

The FAD-directed LSD1-Specific Inhibitor INCB059872 Inhibits Cell Migration and Metastasis by Suppressing Pre-metastatic Niche Formation in a Spontaneous Metastasis Mouse Model

Sang Hyun Lee, Melody Diamond, Antony Chadderton, Huiqing Liu, Alla Volgina, Valerie Roman, Michael Weber, Chunhong He, Rebecca Stewart, Denise Hertel, Phillip Liu, Liangxing Wu, Julian Oliver, Swamy Yeleswaram, Alan Roberts, Wenqing Yao, Gregory Hollis, Reid Huber, Peggy Scherle, Bruce Ruggeri

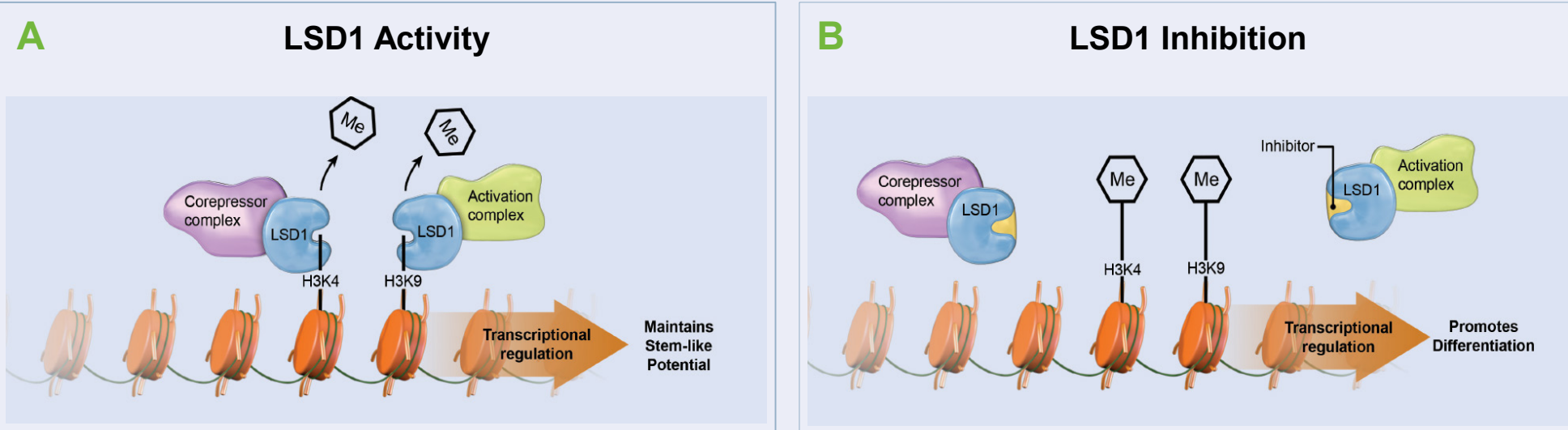
Incyte Research Institute, Wilmington, DE



Abstract

The primary cause of cancer-associated mortality is tumor metastasis. The concept of the tumor pre-metastatic niche is supported by evidence of changes at distal pre-metastatic sites that create a permissive environment to allow disseminated tumor cells to seed. Myeloid-derived suppressor cells (MDSCs) remodel the tumor microenvironment and function as immunosuppressive cells to promote tumor growth. Previously, we demonstrated that the clinical stage LSD1-specific inhibitor, INCB059872, significantly reshaped the myeloid compartment in the murine 4T1 syngeneic murine model of breast cancer. Treatment with INCB059872 significantly reduced the population of MDSCs in the tumor microenvironment. Since it has been reported that MDSCs promote establishment of a pre-metastatic niche, we hypothesized that INCB059872 could suppress or delay metastatic processes in the 4T1 model and thereby could impact spontaneous metastases to the lung. *In vitro*, INCB059872 significantly suppressed cancer cell migration of triple negative breast cancer cells, SUM145PT. *In vivo*, the effect of INCB059872 on forming the metastatic niche using the 4T1 mouse breast tumor model was explored. Vehicle-treated animals exhibited a significant infiltration of MDSCs to the primary tumor and lungs prior to cancer cells metastasizing. In contrast, INCB059872 administration significantly suppressed the infiltration of MDSCs in primary tumor and lung tissues. Histological analyses further demonstrated the reduction of metastatic loci in lung with INCB059872 treatment. Plasma levels of CCL2, a cytokine which is required for the recruitment and functional specialization of MDSCs, were significantly reduced in animals treated with INCB059872. These data suggest a possible mechanism to reduce infiltration of MDSCs into lung tissues. Notably, analyses of molecular pathways using RNA-Seq identified that components of the EMT-associated pathway are also downregulated in tumors treated with INCB059872, which further supports the role of INCB059872 in the inhibition of metastasis. Taken together, these preclinical data suggest that inhibition of LSD1 with INCB059872 can suppress metastasis through multiple molecular and cellular mechanisms, notably by inhibition of the formation of the pre-metastatic niche by modulating the population of MDSCs in the primary tumor and distal tissues.

Biological Effects of LSD1 on Cellular Differentiation



H3K, histone H3 lysine; LSD, lysine-specific demethylase; Me, methylation.

Profile of INCB059872 in Biochemical Assays

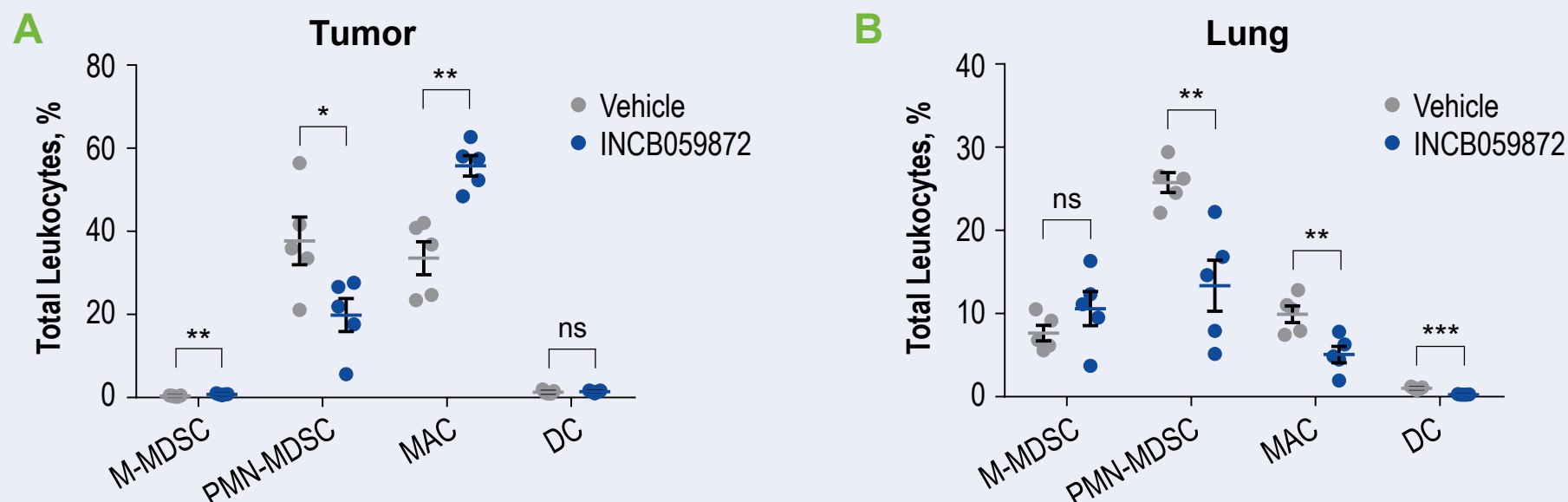
Biochemical Potencies of INCB059872

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

IC₅₀, half maximal inhibitory concentration; MAO, monoamine oxidase; SD, standard deviation.

Enzyme assays were performed with LANCE Ultra LSD1 histone H3K4 demethylase assay formats (PerkinElmer Inc., Waltham, MA). LSD2 and MAO-A/B were used for counter-screening.

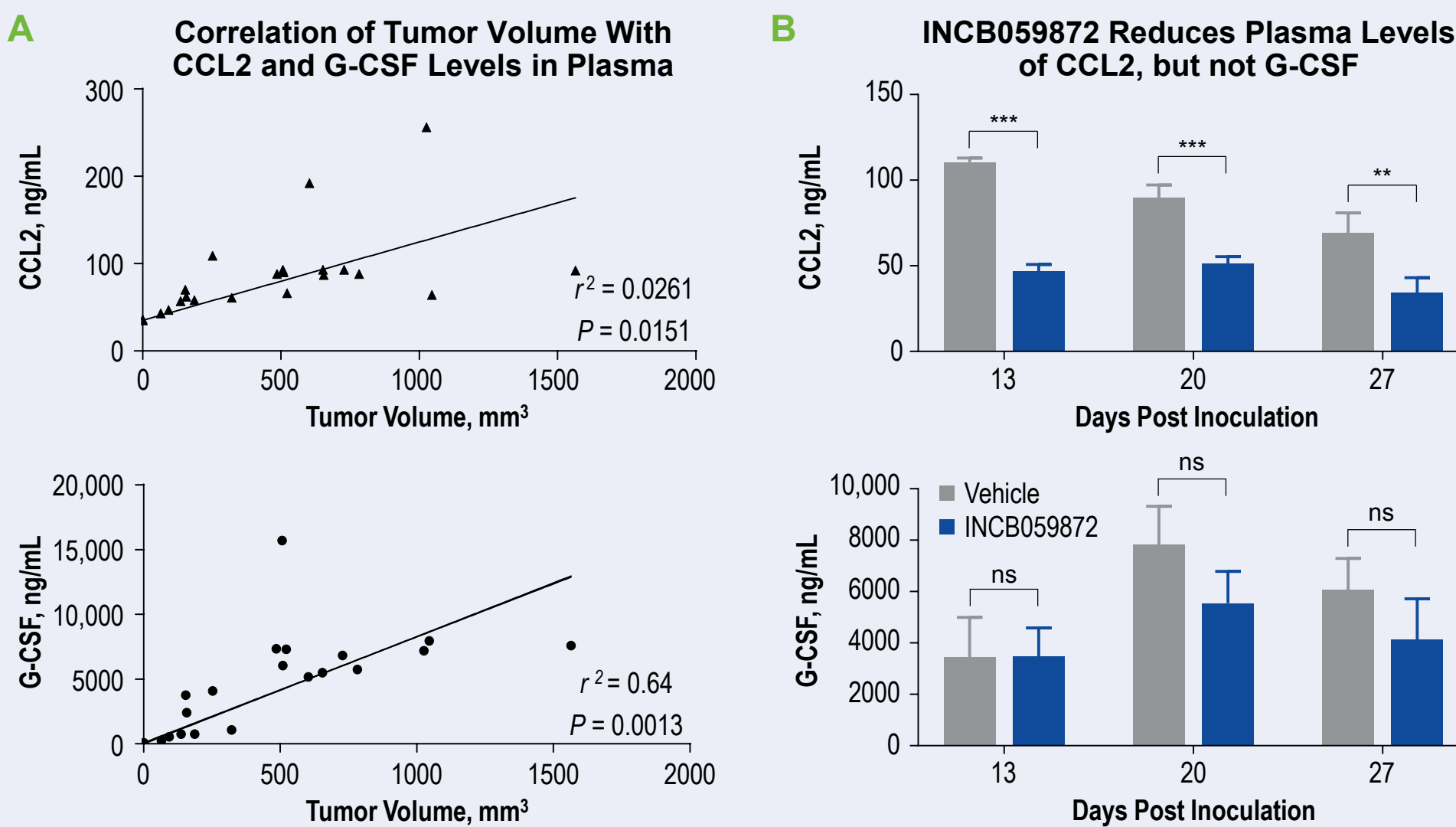
INCB059872 Reshapes the Tumor Microenvironment and Infiltration of MDSC in Lungs of 4T1 Syngeneic Murine Mammary Tumor-Bearing Mice



DC, dendritic cell; MAC, macrophage; M-MDSC, monocytic myeloid-derived suppressor cell; ns, not significant; PMN-MDSC, polymorphonuclear MDSC.

4T1 murine mammary cancer cells (4×10⁵ cells) were implanted in the fat pad of Balb/c mice. When tumor volumes reached approximately 50 mm³, mice were treated for up to 14 days via oral gavage with vehicle or INCB059872 at 1.5 mg/kg once daily (QD; n = 5 mice per group). Myeloid cell accumulation in tissues of tumors (A) and lungs (B) was assessed by flow cytometry (n = 5 mice per group). Data represent mean ± standard error of the mean (SEM). *P* values calculated by *t* test; * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001.

INCB059872 Normalized Plasma CCL2 Levels in 4T1 Tumor-Bearing Mice



CCL2, chemokine ligand 2; G-CSF, granulocyte-colony stimulating factor; *r*, correlation coefficient.

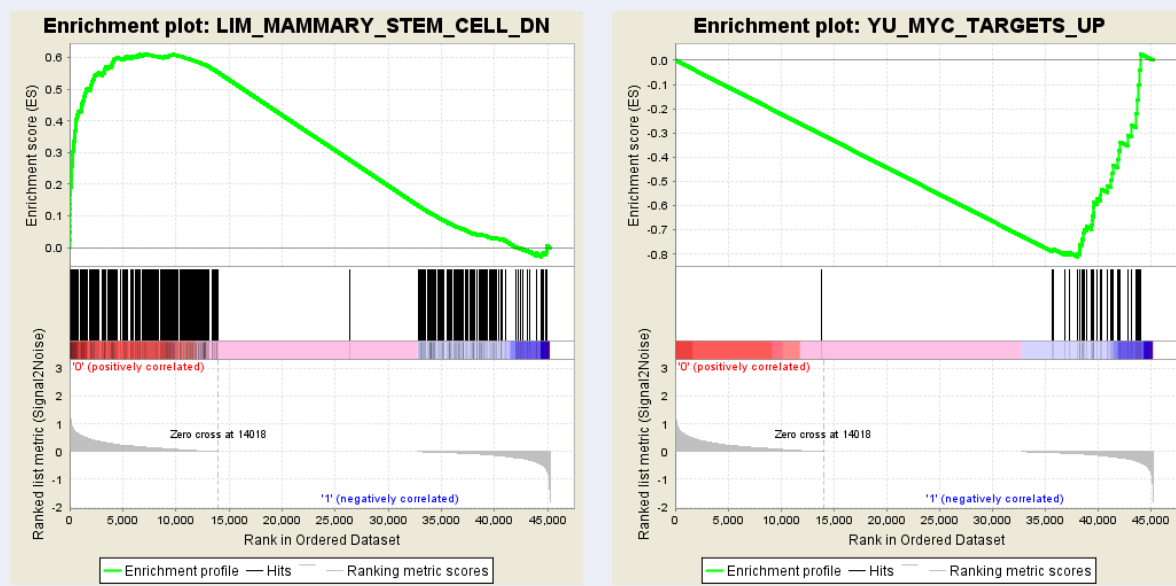
- A. To evaluate the correlation between tumor volume with CCL2 and G-CSF levels in plasma, tumor-bearing animals were established by implanting 4T1 murine mammary cancer cells subcutaneously in the flank of Balb/c mice. CCL2 and G-CSF levels were evaluated using the Quantikine ELISA (R&D Systems, Inc., Minneapolis, MN). Correlation coefficients were calculated by linear regression.
- B. Effects of INCB059872 administration on CCL2 and G-CSF levels were evaluated using the Quantikine ELISA. Mice (n = 4 per group) were inoculated subcutaneously with 4T1 cells, and QD dosing commenced at day 3 post implantation for 20 days. Fresh plasma was drawn 24 hours after dosing. CCL2 was only evaluated at days 13–27. Data represent mean ± SD. *P* values were calculated by *t* test; ** *P* < 0.01, *** *P* < 0.001.

RNA-Seq Analyses of 4T1 Mammary Tumors After Treatment With INCB059872

A Gene Set Enrichment Analysis (GSEA) Provides Insight Into Key Oncogenic Signaling Pathways

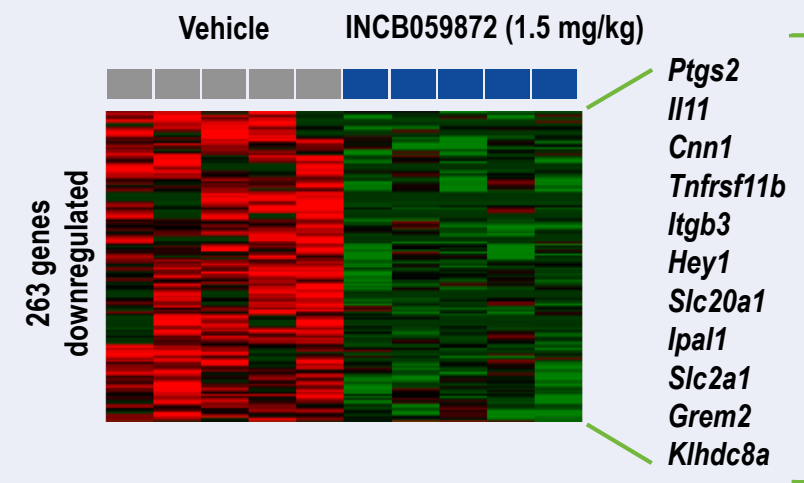
Selected Gene Sets	ES	NES	Nominal <i>P</i> Value
LIM_MAMMARY_STEM_CELL_DN	0.607	1.4665	0.008
YU_MYC_TARGETS_UP	-0.8076	-1.2777	0.008
WANG_IMMORTALIZED_BY_HOXA9_AND_MEIS1_UP	0.756	1.4546	0.0082
GESERICK_TERT_TARGETS_DN	-0.7464	-1.4051	0.0161
MATTHEWS_AP1_TARGETS	-0.6588	-1.3777	0.0317
MULLIGAN_NTF3_SIGNALING_VIA_INSR_AND_IGF1R_UP	-0.7236	-1.5044	0.0332

ES, enrichment score; NES, normalized enrichment score.



GSEA identifies key oncogenic signaling pathways including mammary stem cell and MYC-associated signatures. Notably, many of the conserved genes in the mammary stem cell population are considered epithelial-mesenchymal transition (EMT) signature genes.

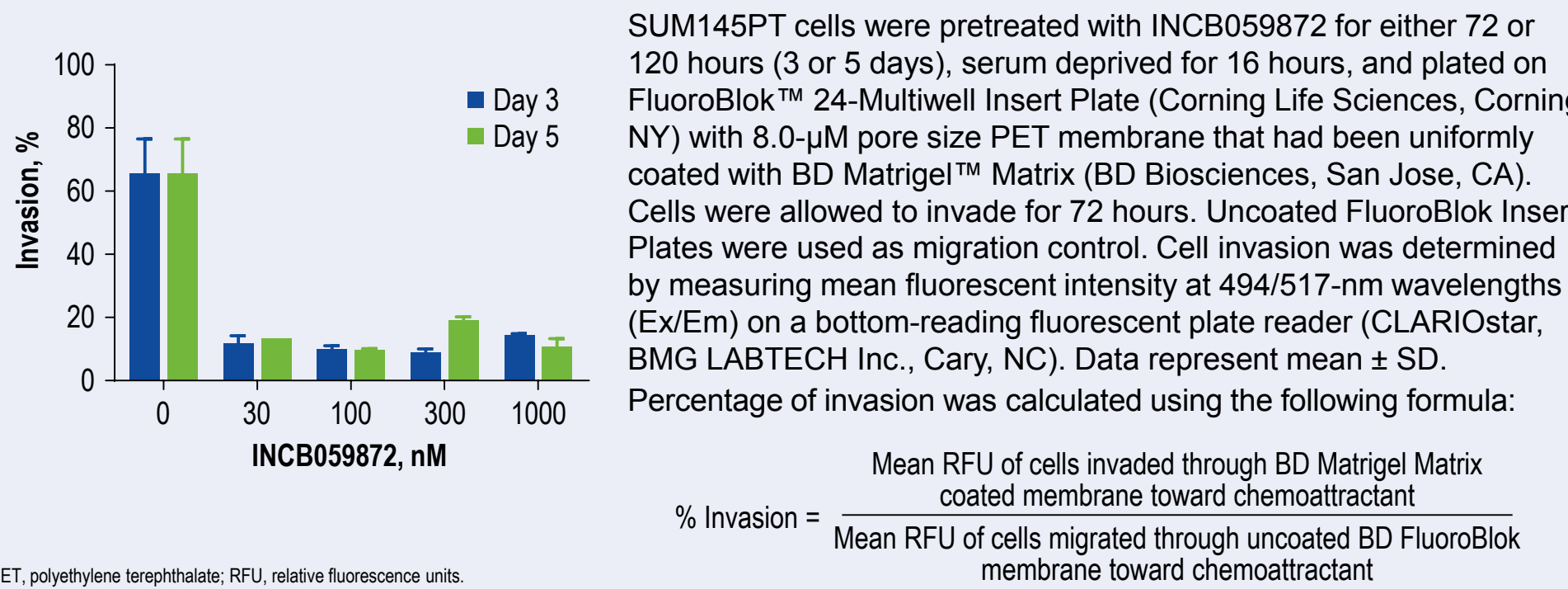
B TGFβ Target Genes Are Significantly Downregulated With the Treatment of INCB059872



INCB059872 treatment downregulated many genes that were induced by TGFβ treatment

A total of 1025 differentially expressed genes were identified with >1.5-fold change and *P* value <0.05 when comparing INCB059872-treated versus vehicle, including 762 upregulated and 263 downregulated genes. A heat map of downregulated genes only from hierarchical clustering shows clear differential expression patterns between INCB059872- and vehicle-treated control mice.

INCB059872 Inhibits Cell Invasion of Triple Negative Breast Cancer Cell Line SUM145PT

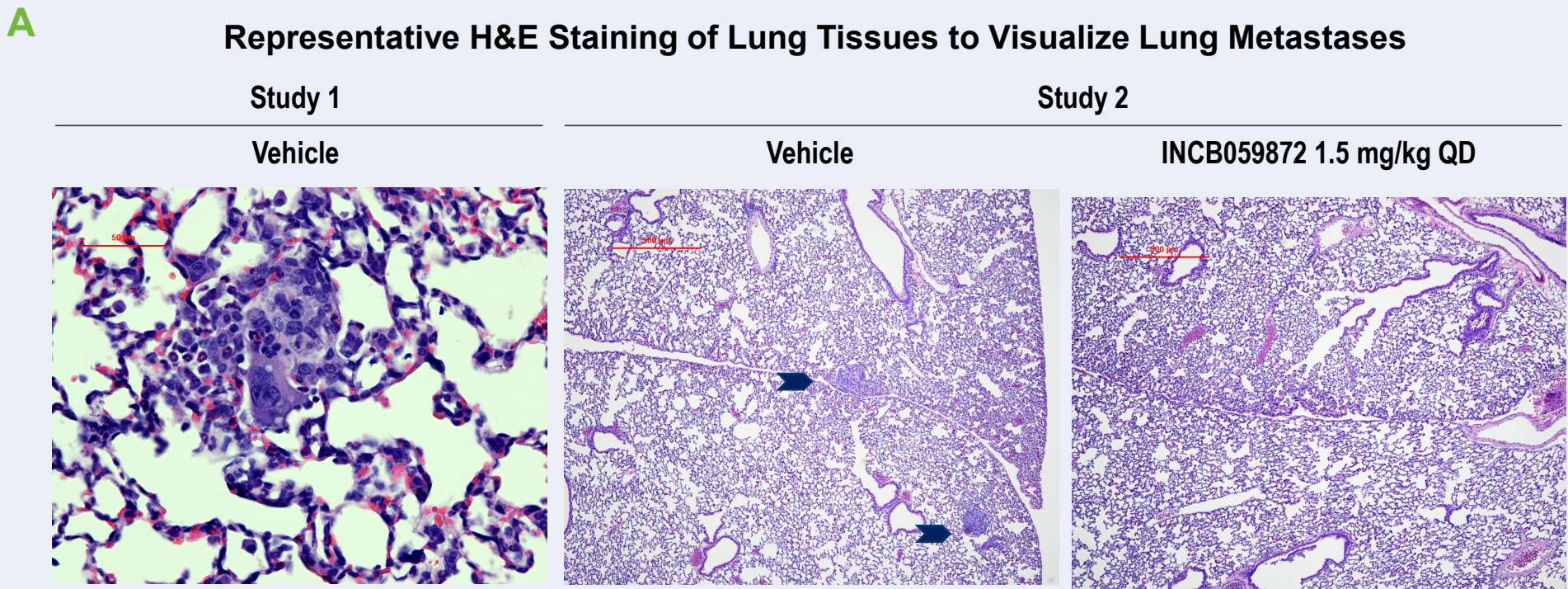


Percentage of invasion was calculated using the following formula:

$$\% \text{ Invasion} = \frac{\text{Mean RFU of cells invaded through BD Matrigel Matrix coated membrane toward chemoattractant}}{\text{Mean RFU of cells migrated through uncoated BD FluoroBlok membrane toward chemoattractant}}$$

PET, polyethylene terephthalate; RFU, relative fluorescence units.

INCB059872 Inhibits Metastases in the 4T1 Model



B Summary of Histological Examination of Lung Tissues

	Vehicle	INCB059872 1.5 mg/kg QD
Study 1 n = 6/group	<ul style="list-style-type: none">• 5 of 6 animals: interstitial infiltrate (1 minimum, 1 mild, 3 moderate)• 1 of 6 animals: focal metastasis	No neoplastic masses
Study 2 n = 3/group	<ul style="list-style-type: none">• Animal 1: 4 small masses poorly differentiated and 2 very small masses on the serosal surface/area• Animal 2: 1 small mass in lung and several masses in the mediastinal region• Animal 3: 6–7 small masses in the lung and 1–2 very small masses on the serosal surface	Animal 1: no neoplastic masses Animal 2: no neoplastic masses Animal 3: 1 small neoplastic mass in the lung

A. & B. 4T1 mammary cancer cells (4×10⁵) were implanted in the fat pad of Balb/c mice. When tumor volumes reached approximately 50 mm³, mice were treated for 14 days via oral gavage with vehicle or INCB059872 1.5 mg/kg QD. At the end of study, tumor tissues were harvested and subjected to H&E staining for histological evaluation. Arrows indicate micrometastases.

Conclusions

- INCB059872 administration significantly reduced the population of MDSCs in the tumor and lung tissue of 4T1 syngeneic murine tumor-bearing mice
- Plasma levels of CCL2, a cytokine that is required for the recruitment and functional specialization of MDSCs, were significantly reduced in animals treated with INCB059872
- RNA-seq data suggest that TGFβ signaling and EMT processes were down-regulated by INCB059872 administration in 4T1 syngeneic murine tumor-bearing mice
- *In vitro*, INCB059872 significantly suppressed cancer cell migration of triple negative breast cancer cells, SUM145PT
- Histological analyses demonstrated the reduction of metastatic loci in the lungs of 4T1 syngeneic murine tumor-bearing mice with INCB059872 treatment

Disclosures

All authors: Employment and stock ownership – Incyte Corporation.

Acknowledgments

Editorial, graphics, and printing support was provided by Evidence Scientific Solutions Inc. (Philadelphia, PA), funded by Incyte Corporation.



To download a copy of this poster, scan code above