DUAL FUNCTION HDAC AND PI3K INHIBITOR CUDC-907 AFFECTS CANCER CELLS AND THE TUMOR MICROENVIRONMENT IN HEMATOLOGICAL MALIGNANCIES

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#1879

Introduction

Histone deacetylases (HDACs) and the phosphatidylinositol 3-kinase (PI3K)/AKT pathways are promising therapeutic targets in hematologic cancers and evidence of synergistic anti-cancer activity has recently emerged. CUDC-907, a small molecule drug candidate that is designed to target HDACs and PI3Ks in a single chemical entity, is currently in Phase 1 clinical testing for the treatment of patients with lymphoma or multiple myeloma. Preclinically, CUDC-907 has been shown to inhibit activation of PI3K/AKT, JAK/STAT and MAPK pathways in solid tumor and hematologic cancer cell lines. In this study, we report that in the setting of hematologic malignancies, CUDC-907 targets not only the cancer cells but also the tumor microenvironment. In the ongoing clinical study, we monitor a panel of 12 cytokines and chemokines. Preliminary results indicate correlative trends between tumor response and baseline TARC levels, and between tumor response and plasma TARC level changes after 15 days of treatments. Based on these results, we are further investigating the potential of utilizing select cytokines and chemokines as predictive markers of CUDC-907 activity.

CUDC-907 Disrupts Key RTK Signaling Pathways

HDAC	IC50 (nM)
1	1.7
2	5.0
3	1.8
6	27
10	2.8
4, 5, 7, 8, 9	>200

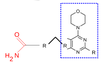
PI3K	IC50 (nM)
Alpha	19
Delta	39
Beta	54
Gamma	311

CUDC-907

- Full HDAC activity
- Full PI3K activity

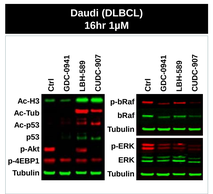
M1

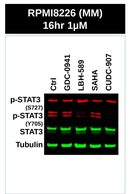
- ~ 30% PI3K activity as compared to parent
- No HDAC activity

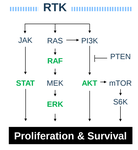


M2

- ~ 80% PI3K activity as compared to parent
- No HDAC activity

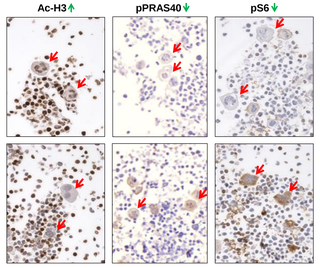






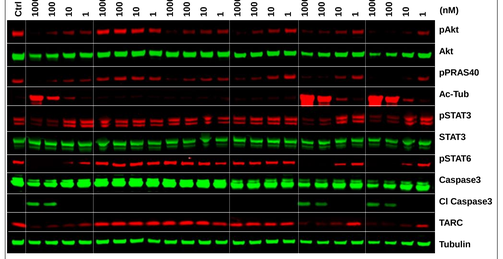
Note: GDC-0941 is a pan PI3K inhibitor; LBH-589 and SAHA are both pan HDAC inhibitors

CUDC-907 Inhibits PI3K & HDAC Activities in Mouse Bone Marrow



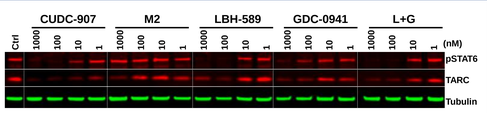
Note: C57BL/6 mice were dosed with CUDC-907 or control vehicle for 5 days through daily oral administration. Marrow of femur bone was isolated for IHC. Red arrows indicate megakarocytes.

CUDC-907 Decreases TARC Levels in a Hodgkin's Lymphoma Cell Line



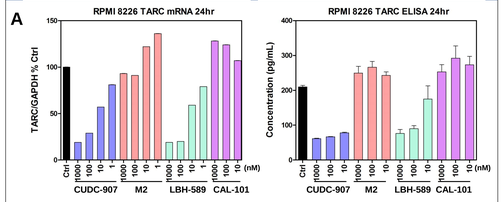
Note: L428 Hodgkin's Lymphoma cells were incubated with indicated compounds for 16 hours prior to Western blot analysis. L+G: LBH-589+GDC-0941

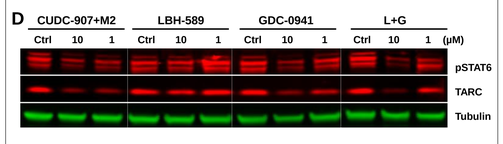
CUDC-907 Decreases TARC Levels in a DLBCL Cell Line

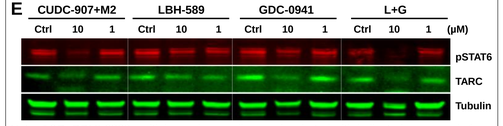


Note: DOHH2 DLBCL cells were incubated with indicated compounds for 24 hours prior to Western blot analysis. L+G: LBH-589+GDC-0941

CUDC-907 Inhibits TARC Production in Multiple Myeloma Cell Lines

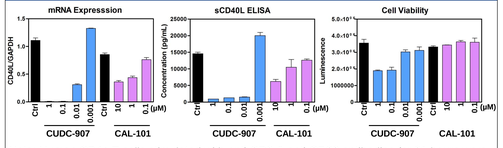






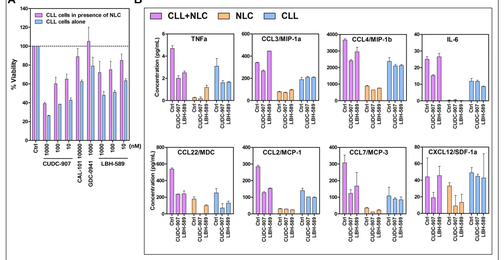
Note: **A&B**. RPMI-8226 multiple myeloma cells were incubated with indicated compounds for 24 hours. Cells were collected for RT-PCR analysis (A), and culture media were collected for TARC ELISA (B). Data are normalized against DMSO control. **C**. Dosing method used in the washout experiments (D&E) to mimic clinical exposure. **D**. RPMI-8226 cells were treated with indicated compounds as in C for a total of 24 hours prior to Western blot analysis. **E**. OPM-2 cells were treated with indicated compounds as in C for a total of 42 hours prior to Western blot analysis. CAL-101: PI3Kδ inhibitor; L+G: LBH-589 + GDC-0941.

CUDC-907 Decreases CD40L Production in Stimulated CD4+ T-Cells



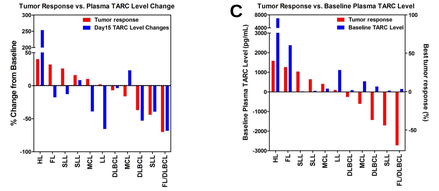
Note: Human CD4+ T-cells stimulated with anti-CD3 & anti-CD28 antibodies for 31 hours were treated with indicated compounds for 16 hours prior to RT-PCR and cell viability testing. Culture media were collected for soluble CD40 ligand ELISA. CD4+ cells from healthy donors were from a commercial source.

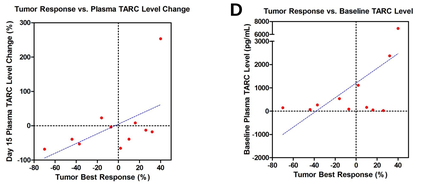
Primary CLL Cells Are Sensitive to CUDC-907



Note: **A**. Primary CLL cells cultured with or without human PBMC-derived nurse-like cells (NLC) were incubated with indicated compounds for 24 hours prior to cell viability assay. Data are normalized against DMSO control. **B**. Primary CLL cells and NLC cells were cultured alone or together, and treated with 1000 nM of CUDC-907 or LBH-589 for 24 hours. Culture medium was collected for MSD multiplex cytokine and chemokine ELISA assays. Primary CLL cells were from a commercial source.

Patient Plasma TARC Level Changes in Phase I Clinical Trial



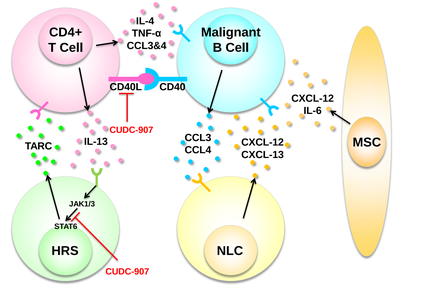


E Correlation Between Tumor Response and Plasma TARC Levels (N=11)

Patient Plasma TARC	Day 15 Percentage Change	Day 15 Absolute Concentration Change	Baseline Concentration
P value (two-tailed)	0.082	0.203	0.095
R square	0.298	0.173	0.279

Note: **A&B**. Clinical tumor response vs. day 15 plasma TARC level changes in CUDC-907 phase I trial. **C&D**. Clinical tumor response vs. baseline plasma TARC levels. **E**. Trend of correlation between plasma TARC level and Best tumor response, (N=11).

CUDC-907 Targets Tumor Microenvironment



Note: CUDC-907 decreases the production of CD40 ligand by CD4+ T cells. It also decreases the STAT6 phosphorylation-medicated TARC production by the HRS Hodgkin's lymphoma cells. TARC stimulates the proliferation, maturation, and migration of T helper 2 cells, which is important for the survival of malignant B cells. CUDC-907 also decreases other cytokines and chemokines in primary CLL and NLC co-cultures. **HRS:** Hodgkin and Reed-Sternberg (HRS) cell of classical Hodgkin's lymphoma; **NLC**: monocyte-derived nurselike cell; **MSC**: mesenchymal stromal cell.

Conclusion

- CUDC-907 decreases the activity of multiple pathways, such as PI3K/AKT, MEK/ERK, and JAK/STAT pathways due to dual PI3K and HDAC inhibition
- In this study, PI3K and HDAC inhibition by CUDC-907 has been demonstrated *in vivo* in mouse bone marrow
- CUDC-907 inhibits STAT6-mediated TARC production in Hodgkin's lymphoma, DLBCL, and multiple myeloma cell lines
- CUDC-907 decreases CD40L production in stimulated human primary CD4+ T-Cells
- CUDC-907 is able to overcome stromal cell-protection of primary CLL cells in *in vitro* co-culture system, where treatment of CUDC-907 decrease cytokine and chemokine levels.
- The ongoing first-in-human Phase 1 trial testing CUDC-907 in the setting of advanced lymphoma and multiple myeloma has yielded preliminary evidence of anti-cancer activity and potential impact on the tumor microenvironment
- These results suggest the potential utility of selected cytokines and chemokines as predictive markers of CUDC-907 activity

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