Dual HDAC and PI3K inhibition with CUDC-907 Downregulates MYC and Suppresses Growth of MYC-dependent Cancers

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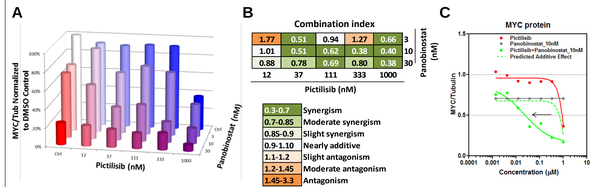
Introduction

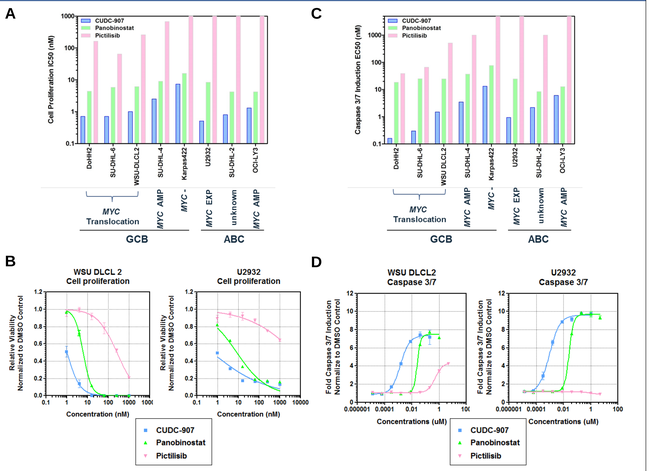
CUDC-907 is a dual-acting inhibitor of class I and II HDACs and class I PI3Ks with potent antitumor activity. Preclinical studies have suggested that individually targeting upstream regulators of MYC such as HDACs and PI3K can reduce MYC expression and suppress the growth of MYC-driven cancers. As HDACs and PI3K regulate MYC protein levels and functions through non-overlapping mechanisms, simultaneous HDAC and PI3K inhibition may further enhance MYC suppression. Here, we evaluate the activity and mechanism of CUDC-907 in cancer types driven by MYC upregulation.

HDAC inhibitor and PI3K inhibitor synergistically downregulate MYC and induce Caspase 3/7 activation in MYC-altered DLBCL cells



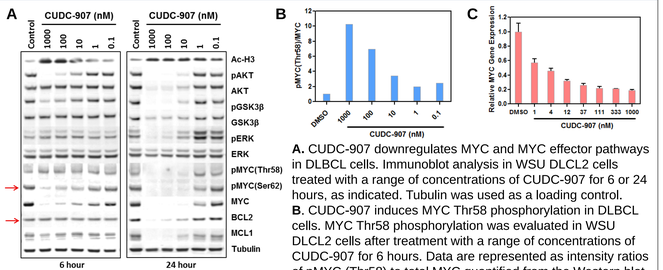
A. Combined treatment with the HDAC inhibitor panobinostat and the PI3K inhibitor pictilisib downregulates MYC protein levels in DLBCL cells. WSU DLCL2 cells were treated with various concentrations of panobinostat and/or pictilisib for 24 hours. Data represent the ratio of MYC to tubulin quantified from the dot Western blot and normalized to DMSO-treated cells.
B,**C**. Panobinostat and pictilisib synergistically decrease MYC protein levels. (B) Combination indexes (CI) from the data presented in (A) were calculated using Compusyn software. Criteria for synergy, additivity, and antagonism are shown. (C) The Bliss independence method was used to determine combinatorial activity between a fixed concentration of panobinostat (10 nM) and increasing concentrations of pictilisib. The effect of pictilisib on MYC protein level relative to tubulin is shown in red. The dashed green line represents the predicted additive effect calculated according to the Bliss independence method, whereas the solid green line shows the observed changes in MYC protein levels induced by the combination of panobinostat and pictilisib.
D. Panobinostat and pictilisib synergistically induce caspase 3/7 activation. The Bliss independence method was used to determine combinatorial activity between a fixed concentration of panobinostat (14 nM) and increasing concentrations of pictilisib. The effect of pictilisib on caspase activation is shown in red. The dashed green line represents the predicted additive effect calculated according to the Bliss independence method, whereas the solid green line shows the observed caspase 3/7 activity induced by the combination of panobinostat and pictilisib. Caspase 3/7 activation was determined by the Caspase-Glo 3/7 assay after treatment with indicated compounds for 24 hours. Data are normalized to DMSO-treated cells. Error bars represent SD of duplicates.
E. Proposed mechanism of MYC suppression induced by CUDC-907. Simultaneous HDAC and PI3K inhibition by CUDC-907 suppresses MYC through non-overlapping transcriptional and post-translational mechanisms.

Antitumor activity of CUDC-907 in DLBCL cell lines



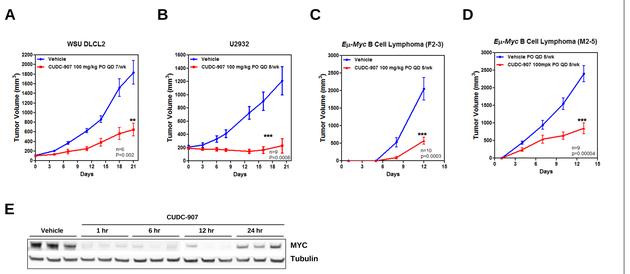
A,**B**. CUDC-907 inhibits growth of DLBCL cell lines. (A) Growth inhibition IC_{50} values of CUDC-907, panobinostat, or pictilisib in a panel of DLBCL cell lines. Cell viability was assessed by the CellTiter-Glo assay after 48-hour incubation with the indicated compound. The MYC status and the cell-of-origin of each cell line are indicated in the graphs. (B) Representative cell viability curves for WSU DLCL2 and U2932 cells treated with CUDC-907, panobinostat, or pictilisib. Data are normalized to DMSO-treated cells. Error bars represent SD of duplicates.
C,**D**. CUDC-907 induces caspase 3/7 activation in DLBCL cell lines. (C) Caspase 3/7 induction EC_{50} values for CUDC-907, panobinostat, or pictilisib in a panel of DLBCL cell lines. Caspase 3/7 activation was determined by the Caspase-Glo 3/7 assay after 24-hour incubation with the indicated compound. (D) Representative caspase 3/7 induction curves for WSU DLCL2 and U2932 cells treated with CUDC-907, panobinostat, or pictilisib. Data are normalized to DMSO-treated cells. Error bars represent SD of duplicates.

CUDC-907 induces MYC Thr58 phosphorylation and downregulates MYC in MYC-altered DLBCL cells



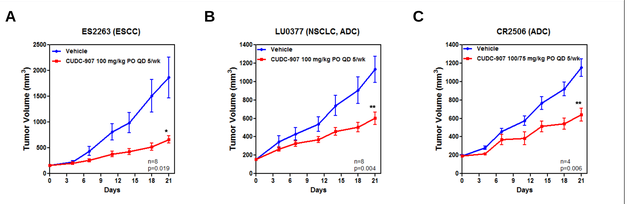
A. CUDC-907 downregulates MYC and MYC effector pathways in DLBCL cells. Immunoblot analysis in WSU DLCL2 cells treated with a range of concentrations of CUDC-907 for 6 or 24 hours, as indicated. Tubulin was used as a loading control.
B. CUDC-907 induces MYC Thr58 phosphorylation in DLBCL cells. MYC Thr58 phosphorylation was evaluated in WSU DLCL2 cells after treatment with a range of concentrations of CUDC-907 for 6 hours. Data are represented as intensity ratios of pMYC (Thr58) to total MYC quantified from the Western blot shown in A and normalized with DMSO-treated cells.
C. CUDC-907 reduces *MYC* gene expression in DLBCL cells. Relative *MYC* gene expression in WSU DLCL2 cells treated with CUDC-907 for 6 hours as calculated by the comparative C_T method (*PPIA* used as a housekeeping gene).

Antitumor activity of CUDC-907 in human DLBCL cell line-derived xenograft models and the *Eμ-Myc* transgenic mouse allograft models



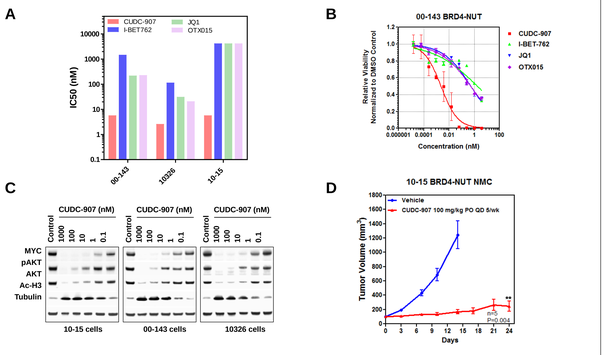
A,**B**. Oral administration of CUDC-907 significantly inhibits the growth of the established WSU DLCL2 (A) and U2932 (B) DLBCL xenograft tumors engrafted in SCID-beige mice. Each graph describes the change in tumor volume (*y* axis) as a function of the time after the initiation of treatment (*x* axis). Each datapoint represents the mean volume of 6 (A) or 9 (B) independent tumors. Error bars represent SEM. WSU DLCL2 xenografts were treated with vehicle control (30% Captisol) or CUDC-907 once a day for 21 days at 100 mg per kg body weight (mg/kg) as indicated. U2932 xenografts were treated with vehicle control or CUDC-907 daily in a 5-days-on and 2-days-off schedule for 20 days at 100 mg/kg as indicated. P values are as indicated.
C,**D**. Oral administration of CUDC-907 significantly inhibits the growth of allograft tumors derived from two independent B-cell lymphoma tumors spontaneously occurring in *Eμ-Myc* transgenic mice. Each graph describes the change in tumor volume (*y* axis) as a function of the time after the initiation of treatment (*x* axis). Each datapoint represents the mean of 10 (C) or 9 (D) independent tumors. Error bars represent SEM. All mice were treated with vehicle control (30% Captisol) or CUDC-907 daily at a 5-days-on, 2-days-off schedule for 12 (C) or 13 (D) days at 100 mg/kg as indicated. P values are as indicated.
*, P<0.05; **, P<0.01; ***, P<0.001.
E. CUDC-907 reduces MYC protein level in DLBCL xenograft tumors *in vivo*. Immunoblot analysis of MYC protein levels in the WSU DLCL2 xenograft tumors treated with 5 oral daily administrations of vehicle control (30% Captisol) or CUDC-907 at 100 mg/kg as indicated. Three independent tumors were analyzed at each timepoint after the fifth administration as indicated. Tubulin was used as a loading control.

CUDC-907 inhibits tumor growth in MYC-amplified patient-derived xenograft (PDX) models



Oral administration of CUDC-907 significantly inhibits the growth of established PDX tumors derived from (A) esophageal carcinoma (ES2263), (B) non-small cell lung carcinoma (LU0377), and (C) colorectal cancer (CR2506), and engrafted in BALB/c nude mice. Each graph describes the change in tumor volume (*y* axis) as a function of the time after the initiation of treatment (*x* axis). Each datapoint represents the mean tumor volume of eight (A,B) or four (C) independent tumors. Error bars represent SEM. The mice from all three PDX models were treated with vehicle control (30% Captisol) or CUDC-907 daily with a 5-days-on, 2-days-off schedule for 21 days at 100 mg/kg as indicated. P values are as indicated.
*, P<0.05; **, P<0.01; ***, P<0.001

Antitumor activity of CUDC-907 against MYC-dependent NUT midline carcinoma (NMC) cells *in vitro* and *in vivo*



A. CUDC-907 inhibits growth of BRD–NUT fusion-positive NMC cell lines more potently than BET inhibitors. Growth inhibition IC_{50} values were determined for CUDC-907 and three BET inhibitors (I-BET-762, JQ1, and OTX015) in three BRD–NUT fusion-positive NMC cell lines. Cell viability after 72-hour incubation was assessed by the CellTiter-Glo assay.
B. CUDC-907 inhibits BRD4-NUT fusion-positive NMC cell growth in a dose-dependent manner. Representative cell viability curves for 00-143 BRD4-NUT fusion-positive NMC cells treated with various concentrations of CUDC-907 or BET inhibitors are indicated. Data are normalized to DMSO-treated cells. Error bars represent SD of duplicates.
C. CUDC-907 downregulates MYC in BRD4-NUT fusion-positive NMC cells. Immunoblot analysis in three BRD–NUT fusion-positive cell lines after treatment with a range of concentrations of CUDC-907 for 24 hours. Tubulin was used as a loading control.
D. Oral administration of CUDC-907 significantly inhibits the growth of 10-15 NMC cell line-derived xenograft tumors engrafted in athymic nude mice. Each graph describes the change in tumor volume (*y* axis) as a function of the time after the initiation of treatment (*x* axis). Each data point represents the mean volume of five independent tumors. Error bars represent SEM. Mice were treated with vehicle control (30% Captisol) or CUDC-907 on a 5-days-on, 2-days-off schedule for 24 days at 100 mg/kg as indicated. The vehicle group had to be terminated earlier (day 14) because the tumor sizes exceeded institutional limitations.

Conclusions

- The combination of HDAC and PI3K inhibition synergistically downregulates MYC in diffuse large B-cell lymphoma (DLBCL) cells. This result provides a rationale of targeting MYC by simultaneously inhibiting HDAC and PI3K, two key upstream regulators of MYC with none-overlapping mechanisms
- CUDC-907, a dual-acting inhibitor of class I and II HDACs and class I PI3Ks, down-regulates MYC through decreasing *MYC* mRNA expression and promoting ubiquitin-proteasome mediated MYC protein degradation in the "double-hit" DLBCL cells, which has very poor prognosis. These results suggest that simultaneous inhibiting HDAC and PI3K with CUDC-907 may be an effective strategy for targeting MYC.
- Our *in vivo* results demonstrate that CUDC-907 effectively inhibits the growth of MYC-driven tumors representing multiple cancer types, including "double hit" DLBCL xenograft models, MYC-dependent NUT midline carcinoma (NMC) xenograft models, *Eμ-Myc* transgenic allograft models of B-cell lymphoma, and *MYC*-amplified solid tumor PDX models. These results suggest the potential of using CUDC-907 as treatment strategy for MYC-dependent cancers across indications.

Acknowledgement

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