

The FAD-Directed LSD1-Specific Inhibitor INCB059872 Is a Promising Epigenetic Agent for AML Therapy by Inducing Differentiation of Leukemic Stem/Progenitor Cells

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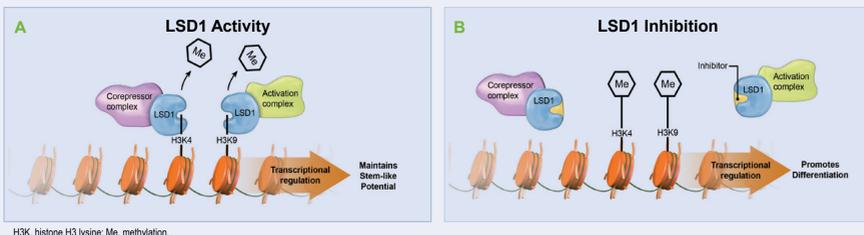
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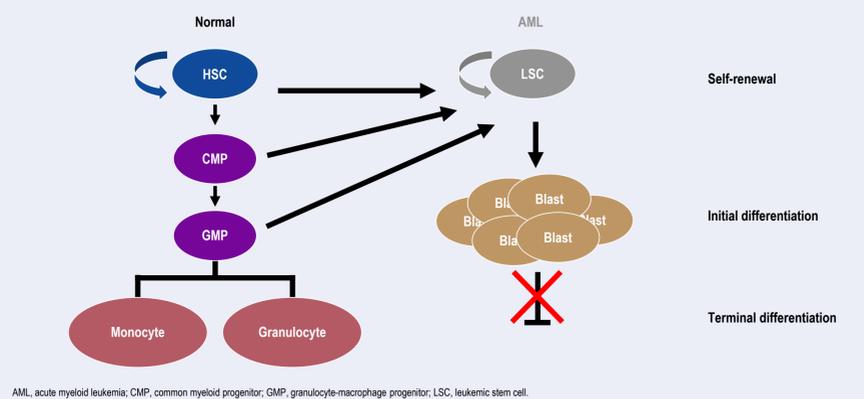
Abstract

Numerous studies have elucidated that the most pivotal functions of lysine-specific demethylase-1 (LSD1) are associated with regulating normal or malignant hematopoiesis by maintaining stem cell self-renewal and regulating myeloid differentiation. In preclinical models, studies with either pharmacological inhibition or genetic knockdown of LSD1 demonstrated that LSD1 is essential for differentiation of progenitor cells during normal hematopoiesis. In the clinic, AML manifests itself via clonal expansion of abnormal differentiation and proliferation of myeloid cells and, therefore, the inhibition of LSD1 activity with small molecule inhibitors could be a promising therapeutic approach for AML. Previously, we reported upon the identification of a flavin adenine dinucleotide (FAD)-directed LSD1 specific inhibitor, INCB059872, which is efficacious in preclinical mouse models utilizing human AML cell lines and primary AML cells by inducing cell differentiation as indicated by the induction of CD11b and CD86 markers. Using a larger panel of myeloid and hematopoietic stem cell (HSC) flow cytometry markers, our current efforts expanded upon these observations to ascertain whether INCB059872 enhanced lineage commitment at HSC and/or promoted monocytic/granulocytic differentiation of human primary AML cells *ex vivo* and in human systemic AML PDX models. In both human AML PDX models and human primary AML samples, INCB059872 increased myeloid differentiation with increasing populations of monocytes (CD14⁺) and granulocytes (CD15⁺). Furthermore, INCB059872 induced the differentiation of early hematopoietic progenitors, CD34⁺/CD38⁻ to more committed CD34⁺/CD38⁺ multipotent/oligopotent progenitors, which in turn gave rise to lineage-specific progenitors in the human AML PDX models. These studies support further exploration of INCB059872 as a promising novel epigenetic agent for AML therapy whose mechanism of action lies in part through the induction of differentiation of leukemic stem/progenitor cells to more committed hematopoietic lineages.

Biological Effects of LSD1 on Cellular Differentiation



Normal Human Hematopoietic Stem Cell Differentiation Is Disrupted in AML and Is Manifested by a Buildup of Blast Cells



INCB059872 Is a Potent and Selective LSD1 Inhibitor and Is Efficacious Against AML Cells *In Vitro*

Biochemical Assay		Cellular Proliferation Assay <i>In Vitro</i>			
Enzyme	INCB059872 IC ₅₀ (Mean ± SD, nM)	Cell Line	FAB Subtype	Mutations	INCB059872 GI ₅₀ (Mean, nM)
LSD1	18 ± 3	HL-60	M2	MYC-amplified, NRAS	7
LSD2	>5000	THP-1	M5	MLL-AF9	17
MAO-A	>20,000	SKM-1	M5	EZH2 Y641C	21
MAO-B	>20,000	MOLM-13	M5	MLL-AF9, FLT3-ITD	103
		MOLM-16	M0	Not specified	137

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

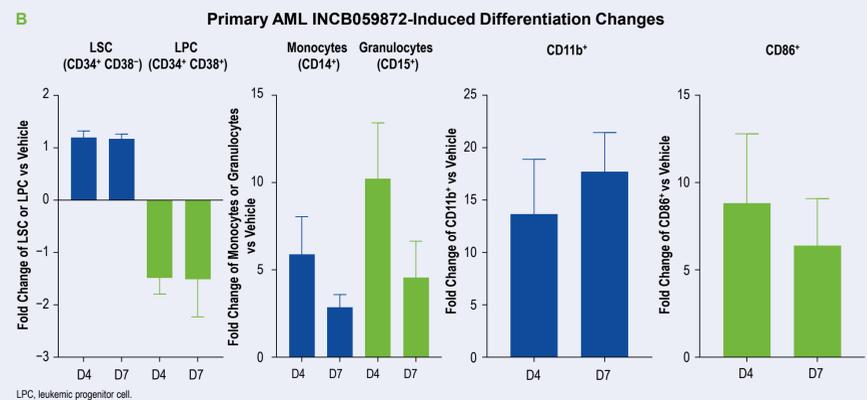
FAB, French-American-British; GI₅₀, concentration required for 50% growth inhibition.

- A. Enzyme assays were performed using the LANCE Ultra LSD1 histone H3K4 demethylase assay formats (PerkinElmer Inc., Waltham, MA). LSD2 and MAO-A/B were used for counter-screening.
- B. Cell proliferation was evaluated using the chemiluminescent CellTiter-Glo™ reagent (Promega, Madison, WI) after 10 days in culture.

INCB059872 Drives Early Progenitor Cell Differentiation and Increases Myeloid Cell Differentiation Within Primary Human AML Cells *In Vitro*

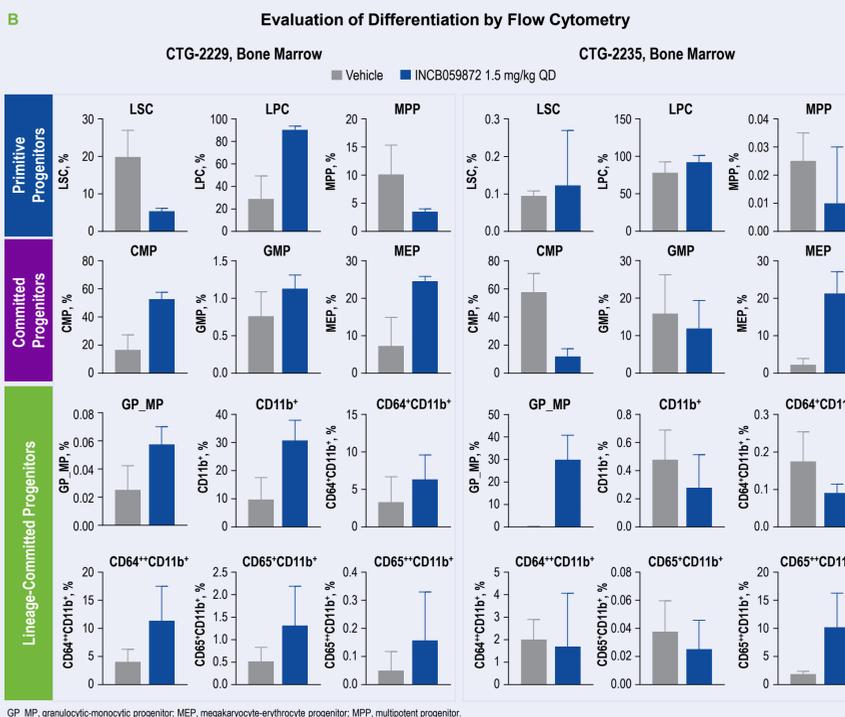
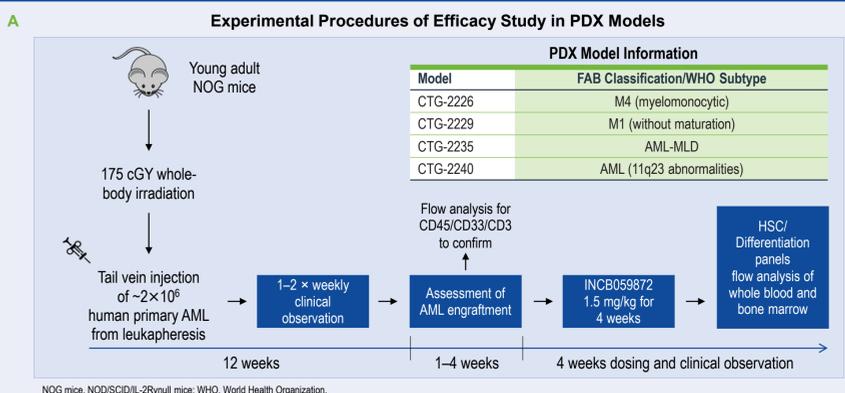
Primary AML Sample Subtype Information and Blast Cell Content			
Sample ID	Diagnosis	Subtype	Blast Cells, %
1	AML	AML-MLD with prior MPN	100
2	AML	No subtype defined	96
3	AML	AML with 11q23 abnormalities	97
4	AML	AML-MLD with prior MDS/MPN	98
5	AML	No subtype defined	65
6	AML	M4 [myelomonocytic]	73

MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; MLD, multilineage dysplasia.



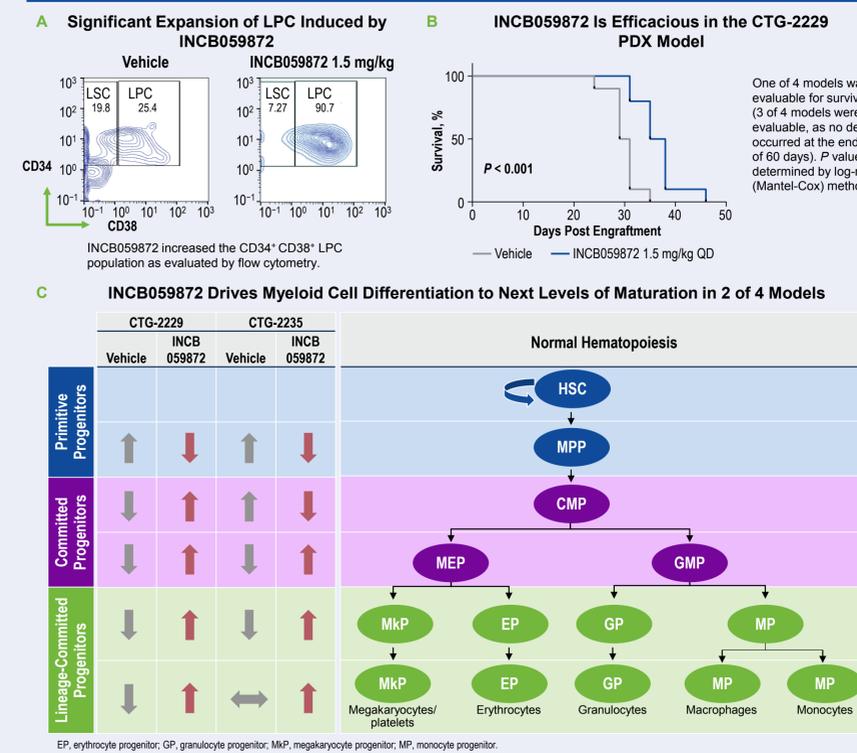
A. & B. Human AML cells were expanded for 7 days in the presence of stem cell factor, IL-3, IL-6, and 100 nM INCB059872. On day 4 (D4) and day 7 (D7), cells were stained with appropriate fluorescent conjugated antibodies and analyzed by flow cytometry. The fold change was calculated relative to DMSO control for all 6 samples. Values are presented as mean ± standard error of the mean.

INCB059872 Drives Cellular Differentiation in Primary AML Patient-Derived Xenograft (PDX) Models



A. & B. Bone marrow and whole blood were collected after 28 days of INCB059872 dosing and clinical observation and analyzed by flow cytometry for human progenitor cells and differentiated myeloid cells. Progenitor cells are described as follows: LSC = CD34⁺ CD38⁻, LPC = CD34⁺ CD38⁺, MPP = LSC⁺ CD45RA⁺ CD133⁻, MEP = LSC⁺ CD45RA⁺ CD133⁺, GP_MP = LPC⁺ CD45RA⁺ CD133⁻, CMP = LPC⁺ CD45RA⁺ CD133⁺, and GMP = LPC⁺ CD45RA⁺ CD133⁺. Differentiated myeloid cells are as described (n = 4 per group). Values are presented as mean ± SD.

Summary of Efficacy of INCB059872 in Human AML Systemic PDX Models



INCB059872 drives myeloid cell differentiation to next levels of maturation. Gray arrows = vehicle, red arrows = INCB059872 treated, up arrows = relatively higher percentage of cells, down arrows = relatively lower percentage of cells, horizontal arrow = no change.

Conclusions

- INCB059872 significantly inhibits cell proliferation in human primary AML cells both *in vitro* and *in vivo*
- In primary AML *ex vivo* differentiation studies, INCB059872 significantly altered the hematopoietic differentiation status of early progenitor cells
 - LSCs (CD34⁺ CD38⁻) were increased and LPCs (CD34⁺ CD38⁺) were decreased
 - Monocytes and granulocytes were further differentiated along with an increase in CD11b⁺ and CD86⁺ cells
- In contrast to the primary AML cells, the LPC (CD34⁺ CD38⁺) fraction was induced by INCB059872 in 2 of 4 AML systemic PDX models. This induction led to changes in the more differentiated and lineage-committed progenitors
 - INCB059872 induced both monocytic and granulocytic differentiation through induction of CD64 and CD65 within both the blast and non-blast cell compartments of the PDX AML models
- INCB059872 is a promising novel epigenetic agent for AML therapy; its mechanism of action lies in part in the induction of differentiation of leukemic stem/progenitor cells to more committed hematopoietic lineages

Disclosures

All authors: Employment and stock ownership – Incyte Corporation.

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