Discovery of first-in-class YTHDC1 small molecule inhibitors for the treatment of MYC-driven cancers

Presentation # 7101

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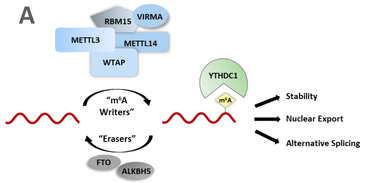
YTHDC1 is a targetable driver of MYC-driven cancers



- 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 (such as *MYC*) thus protecting them from degradation[1]

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

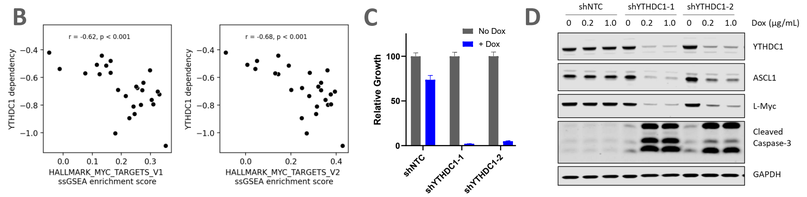
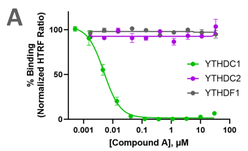
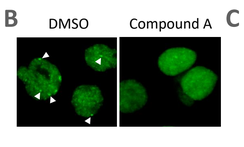


Figure 1. A. Schematic demonstrating the RNA processing role of YTHDC1 elicited through m^{6}A-RNA binding. **B. YTHDC1 dependency correlates with ssGSEA enrichment of MYC target gene expression** across a panel of AML cell lines. Data downloaded from Depmap.org (Data release 24Q2). **C.** Knockdown of YTHDC1 in small cell lung cancer (SCLC) SCLC #1 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**.

Potent & selective YTHDC1 small molecule inhibitors dissolve YTHDC1 condensates



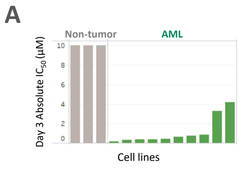


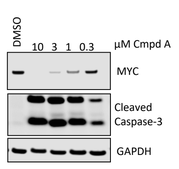
C

Compound	A	B	C	D
YTHDC1 HTRF IC_{50} (μM)	0.006	0.037	0.009	0.013
Cellular YTHDC1 Condensate IC_{50} (μM)	0.647	2.268	0.790	0.400
MOLM13 Viability IC_{50} (μM)	0.115	1.254	0.201	0.236
NEPC #1 Prolif GI_{50} (μM)	0.226	-	0.436	0.253

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 Compound A. **C.** Table of biochemical and cellular potencies for Compounds A, B, C & D.

YTHDC1 small molecule inhibitors downregulate MYC & cause AML cell death





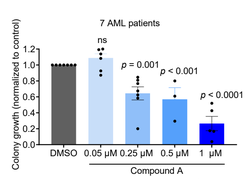


Figure 3. A. Compound A impacts proliferation of a panel of AML cell lines, with **no effect on non-cancer cells**. Absolute IC_{50} values are plotted after a 3-day CTG assay. **B.** MV411 cells were treated with Compound A for 48 hours and Western blot was performed, demonstrating **reduction in MYC and induction of the apoptotic marker, Cleaved Caspase-3**. **C.** Primary samples from AML patients were treated with Compound A and their ability to form colonies assessed; **a clear dose-dependent reduction in CFUs** was seen.

YTHDC1 inhibition leads to dramatic tumor growth inhibition in AML as single agent…

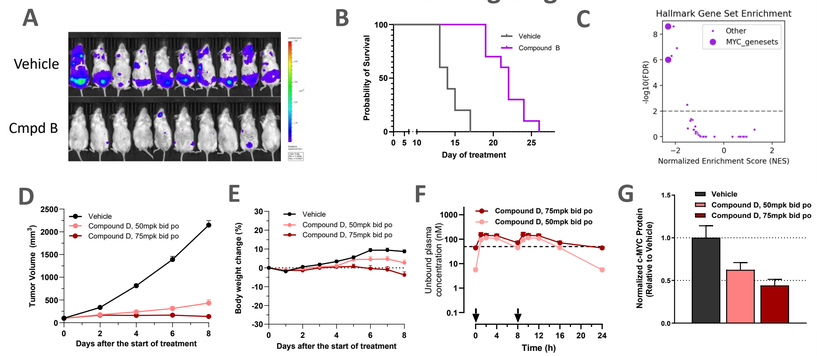


Figure 4. A. NOD/SCID mice were inoculated with MOLM13-Luc human leukemia cells and treated with Vehicle or Compound B. Bioluminescent images taken after 10 days of dosing, demonstrated **reduced tumor burden in treated animals**. **B.** Kaplan-Meier plot demonstrates **significant survival benefit in 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-G.** BALB/c nude mice were inoculated subcutaneously with MOLM13-Luc cells and treated with Vehicle or Compound D. **D.** Tumor volume measurements demonstrate **dose-dependent tumor growth inhibition with Compound D**. **E.** Percent body weight change shows treatment with **Compound D was well-tolerated**. **F.** Unbound plasma PK levels of Compound D demonstrate coverage over unbound MOLM13 IC_{50} (dotted line). **G.** Tumor samples were collected, and Western blot was performed. **Dose-dependent cMYC suppression correlated with unbound compound exposure in plasma, demonstrating PK/PD correlation**.

……and tumor regression in combination with SoC agents

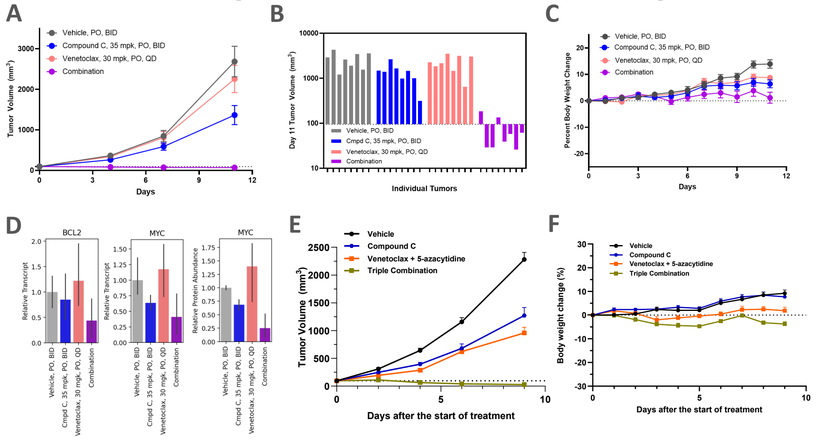


Figure 5. A. BALB/c nude mice were inoculated subcutaneously with MOLM13-Luc cells and treated with vehicle, suboptimal doses of Compound C or Venetoclax, alone or in combination to test synergy. Tumor volume measurements demonstrate **synergistic tumor growth inhibition with the combination treatment**. **B.** Waterfall plot demonstrated **tumor regression with the combination treatment** of Compound C + Venetoclax. **C.** Percent bodyweight change shows Compound C, Venetoclax, and the combination treatments were **well-tolerated**. **D. Reduction of *BCL2* and *cMYC* transcripts (RT-qPCR), or cMYC protein (Western blot), in tumor samples** following Compound C treatment, and further reduction upon combination treatment with Venetoclax. **E-F.** BALB/c nude mice were inoculated subcutaneously with MOLM13-Luc cells and treated with Vehicle, a suboptimal dose of Compound C, the Venetoclax + 5-azacytidine doublet, or the triplet combination to test synergy. Tumor volume measurements (**E**) demonstrate **synergistic tumor growth inhibition with the combination treatment leading to regressions,** whilst percent bodyweight change (**F**) shows the treatments were well-tolerated.

YTHDC1 inhibition leads to robust tumor growth inhibition in neuroendocrine prostate cancer as single agent…

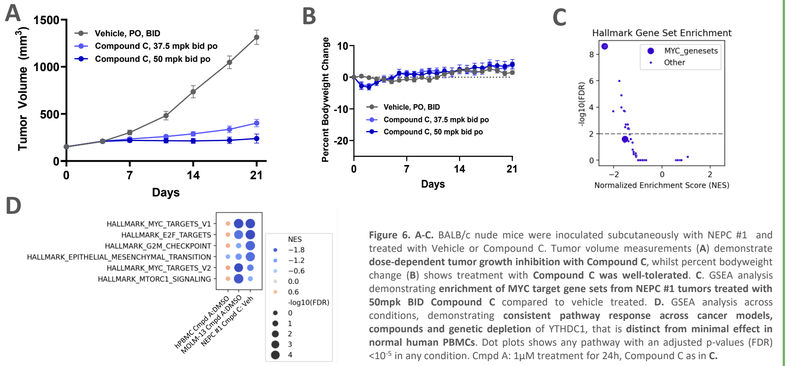


Figure 6. A-C. BALB/c nude mice were inoculated subcutaneously with NEPC #1 and treated with Vehicle or Compound C. Tumor volume measurements (**A**) demonstrate **dose-dependent tumor growth inhibition with Compound C**, whilst percent bodyweight change (**B**) shows treatment with **Compound C was well-tolerated**. **C**. GSEA analysis demonstrating **enrichment of MYC target gene sets from NEPC #1 tumors treated with 50mpk BID Compound C** compared to vehicle treated. **D.** GSEA analysis across conditions, demonstrating **consistent pathway response across cancer models, compounds and genetic depletion** of YTHDC1, that is **distinct from minimal effect in normal human PBMCs**. Dot plots shows any pathway with an adjusted p-values (FDR) $<10^{-5}$ in any condition. Cmpd A: 1μM treatment for 24h, Compound C as in **C.**

……and tumor regression in combination with SoC agents

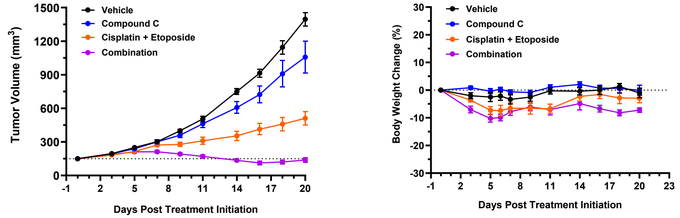


Figure 7. A-B. BALB/c nude mice were inoculated subcutaneously with NEPC #1 and treated with Vehicle, a suboptimal dose of Compound C, the Cisplatin + Etoposide doublet, or the triplet combination to test synergy. Tumor volume measurements (**A**) demonstrate **synergistic tumor growth inhibition with the combination treatment leading to tumor regression,** whilst percent bodyweight change (**B**) shows the treatments were well-tolerated.

Summary

- YTHDC1 is a targetable driver of MYC-driven AML & neuroendocrine prostate cancer
- Transition Bio has developed **potent, selective, and orally-bioavailable small molecule inhibitors of YTHDC1 and have nominated a development candidate**
- YTHDC1 inhibitors dissolve biomolecular condensates containing m6A-modified RNAs
- YTHDC1 inhibition leads to tumor stasis/regression in AML & NEPC models as a single agent
- YTHDC1 inhibition demonstrates synergistic response with SoC agents in AML and NEPC and the combination are well-tolerated.