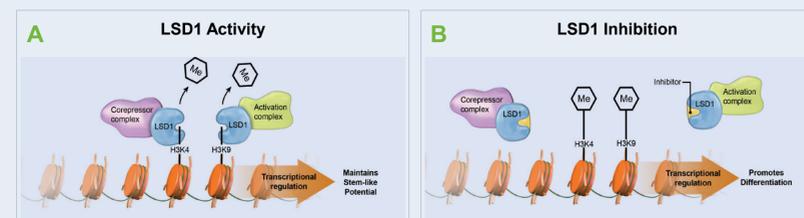


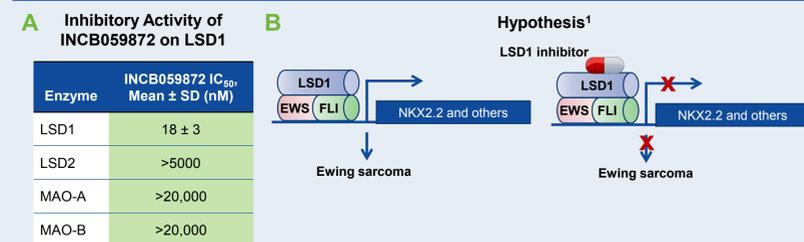
Abstract

Ewing sarcoma is a rare bone cancer affecting predominantly children. The chromosomal translocation of chromosomes 11 and 22 results in the EWS/FLI gene fusion oncoprotein that is associated with ~85% of Ewing sarcoma cases. The EWS/FLI fusion protein is involved in deregulating gene expression and consequently causing cellular transformation. It was previously reported that lysine specific demethylase 1 (LSD1) regulates EWS/FLI transcriptional activity via its functional interaction through the NuRD co-repressor complex. We therefore evaluated whether inhibition of LSD1 could have anti-tumor effects in Ewing sarcoma that express the EWS/FLI fusion oncoprotein. INCB059872 is a potent, selective, and orally available FAD-directed covalent inhibitor of LSD1. To investigate the potential utility of INCB059872 in Ewing sarcoma, the A-673 cell line having the characteristic chromosomal translocation was chosen as the experimental model system. INCB059872 inhibition of LSD1 did not significantly alter A673 proliferation *in vitro*. However, INCB059872 inhibited oncogenic transformation as determined by colony formation clonogenicity assays. NKX2.2 was previously identified as a critical downstream target molecule of the EWS-FLI fusion oncoprotein that is required for transformation. A significant downregulation of NKX2.2 was observed in A673 cells treated with INCB059872, suggesting that INCB059872 mediates its effects through modulation of the EWS/FLI-NKX2.2 axis. Oral administration of INCB059872 significantly suppressed the growth of both A673 and SK-ES Ewing sarcoma xenografts *in vivo*. In addition, *in vivo* efficacy was evaluated in patient derived xenograft (PDX) models that were developed from relapsed tumor tissues of Ewing sarcoma patients. Notably, a subset of PDX models having EWS/FLI translocations (3/6) exhibited significant tumor growth inhibition at well-tolerated doses of INCB059872. Molecular signatures obtained from RNA sequencing data with these PDX models exhibited intrinsic differences between responders and nonresponders, suggesting additional molecular or genetic variations may contribute to their sensitivity to INCB059872. Studies identifying potential candidate molecular mechanisms are underway. Together, these data suggest that INCB059872 may be therapeutically efficacious in a subset of Ewing sarcoma patients.

General Biological Effects of LSD1 in Cellular Differentiation

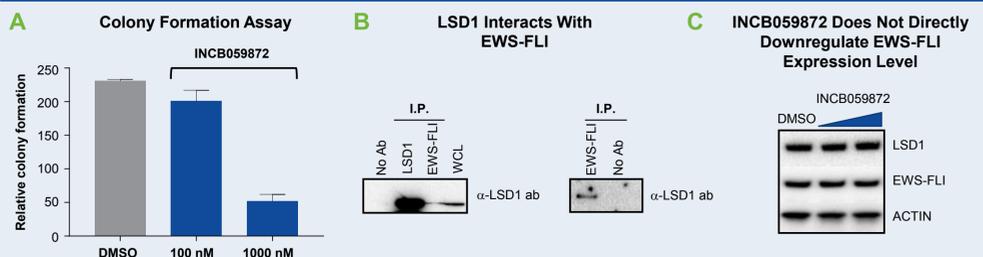


Profile of INCB059872 in Biochemical Assays and Therapeutic Hypothesis for Evaluation in Ewing Sarcoma



- A.** Enzyme assays were performed with LANCE Ultra LSD1 histone H3K4 demethylase assay format. LSD2 and MAO-A/B were used as counterscreens.
- B.** Hypothesis: EWS-FLI translocations are associated with approximately 85% of Ewing sarcomas. The interaction of LSD1 and EWS-FLI oncogenic transcription factor deregulates gene expression, contributing to the tumorigenic phenotype. Inhibition of LSD1 normalizes deregulated gene expression caused by EWS-FLI translocation and inhibits Ewing sarcoma growth.

In Vitro Characterization of INCB059872 in Ewing Sarcoma Cell Line, A-673



- A.** Colony formation assay to evaluate the effect of INCB059872 on cellular transformation: Five-hundred A-673 human Ewing sarcoma cells were plated in semi-solid media in the presence of INCB059872 or DMSO and incubated for 2 weeks. Colonies were counted.
- B.** Whole cellular lysates (WCL) of A-673 cells were immunoprecipitated with anti-LSD1 or anti-EWS-FLI antibody as shown. Immunoblots were conducted with anti-LSD1 antibody.
- C.** A-673 cells were treated with DMSO or INCB059872 (100, 1000 nM) for 24 hours and immunoblot assays were conducted.

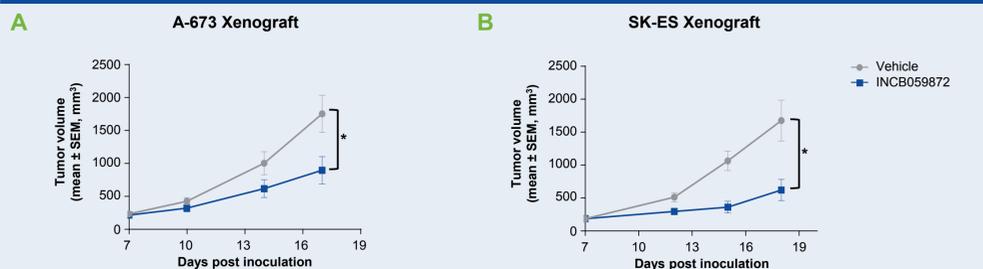
In Vitro and In Vivo Pharmacodynamics of INCB059872



RT-PCR was conducted to determine the change in the NKX2.2 expression level upon INCB059872 treatment. Graphs show the NKX2.2 expression level relative to DMSO-treated cells (**A**) or vehicle-treated animals (**B**). Normalized NKX2.2 gene expression level = 2^{-CtΔΔCt}.

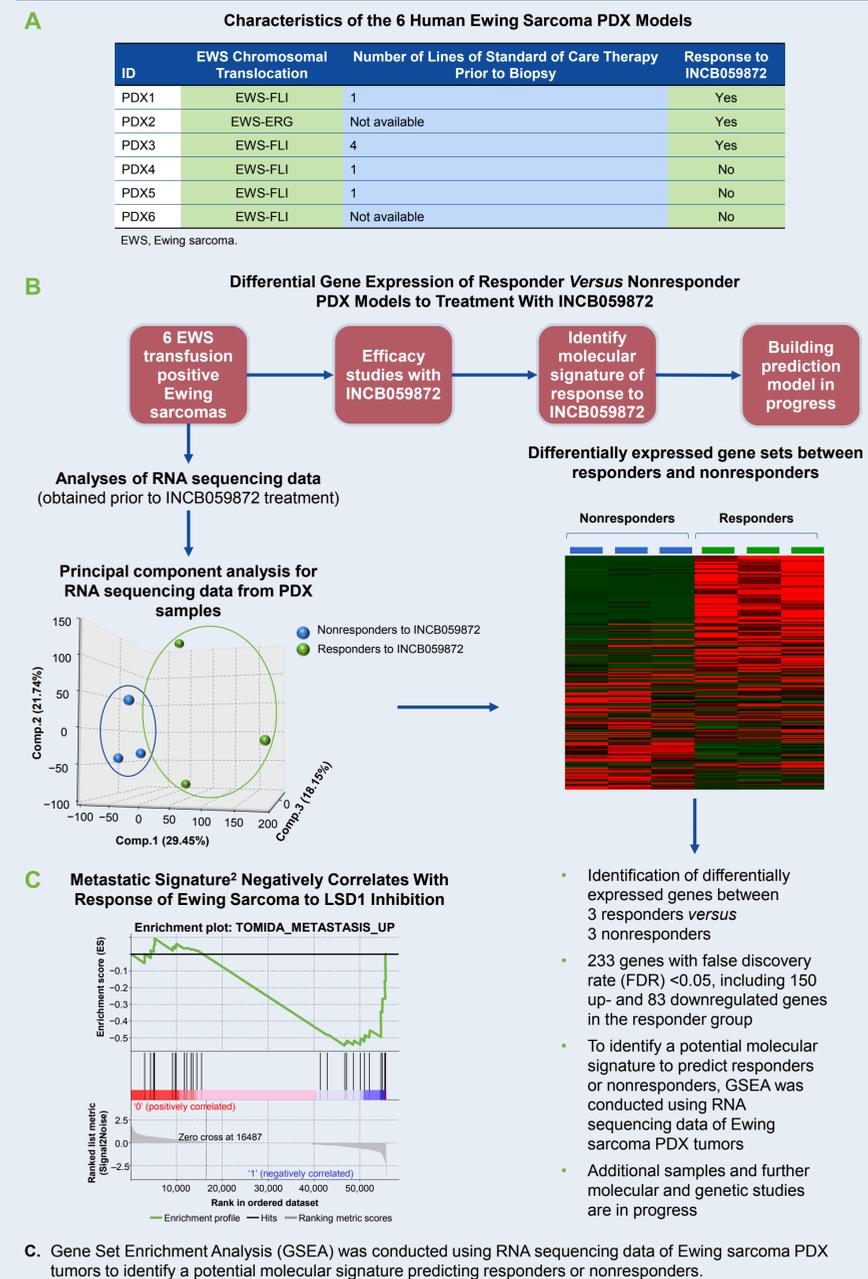
- A.** *In vitro*: A-673 cells were treated with DMSO or various concentrations of INCB059872 and incubated for 72 hours.
- B.** *In vivo*: Animals bearing A-673 xenograft were dosed with INCB059872, 1.5 mg/kg, PO, QD for 24 hours or 72 hours.

INCB059872 Is Efficacious as a Single Agent Against Human Ewing Sarcoma Xenografts

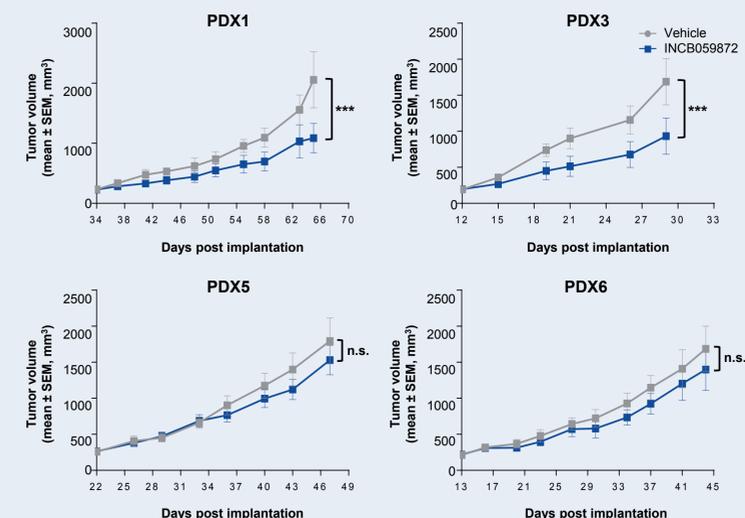


Mice bearing human Ewing sarcoma of A-673 (**A**) or SK-ES (**B**) xenografts were dosed with INCB059872 (1.5 mg/kg, QD, PO) for 2 weeks. Significant efficacy (* *P* < 0.05) was observed in both xenograft models. *P* values among groups were determined by unpaired *t* test using GraphPad Prism. Dosing regimens were well tolerated.

RNA Sequencing Data Suggest That Intrinsic Molecular Mechanisms Determine the Sensitivity of Ewing Sarcoma PDX Models to INCB059872



INCB059872 Is Efficacious in a Subset of Human Ewing Sarcoma PDX Models Possessing EWS-Translocations



n.s., non-significant.

Mice were implanted subcutaneously into the left flank with PDX tumor fragments from the various models indicated. Upon achieving tumor volumes of approximately 300 mm³, mice were treated with vehicle or INCB059872 at 1.5 mg/kg QD via oral gavage for up to 32 days. Significant efficacy (***) *P* < 0.001 was observed in models PDX1 and PDX3 but not in models PDX5 and PDX6. Statistical analyses of tumor volumes were conducted using 2-way ANOVA followed by Bonferroni multiple comparison test comparing treatments to control. Dosing regimens were well tolerated.

Conclusions

- INCB059872 significantly inhibited cell growth *in vitro*
- INCB059872 reduced expression of NKX2.2 in A-673 cells *in vitro* and *in vivo*
- Efficacy studies in Ewing sarcoma PDX models demonstrated that INCB059872 was efficacious and well tolerated and not solely dependent on tumor EWS-FLI fusion status, suggesting that additional yet unknown molecular/genetic mechanisms contribute to the sensitivity to LSD1 inhibition
- Combination studies of INCB059872 and standard of care agents are under investigation
- Studies of the molecular and genetic profiles of Ewing sarcomas to predict their sensitivity to INCB059872 are in progress

Reference

- Smith R, et al. *Cancer Cell*. 2006;9:405–416.
- Tomida S, et al. *Oncogene*. 2007;26(31):4600–4608.

Author Disclosures

All Authors: Incyte Corporation: Employment and Stock Ownership.

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