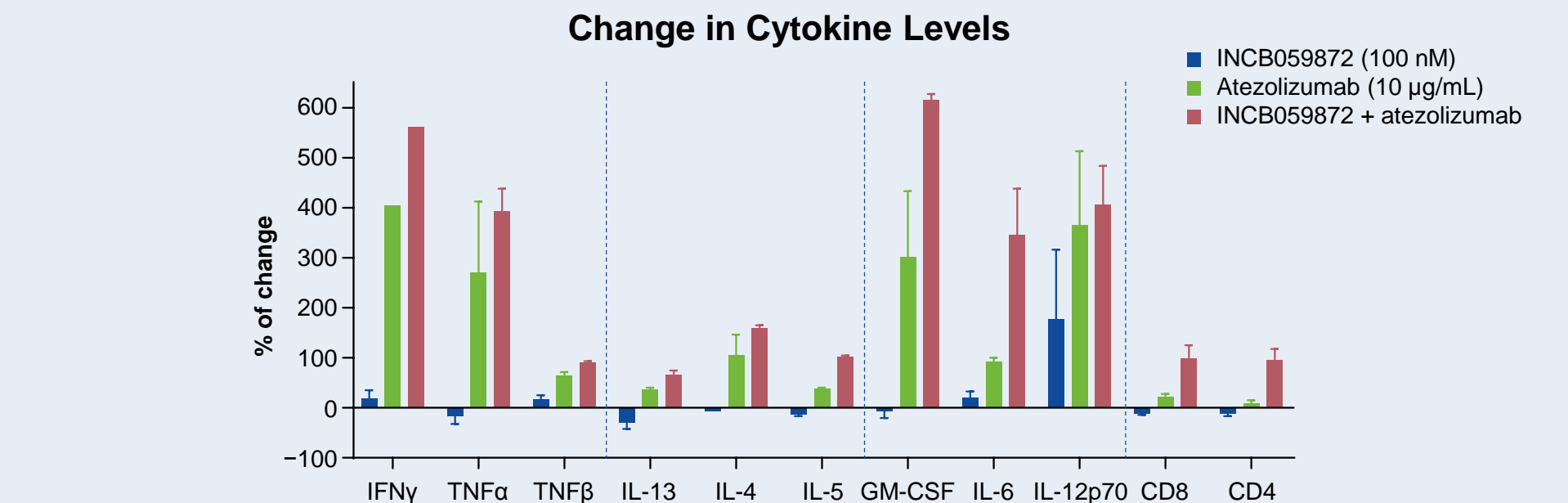
INCB059872 normalizes a subset of plasma cytokine levels in 4T1 tumor bearing mice. 4T1 murine mammary cancer cells (10⁵) were implanted into the mammary fat pad of Balb/c mice. Upon achieving tumor volumes of approximately 50 mm³, mice were treated up to 5 days via oral gavage of vehicle daily (QD) or INCB059872 at 0.5 mg/kg QD. Plasma samples were collected and Luminex analyses were used to determine changes of cytokines. A significant subset of cytokine levels were altered in 4T1 tumor bearing mice treated with vehicle and INCB059872.

LSD1 Inhibition Has No Direct Effect on T Cell Function



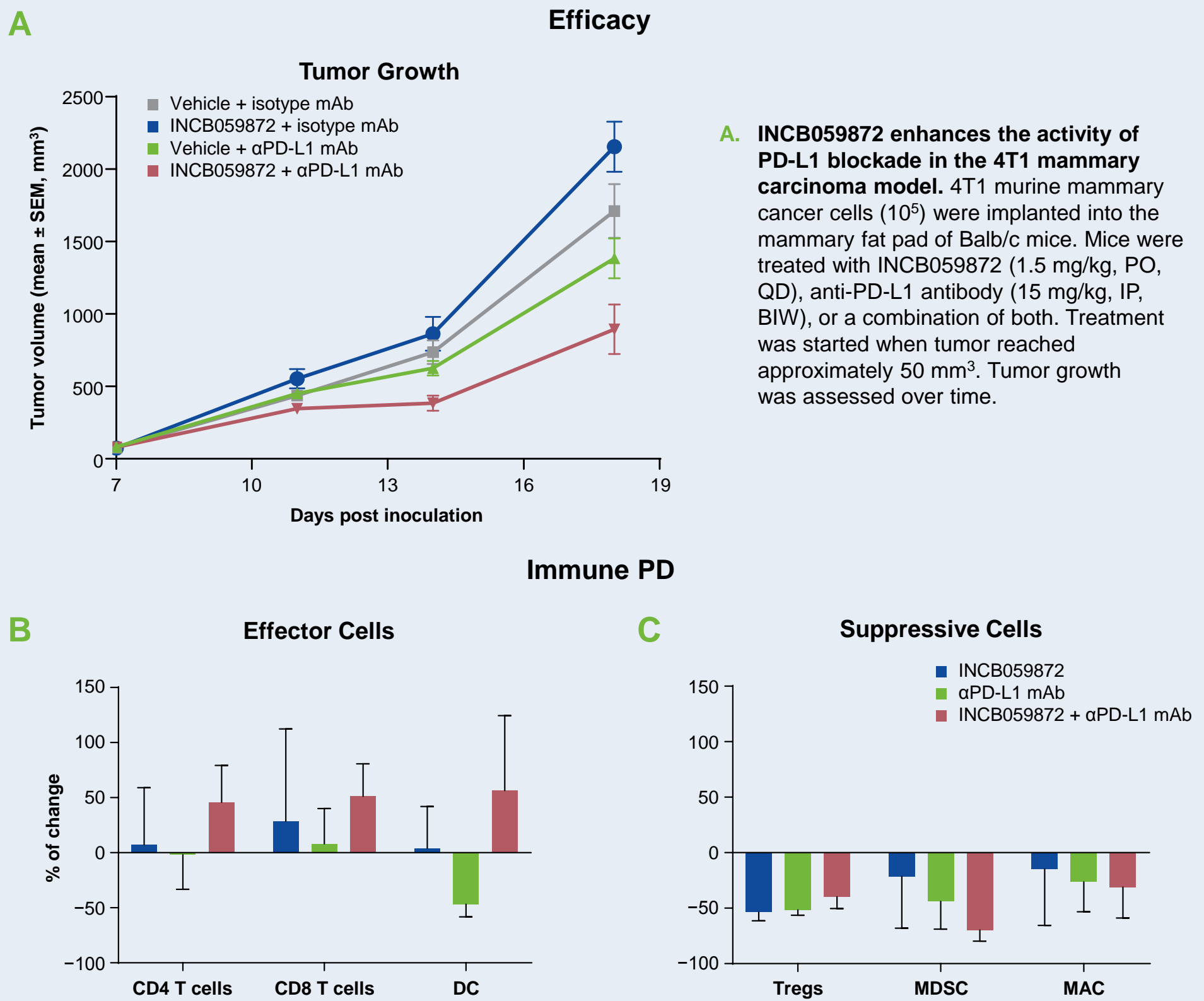
INCB059872 does not directly affect T cell function *in vitro*. The effect of INCB059872 on T cell function was assessed on healthy donor peripheral blood mononuclear cells (PBMC) stimulated with staphylococcal enterotoxin-A (SEA superantigen). The cells were treated with various concentrations of INCB059872 (1–1000 nM) for 3 days and secreted cytokine levels were measured using Luminex technology (Ebiosciences, San Diego, CA). No significant effect of INCB059872 on T cell function was observed. Average percent changes observed in 2 different healthy donors are depicted.

LSD1 Inhibition Synergizes With PD-L1 Blockade *In Vitro*



INCB059872 enhances the effect of atezolizumab (Genentech PD-L1 mAb) in a mixed lymphocyte reaction assay. Monocyte-derived DC from healthy volunteers were generated using standard GM-CSF + IL-4 culture. DC were generated in the presence or absence of INCB059872 for 7 days. The resulting DC were then used to stimulate allogeneic PBMC with or without 10 μ g/mL of anti-PD-L1 mAb (atezolizumab). After 5 days of culture, proliferation was assessed by flow cytometry and cytokines were analyzed using Luminex technology (Ebioscience, San Diego, CA). Results from 1 representative experiment are depicted.

LSD1 Inhibition Enhances the Effect of PD-L1 Blockade on Tumor Growth Inhibition and Immune Cell Infiltration



The combination of INCB059872 and anti-PD-L1 antibody increases the infiltration of effector T cells as well as DC in the 4T1 orthotopic tumor bearing mice (B). These increases were associated with a decreased infiltration in Tregs, MDSC, and macrophages (C). Tumor bearing animals were treated with INCB059872 (1.5 mg/kg, PO, QD), anti-PD-L1 antibody (15 mg/kg, IP, BIW), or a combination of both for 7 days.

Conclusions

- LSD1 inhibition has a profound effect on myeloid cell differentiation and promotes the accumulation of monocytic cells
- LSD1 inhibition monotherapy does not affect tumor growth in the orthotopic 4T1 mammary carcinoma model but reshapes the myeloid compartment, normalizes the levels of several plasma cytokines and leads to an increased proportion of PD-1⁺ TILs, suggesting a potential for synergy with PD-1/PD-L1 targeting therapy
- LSD1 inhibition enhances the activity of PD-L1 targeting therapy both *in vitro* and *in vivo*
- The combination of LSD1 and PD-L1 inhibition leads to an increased infiltration in effector T cells and DC associated with a decrease in suppressive cells

Author Disclosures
All Authors: Incyte Corporation: Employment and Stock Ownership.

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