

Presented at the

American Association for Cancer  
Research 109th Annual Meeting  
Chicago, IL, USA • April 14–18, 2018

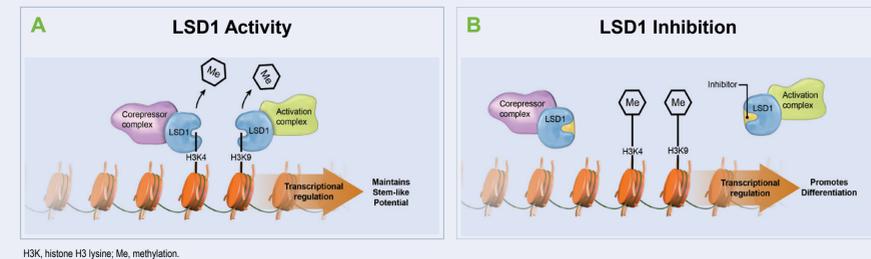
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## Abstract

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological tumor that is derived from the clonal expansion of immature T-cell progenitors. Multiple genetic and epigenetic alterations are attributed to the development of malignant T-cell transformation. Among these, there is supporting evidence for a role of lysine-specific demethylase (LSD1) in T-ALL. Oncogenic transcription factors, such as TAL-1, Notch, and ZEB2, form a complex with LSD1 to alter gene expression in T-ALL cells. In addition, LSD1 is aberrantly expressed in ALL, including B-ALL and T-ALL. Furthermore, the overexpression of LSD1 under control of the Sca-1 promoter in transgenic mice triggered T leukemogenesis via acquisition of self-renewal activity and alteration in the differentiation program to T-cell lineages. Together with the known function of LSD1 in regulating the activity of self-renewal in hematological malignancies, these studies prompted evaluation of the efficacy of the potent, selective, and orally bioavailable FAD-directed LSD1 inhibitor, INCB059872, in preclinical models of T-ALL. Expression of LSD1 was abundant in human T-ALL cell lines as detected by immunoblotting. *In vitro*, INCB059872 treatment significantly inhibited the proliferation of a subset of human T-ALL cell lines. *In vivo*, once-daily oral administration of INCB059872 inhibited tumor growth significantly in multiple human T-ALL subcutaneous xenograft models including Molt-4, RPMI-8402, CCRF-HSB-2, and CCRF-CEM, but was ineffective against DND-41 xenografts. The anti-tumor efficacy observed with INCB059872 had no clear genetic correlation with Notch mutation status of T-ALL tumors. Combination efficacy studies of INCB059872 with standard-of-care agents or targeted therapeutic agents in T-ALL models are currently being evaluated. These data suggest exploring the potential clinical development of INCB059872 as a therapy for T-ALL patients.

## Biological Effects of LSD1 on Cellular Differentiation



## Profile of INCB059872 in Biochemical Assays

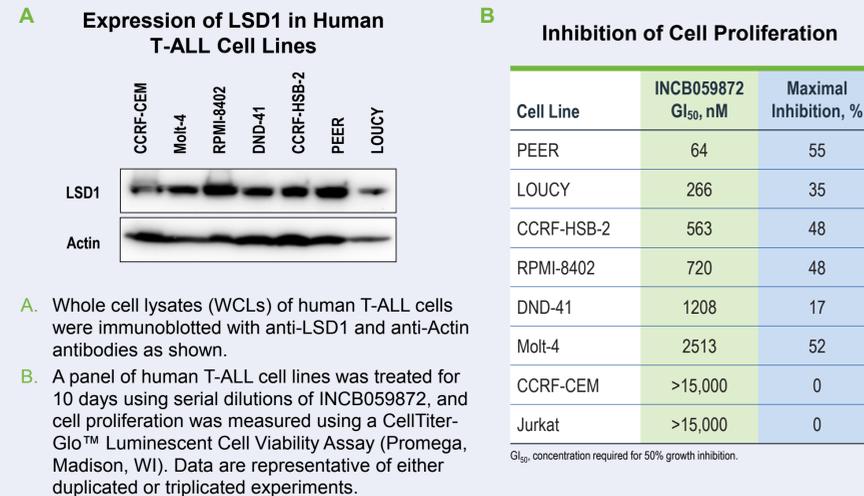
### Biochemical Potencies of INCB059872

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

IC<sub>50</sub>, half maximal inhibitory concentration; MAO, monoamine oxidase; SD, standard deviation.

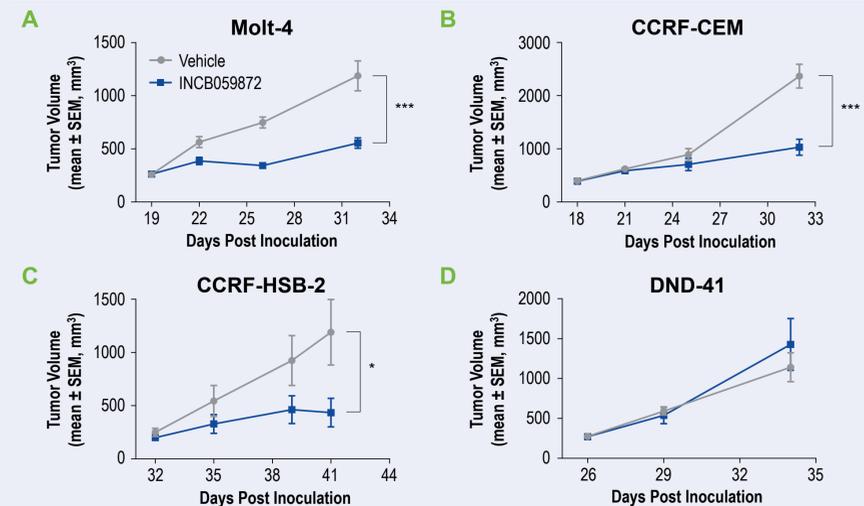
Enzyme assays were performed with LANCE Ultra LSD1 histone H3K4 demethylase assay formats (PerkinElmer Inc., Waltham, MA). LSD2 and MAO-A/B were used for counter-screening.

## Characterization of INCB059872 in Human T-ALL Cell Lines



- A.** Whole cell lysates (WCLs) of human T-ALL cells were immunoblotted with anti-LSD1 and anti-Actin antibodies as shown.
- B.** A panel of human T-ALL cell lines was treated for 10 days using serial dilutions of INCB059872, and cell proliferation was measured using a CellTiter-Glo™ Luminescent Cell Viability Assay (Promega, Madison, WI). Data are representative of either duplicated or triplicated experiments.

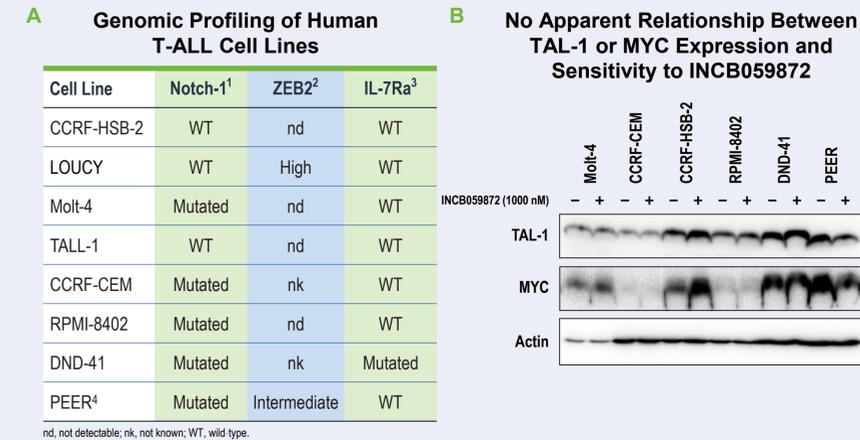
## INCB059872 Is Efficacious as a Single Agent in Various Human T-ALL Xenografts



SEM, standard error of the mean.

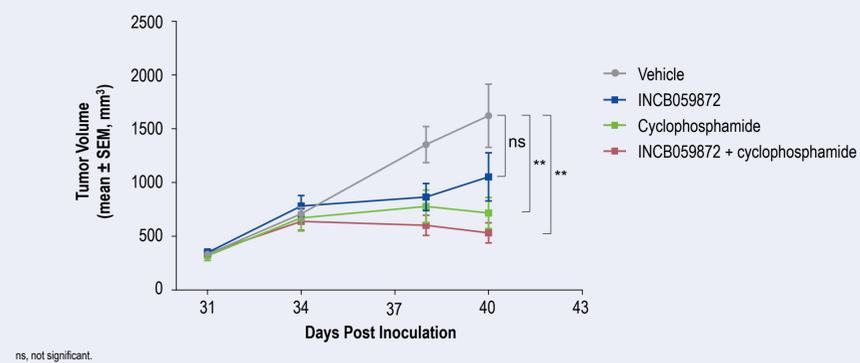
(A) Molt-4, (B) CCRF-CEM, (C) CCRF-HSB-2, and (D) DND-41 subcutaneous xenograft-bearing mice were dosed orally with INCB059872 1.5 mg/kg once daily for 14 days. INCB059872 was well tolerated. \*  $P < 0.05$ ; \*\*\*  $P < 0.001$  versus vehicle group determined by *t* test.

## Correlation of T-ALL Genetic Profiles and Sensitivity to INCB059872



- A.** Summary of Notch-1 mutations, ZEB2 expression, and IL-7Ra mutations from published literature.
- B.** Human T-ALL cell lines were treated with DMSO or 1000 nM of INCB059872 for 4 hours. WCLs of human T-ALL cells were immunoblotted with antibodies as shown. No significant correlation was found between Notch mutation status, expression of TAL-1 level, or ZEB2 expression to sensitivity of T-ALL cell lines to INCB059872. No change in expression of these genes was observed after treatment with INCB059872.

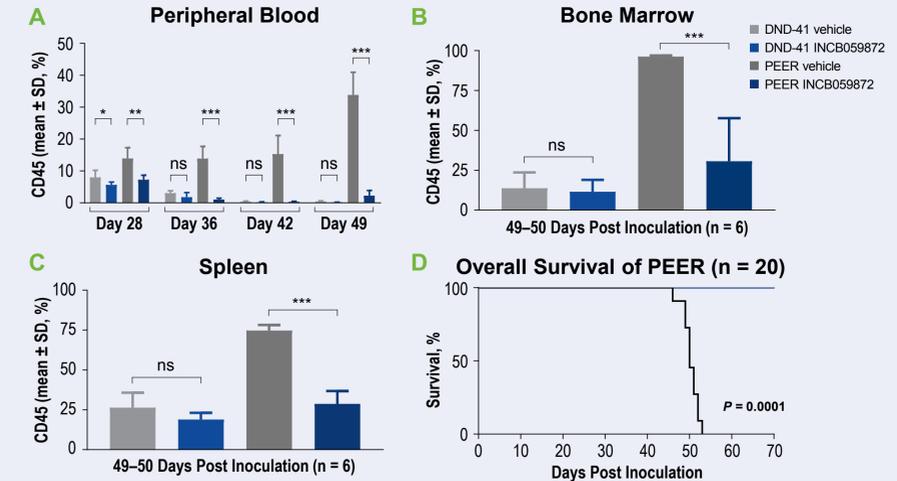
## INCB059872 Enhances the Efficacy of Cyclophosphamide in the Molt-4 Xenograft Model



ns, not significant.

Mice bearing Molt-4 xenografts were dosed orally with vehicle, INCB059872 1.5 mg/kg orally once daily, cyclophosphamide 5 mg/kg intraperitoneally twice weekly, and the combination of both agents. The combination of INCB059872 and cyclophosphamide resulted in enhanced tumor growth inhibition compared with single-agent activity and vehicle. Both agents alone and in combination were well tolerated. \*\*  $P < 0.01$  versus vehicle group determined by analysis of variance.

## INCB059872 Significantly Reduces Tumor Burden of Human T-ALL Cells in Systemic T-ALL Xenografts



Mice were intravenously injected with  $1 \times 10^5$  DND-41 or PEER cells. Tumor-bearing animals were randomized and dosed orally with vehicle or INCB059872 1.5 mg/kg once daily for the course of the study. At day 49 after inoculation, peripheral blood (A), bone marrow (B), and spleen (C) were collected and single cells were subjected to flow cytometry. No therapeutic benefit was seen in the DND-41 model. Overall survival of PEER systemic T-ALL models was determined by log-rank (Mantel-Cox) test (D). \*  $P < 0.05$ ; \*\*  $P = 0.01$ ; \*\*\*  $P < 0.001$  versus vehicle group determined by *t* test.

## Conclusions

- LSD1 is abundantly expressed in a panel of human T-ALL cell lines
- INCB059872 significantly inhibited cell growth of a subset of human T-ALL cell lines *in vitro* and T-ALL xenografts *in vivo*, and was well tolerated
- The status of Notch-1 mutation, TAL-1 expression, or MYC expression is not related to the sensitivity of T-ALL cells to INCB059872 *in vitro* or *in vivo*
- The combination of INCB059872 and the standard-of-care agent, cyclophosphamide, enhanced anti-tumor efficacy compared with single agent alone in the Molt-4 xenograft model
- INCB059872 significantly reduced tumor burden in the PEER systemic T-ALL model based on engraftment of CD45<sup>+</sup> cells in the peripheral blood and bone marrow of tumor-bearing mice, and significantly increased overall survival
- These data support the potential clinical development of INCB059872 as a therapy for patients with T-ALL

### 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.

### References

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