Discovery of potent, selective, and orally bioavailable small molecule inhibitors of YTHDC1 for the treatment of MYC-driven cancers

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Abstract 453

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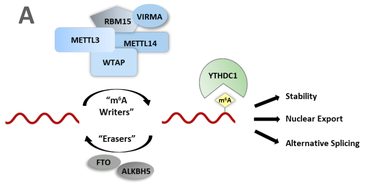
YTHDC1 is a targetable driver of MYC-driven AML & SCLC



- Nuclear epigenetic "reader" of m^{6}A-modified RNA
- Forms nuclear condensates by binding to m^{6}A RNAs and through its intrinsically disordered regions (IDRs)
- These condensates drive the proliferation of Acute Myeloid Leukemia (AML) cells through sequestration of onco-protective m^{6}A RNAs (i.e., *MYC*) thus protecting them from degradation[1]

[1] Cheng, Y., *et al. Cancer Cell*. 2021. 39, 958–972.

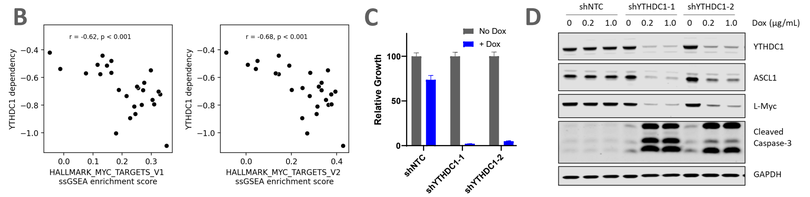
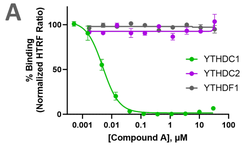
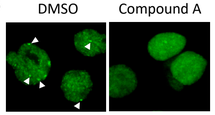


Figure 1. A. Schematic depicting the RNA processing roles of YTHDC1 elicited through m^{6}A-RNA binding. **B. YTHDC1 dependency correlates with ssGSEA enrichment of MYC target gene expression** across a panel of AML cell lines. Data downloaded from Depmap.org (Data release 24Q2). **C.** Knockdown of YTHDC1 in small cell lung cancer (SCLC) cell line "X" demonstrate **strong viability defect** 7 days post-induction. **D.** Cells were treated as in (C) and Western blot was performed after 48h of shRNA induction, demonstrating **reduction in L-MYC and ASCL1 with concomitant induction of the apoptotic marker, Cleaved caspase-3**.

Selective YTHDC1 small molecule inhibitor dissolves YTHDC1 condensates





C

Compound	A	B	C
HTRF IC_{50} (μM)	0.006	0.037	0.009
Cellular YTHDC1 Condensate IC_{50} (μM)	0.647	2.268	0.790
MOLM13 Viability IC_{50} (μM)	0.115	1.254	0.201

Figure 2. A. Homogenous Time-Resolved Fluorescence (HTRF) assay demonstrates **selective effects of Compound A in disrupting the interaction of m^{6}A-RNA with YTHDC1**, but not with other YTH-domain family members. **B.** KG1 AML cells harboring Emerald-YTHDC1 demonstrate a **reduction of YTHDC1 condensates** 24h after treatment with 1.25 μM Compound A. **C.** Table of properties for Compounds A, B & C.

Summary

- YTHDC1 is a targetable driver of MYC-driven AML & Small Cell Lung Cancer
- Transition Bio has developed **potent, selective, and orally-bioavailable small molecule inhibitors of YTHDC1**
- YTHDC1 inhibitors dissolve biomolecular condensates containing m6A-modified RNAs
- Inhibition of YTHDC1 results in potent anti-cancer effects in MYC-driven AML and L-MYC and ASCL1-driven SCLC
- Inhibition of YTHDC1 leads to reduced expression of oncogenic transcription factors, correlating with anti-tumor activity in primary AML patient samples as well as in AML and SCLC xenograft models

YTHDC1 small molecule inhibitors downregulate MYC & cause AML cell death & differentiation

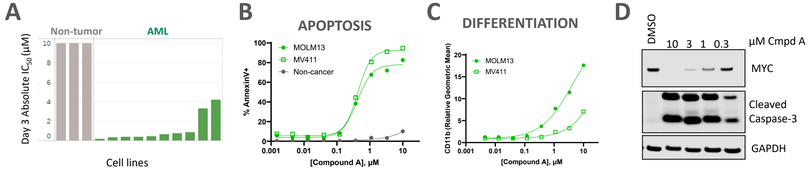


Figure 3. A. Compound A impacts proliferation of a panel of AML cell lines, with **no effect on non-cancer cells**. Absolute IC_{50} values are plotted after a 3-day CTG assay. **B-C. Dose-dependent induction of apoptosis and differentiation with Compound A. B.** After 5 days, the percentage of Annexin V positive cells was measured by flow cytometry **C.** Myeloid differentiation was evaluated by flow cytometry of CD11b positive cells. **D.** MV411 cells were treated with Compound A for 48 hours and Western blot was performed, demonstrating **reduction in MYC and induction of the apoptotic marker, Cleaved Caspase-3**.

YTHDC1 inhibitors cause reduced cell growth and MYC expression in primary AML patient cells, while sparing normal CD34$^+$ cells

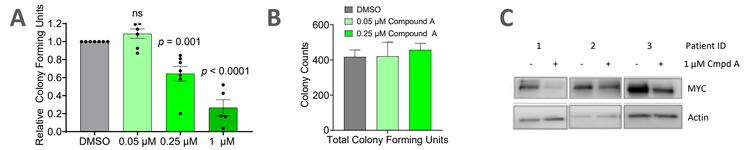


Figure 4. A. Colony formation assay of peripheral blood mononuclear cells (PBMCs) from 5-7 AML patients demonstrates **dose-dependent proliferation defect with Compound A**. Colony numbers are normalized to DMSO-treated controls and each dot represent one patient. **B.** Fresh cord blood was obtained and normal CD34+ cells were enriched. Cells were plated in methylcellulose with cytokines and Compound A for 7 days, and colony forming units (CFU) were counted. Compound A had **no effect on the total number of CFU of normal CD34+ cells**, and no significant differences were detected in the differentiated phenotypic colony subtypes in response to the treatment. **C.** PBMCs from 3 AML patient samples were treated with Compound A for 3 days and Western blot was performed, demonstrating **reduction of MYC protein expression**.

Tumor growth inhibition and reduction of MYC *in vivo*

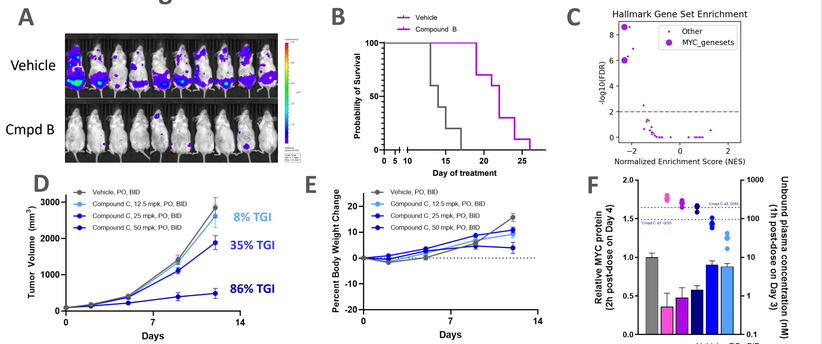
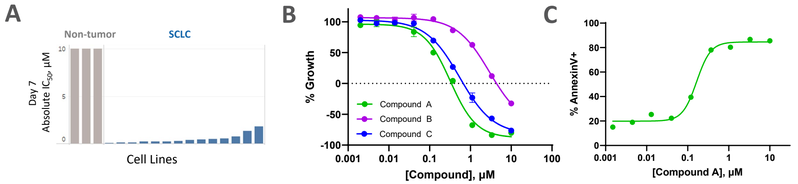


Figure 5. A. NOD/SCID mice were inoculated with MOLM13-Luc human leukemia cells and treated with Vehicle or Compound B. Bioluminescent images taken after 10 days of dosing, demonstrating **reduced tumor burden in treated animals. B.** Kaplan-Meier plot demonstrates **significant survival benefit in animals from (A) treated with Compound B** as compared to vehicle-treated animals. **C.** GSEA analysis demonstrating **enrichment of MYC target gene sets from tumors treated with Compound B** compared to vehicle arm. **D.** BALB/c nude mice were inoculated with MOLM13-Luc cells and treated with Vehicle or Compound C. Tumor volume measurements demonstrate **dose-dependent tumor growth inhibition with Compound C**. **E.** Percent body weight change shows treatment with **Compound C was well-tolerated. F.** Animals were treated as in (D) and were sacrificed on day 4. Tumor samples were collected, and Western blot was performed. **Dose-dependent MYC suppression correlates with unbound compound exposure in plasma, demonstrating PK/PD correlation.**

YTHDC1 small molecule inhibitors downregulate L-MYC & ASCL1, and cause SCLC cell death



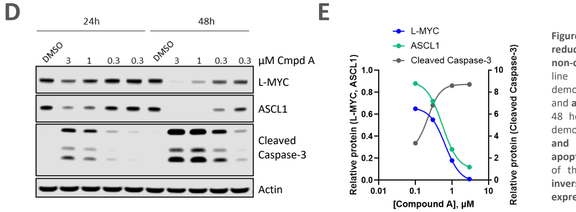


Figure 6. A. A panel of SCLC cell lines **demonstrated reduced viability** in the presence of Compound A while **non-cancer cell lines was not impacted**. **B-C.** SCLC cell line "X" was treated with YTHDC1 inhibitors, demonstrating **dose-dependent effects on viability** (**B**) and **apoptosis** (**C**). **D.** Cell line "X" was treated for 24 or 48 hours with Compound A. Western blot analysis demonstrates **dose-dependent reduction in L-MYC and ASCL1**, with concomitant **induction of the apoptotic marker, Cleaved caspase** 3. **E.** Quantification of the Western blot data from (D) demonstrating **inverse correlation between L-MYC & ASCL1 expression levels and induction of apoptosis.**

Tumor growth inhibition and reduction of MYC & ASCL1 *in vivo*

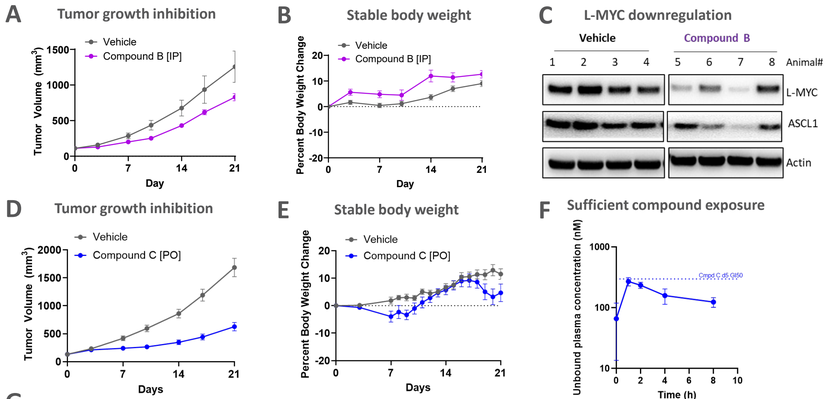


Figure 7. A. BALB/c nude mice were xenografted with cell line "X" human SCLC cells and treated with Vehicle or Compound B. Tumor volume measurements demonstrate **tumor growth inhibition with Compound B**. **B.** Percent body weight change shows Compound B was **well-tolerated C.** Western blot demonstrates **suppression of L-MYC in tumor samples** from animals treated with Compound B in (A). **D.** CB17/SCID mice were xenografted with cell line "Y" human SCLC cells and treated with Vehicle or Compound C. Tumor volume measurements **demonstrate tumor growth inhibition with Compound C dosed orally. E.** Percent body weight change shows treatment was **well-tolerated**. **F.** PK data from (D) was collected after last dose. **G.** At the end of the study, tumor samples were collected and Western blot performed, demonstrating **downregulation of ASCL1**.