Selective inhibition of the m^{6}A RNA reader, YTHDC1, as a novel therapeutic strategy for MYC-driven Acute Myeloid Leukemia

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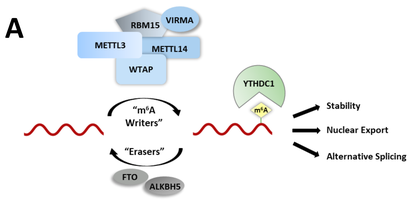
YTHDC1 is a targetable driver of MYC-driven AML

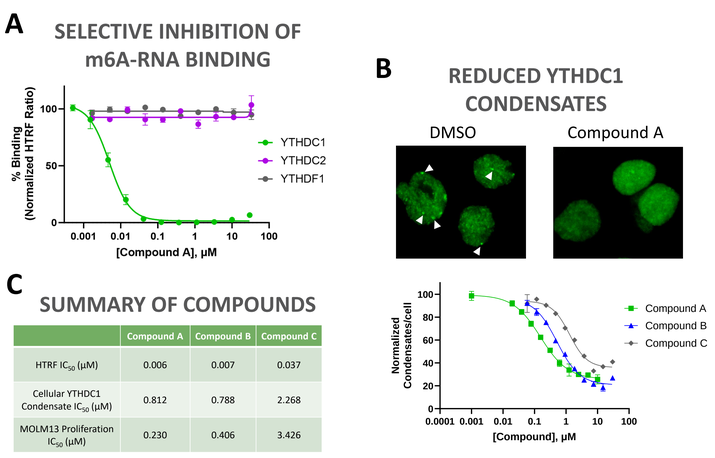


- YTHDC1 is a nuclear epigenetic "reader" of m^{6}A-modified RNA
- YTHDC1 forms nuclear condensates by binding to m^{6}A RNAs and through its intrinsically disordered regions (IDRs)
- YTHDC1 condensates drive the proliferation of Acute Myeloid Leukemia (AML) cells through sequestration of onco-protective m^{6}A RNAs (i.e., *MYC*) thus protecting them from degradation[1]

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. Dependency and MYC target enrichment data were downloaded from Depmap.org (Data release 24Q2).

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

Selective YTHDC1 small molecule inhibitor dissolves YTHDC1 condensates in AML cells



C

SUMMARY OF COMPOUNDS

	Compound A	Compound B	Compound C
HTRF IC_{50} (µM)	0.006	0.007	0.037
Cellular YTHDC1 Condensate IC_{50} (µM)	0.812	0.788	2.268
MOLM13 Proliferation IC_{50} (µM)	0.230	0.406	3.426

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 the YTH domain of YTHDC1, but not with other YTH-domain family members. **B.** (Top) KG1 AML cells harboring Emerald-YTHDC1 (endogenous) were imaged for condensates 24h after treatment with DMSO or 1.25 µM Compound A. (Bottom) Experiment performed as in Top panel, and quantified dose-response of Compounds A & B is plotted. **C.** Table of properties for Compounds A, B & C.

YTHDC1 inhibition reduces AML cell viability and stemness, and downregulates MYC expression

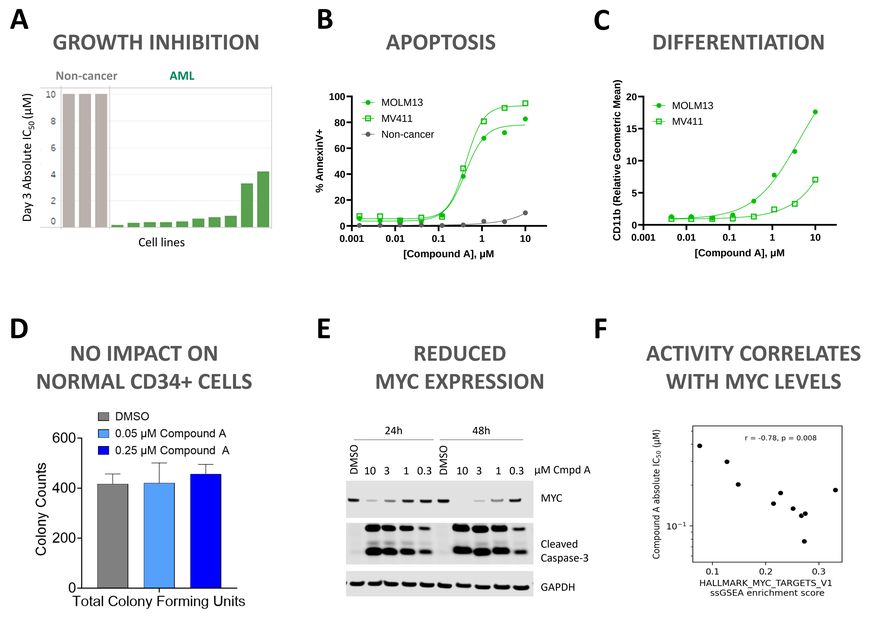


Figure 3. A. A panel of non-cancer or AML cell lines was treated with dose-titration of Compound A, and relative growth was evaluated after 3 days with Cell-titer glo. Absolute IC_{50} values are plotted. **B.** Cells were treated as in (A) for 5 days, and the percentage of Annexin V positive cells was measured by flow cytometry **C.** Cells were treated with Compound A, and myeloid differentiation was evaluated by flow cytometry of CD11b positive cells. **D**. Fresh cord blood was obtained and CD34+ cells were enriched with magnetic bead selection. Cells were plated in methylcellulose with cytokines and DMSO or Compound A for 7 days, and colony forming units (CFU) were counted. Compound A had no effect on the total number of CFU, and no significant differences were detected in the differentiated phenotypic colony subtypes in response to the treatment. **E**. MV411 cells were treated with Compound A and Western blots performed at the indicated timepoints, demonstrating reduction in MYC and induction in the apoptotic marker, Cleaved Caspase-3. **F.** Sensitivity of AML cell lines to Compound A correlates with ssGSEA enrichment of MYC target genes at baseline. MYC target enrichment data were downloaded from Depmap.org (Data release 24Q2). **G.** NOD/SCID mice were inoculated with MOLM13-Luc human leukemia cells and treated with Vehicle or Compound C. Bioluminescent images taken after 10 days of dosing, demonstrating reduced tumor burden in treated animals. **H.** Kaplan-Meier plot demonstrating significant survival benefit in animals from (G) treated with Compound C as compared to vehicle-treated animals. **I.** Gene set enrichment analysis (GSEA) demonstrating enrichment of MYC target gene sets from MOLM13-Luc tumors treated with Compound C compared to vehicle-treated animals.

PK/PD with an orally bioavailable molecule demonstrates dose- & exposure-dependent reduction of MYC *in vivo*

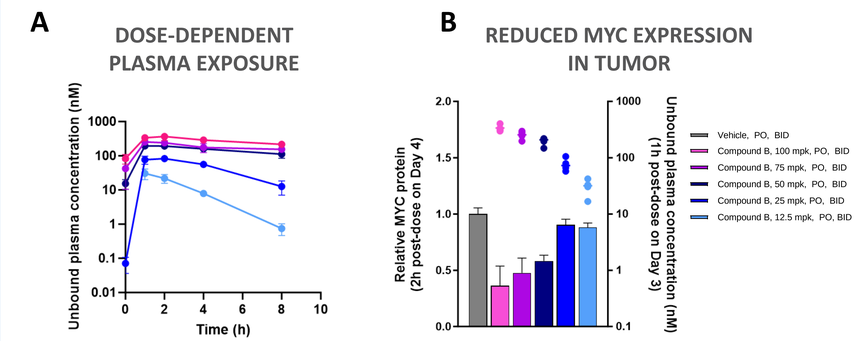


Figure 4. A. BALB/c nude mice were inoculated with MOLM13-Luc cells and treated with Vehicle or Compound B. Plasma was collected from animals after 3 days of dosing, and unbound concentration of Compound B was measured. Free compound exposure is dose-dependent. **B.** Animals from (A) and were sacrificed on day 4. Tumor samples were collected and Western blot for MYC and Actin was performed. Dose-dependent MYC suppression correlates with unbound compound exposure in plasma, demonstrating PK/PD correlation.

Dose-dependent AML tumor growth inhibition with well-tolerated & orally-dosed YTHDC1 inhibitor *in vivo*

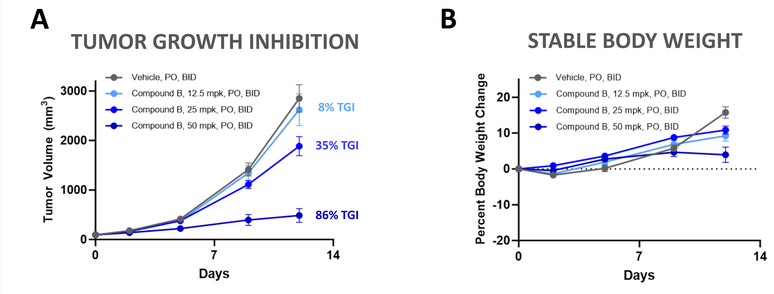


Figure 5. A. BALB/c nude mice were inoculated with MOLM13-Luc human leukemia cells and treated with Vehicle or Compound B. Tumor volume measurements demonstrate dose-dependent tumor growth inhibition with Compound B. **B.** Percent body weight change from (A) shows treatment with Compound B was well-tolerated.

Summary

- Transition Bio has developed novel, selective, orally bioavailable small molecule inhibitors of epigenetic reader, YTHDC1
- YTHDC1 inhibition disrupts YTHDC1 condensate formation in AML cells, leading to reduction in levels of MYC protein and MYC target gene expression
- This leads to dose-dependent reduction of AML cell proliferation, and induces both apoptosis and myeloid differentiation, while sparing normal cells from CD34+ cord blood
- YTHDC1 inhibition leads to reduction of MYC protein in an exposure-dependent manner *in vivo*
- Orally-dosed YTHDC1 inhibitor demonstrates dose-dependent tumor growth inhibition in an AML CDX model at well-tolerated doses, providing proof-of-concept for YTHDC1 condensate inhibitors as treatment for AML and other MYC-driven cancers