# Pharmacological inhibition of the m6A-RNA reader, YTHDC1, as a novel approach to targeting biomolecular condensates in cancer

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Transition Bio

Abstract 3231

Introduction
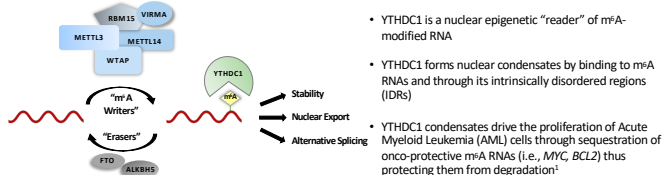
- YTHDC1 is a nuclear epigenetic "reader" of m6A-modified RNA
- YTHDC1 forms nuclear condensates by binding to m6A RNAs and through its intrinsically disordered regions (IDRs)
- YTHDC1 condensates drive the proliferation of Acute Myeloid Leukemia (AML) cells through sequestration of onco-protective m6A RNAs (i.e., MYC, BCL2) thus protecting them from degradation[1]
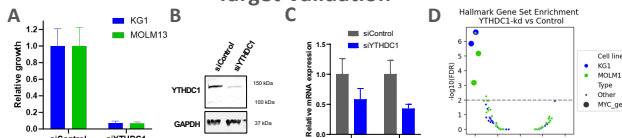
Target Validation

Figure 1. A. YTHDC1 is required for AML cell viability. KG1 and MOLM13 cells were treated with siControl or siYTHDC1 and growth was measured after 5 days using Cell-Titer Glo (CTG). B. KG1 cells from (A) were collected 48 hours after electroporation, and YTHDC1 levels were assessed by Western Blot. C. RNA was extracted from KG1 cells from (A) and levels of MYC or BCL2 transcripts were detected by qRT-PCR. D. Samples from (A) were collected 48 hours after knockdown and RNA-seq was performed. Hallmark gene sets enriched in siYTHDC1- vs. siControl-treated cells are plotted.

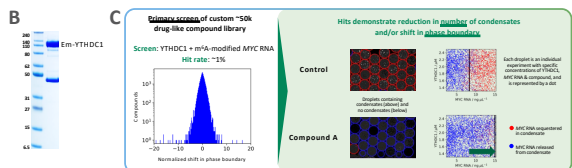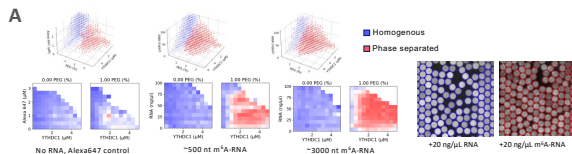## PhaseScan: Microfluidics-based screening for modulators of phase separation[2]

Figure 2. A. MYC m6A-RNA induces liquid-phase separation of YTHDC1. Phase diagrams (left) and PhaseScan microfluidics droplet images & phase diagrams (right) in the presence or absence of MYC m6A-RNA. B. Purified Emerald-YTHDC1 from Sf9 cells for PhaseScan screening. C. Primary PhaseScan screening of ~50k small molecule compound library identified compounds that reduce the number of condensates triggered by MYC m6A-RNA and/or shift the phase boundary.

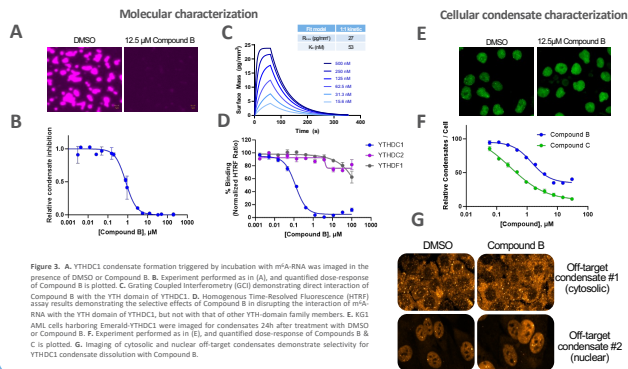## YTHDC1 inhibitors selectively dissolve condensates through disrupting m6A-RNA binding

Figure 3. A. YTHDC1 condensate formation triggered by incubation with m6A-RNA was imaged in the presence of DMSO or Compound B. B. Experiment performed as in (A), and quantified dose-response of Compound B is plotted. C. Grating Coupled Interferometry (GCI) demonstrating direct interaction of Compound B with the YTH domain of YTHDC1. D. Homogenous Time-Resolved Fluorescence (HTRF) assays demonstrating the selective effects of Compound B in disrupting the interaction of m6A-RNA with the YTH domain of YTHDC1, but not with that of other YTH domain family members. E. KG1 AML cells harboring Emerald-YTHDC1 were imaged for condensates 24h after treatment with DMSO or Compound B. F. Experiment performed as in (E), and quantified dose-response of Compounds B & C is plotted. G. Imaging of cytosolic and nuclear off-target condensates demonstrate selectivity for YTHDC1 condensate dissolution with Compound B.

YTHDC1 inhibitors impact AML cell viability, stemness, and oncogene expression
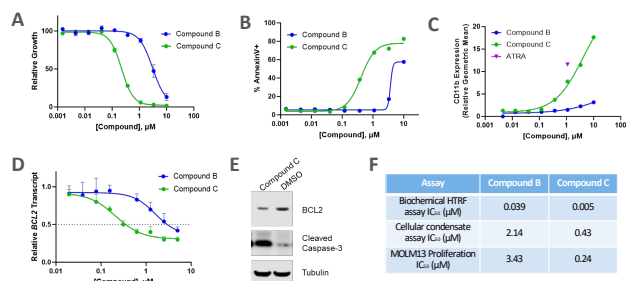

Figure 4. A. MOLM13 (AML) cells were treated YTHDC1 inhibitors for 3 days and effects on cell proliferation were measured by CTG. B-C. MOLM13 AML cells were treated with increasing concentrations of YTHDC1 inhibitors and apoptosis (B) or differentiation (C) was measured after 5 days by flow cytometric quantification of Annexin V positivity or CD11b expression, respectively. For differentiation experiments, 1 µM ATRA was used as a positive control. D. MOLM13 cells were treated for 24 hours with Compounds B-C and relative expression of BCL2 was measured by qRT-PCR. E. MOLM13 cells were treated for 24 hours with DMSO or 0.3 µM Compound C, and levels of BCL2 and Cleaved Caspase-3 were measured by Western blot. F. Table comparing the biochemical and cellular potencies of Compounds B-C.

YTHDC1 inhibition results in reduced AML tumor burden and prolonged survival in multiple in vivo models
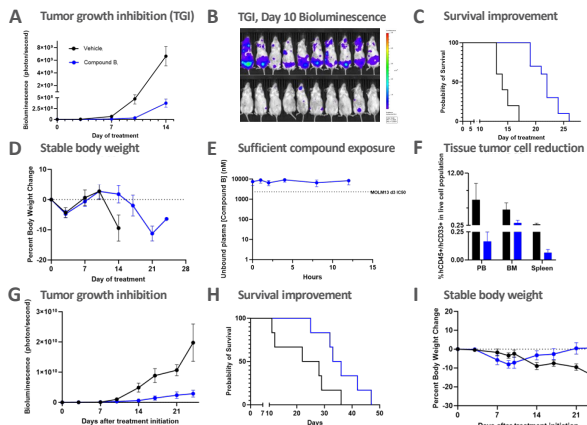

Figure 5. A. NOD/SCID mice were inoculated with MOLM13-Luc human leukemia cells and treated with Vehicle or Compound B. Bioluminescence measurements quantifying tumor burden over time demonstrate tumor growth inhibition with Compound B. B. Bioluminescent images from (A) taken after 10 days of dosing, demonstrating reduced tumor burden in animals treated with Compound B. C. Kaplan-Meier plot demonstrating significant survival benefit in animals from (A) treated with Compound B as compared to vehicle-treated animals. D. Percent body weight change from (A) showing that treatment with Compound B was well-tolerated in mice. E. Plasma was collected from animals from (A), and unbound concentration of Compound B was measured at day 12 after treatment initiation. Free compound exposure exceeds the proliferation IC50 for MOLM13. F. Animals were inoculated and treated as in (A) and were sacrificed on day 12 and human AML cells from peripheral blood (PB), bone marrow (BM), and spleen were quantified by flow cytometry of human CD45+/ human CD33+ cells. G. NOG mice were inoculated with MV411-Luc human leukemia cells and treated with Vehicle or Compound B. Bioluminescence measurements quantifying tumor burden over time demonstrate tumor growth inhibition with Compound B. H. Kaplan-Meier plot demonstrating significant survival benefit in animals from (G) treated with Compound B as compared to vehicle-treated animals. H. Percent body weight change from (G) showing that treatment with Compound B was well-tolerated in mice.

Summary

- YTHDC1 is an epigenetic nuclear reader of m6A-modified RNA that plays a critical role in the survival of AML cells
- PhaseScan technology provides a novel approach to screening modulators of liquid-liquid phase separation
- Transition Bio has identified potent and selective inhibitors of YTHDC1, which dissolve condensates at the molecular and cellular level
- YTHDC1 inhibition leads to downregulation of important oncogenic transcripts (e.g., MYC, BCL2) and ultimately causes AML cell death and differentiation
- YTHDC1 inhibition causes reduction of tumor burden and prolonged survival in in vivo models of AML

References

[1] Cheng, Y., et al. N6-Methyladenosine on mRNA facilitates a phase-separated nuclear body that suppresses myeloid leukemic differentiation. Cancer Cell. 2021. 39, 958–972.
[2] Arter, W.E., et al. Biomolecular condensate phase diagrams with a combinatorial microdroplet platform. Nature Communications. 2022. 13, 7845.