Pharmacological inhibition of YTHDC1 biomolecular condensates as a novel approach to targeting L-MYC driven small cell lung cancer

TransitionBio

Abstract 244

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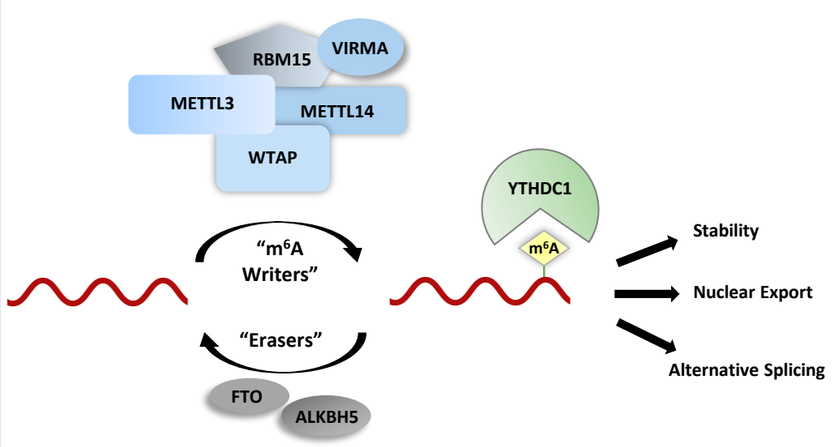
Introduction to YTHDC1



- Nuclear epigenetic "reader" of m6A-modified RNA
- Forms nuclear condensates by binding to m6A RNAs and through its intrinsically disordered regions (IDRs)
- These condensates drive proliferation of cancer cells through sequestration of onco-protective m6A RNAs (*i.e.*, *MYC, BCL2*) thus protecting them from degradation[1]

Validation of YTHDC1 dependency in small cell lung cancer (SCLC)

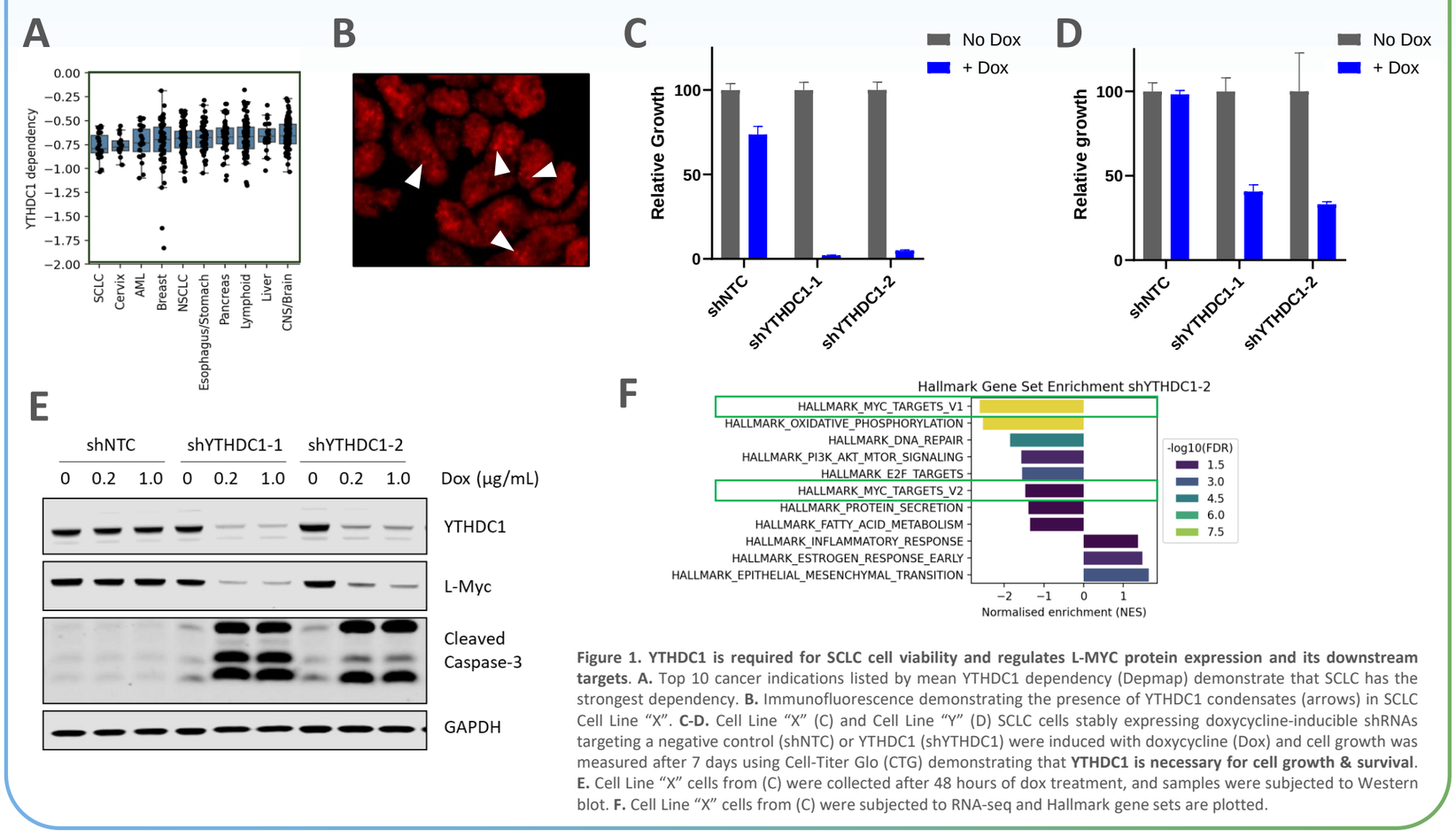


Figure 1. YTHDC1 is required for SCLC cell viability and regulates L-MYC protein expression and its downstream targets. **A.** Top 10 cancer indications listed by mean YTHDC1 dependency (Depmap) demonstrate that SCLC has the strongest dependency. **B.** Immunofluorescence demonstrating the presence of YTHDC1 condensates (arrows) in SCLC Cell Line "X". **C-D.** Cell Line "X" (C) and Cell Line "Y" (D) SCLC cells stably expressing doxycycline-inducible shRNAs targeting a negative control (shNTC) or YTHDC1 (shYTHDC1) were induced with doxycycline (Dox) and cell growth was measured after 7 days using Cell-Titer Glo (CTG) demonstrating that **YTHDC1 is necessary for cell growth & survival**. **E.** Cell Line "X" cells from (C) were collected after 48 hours of dox treatment, and samples were subjected to Western blot. **F.** Cell Line "X" cells from (C) were subjected to RNA-seq and Hallmark gene sets are plotted.

Summary

- YTHDC1 plays a critical role in small-cell lung cancer (SCLC) cell survival
- Transition Bio has identified potent and selective small molecule YTHDC1 inhibitors that dissolve condensates[2]
- YTHDC1 inhibition leads to downregulation of L-MYC and ultimately causes SCLC cell death
- Small molecule inhibition of YTHDC1 leads to tumor growth inhibition *in vivo*, and provides a novel therapeutic approach for the treatment of MYC-driven SCLC

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.

YTHDC1 small molecule inhibitors dissolve condensates, downregulate L-MYC, and cause SCLC cell death

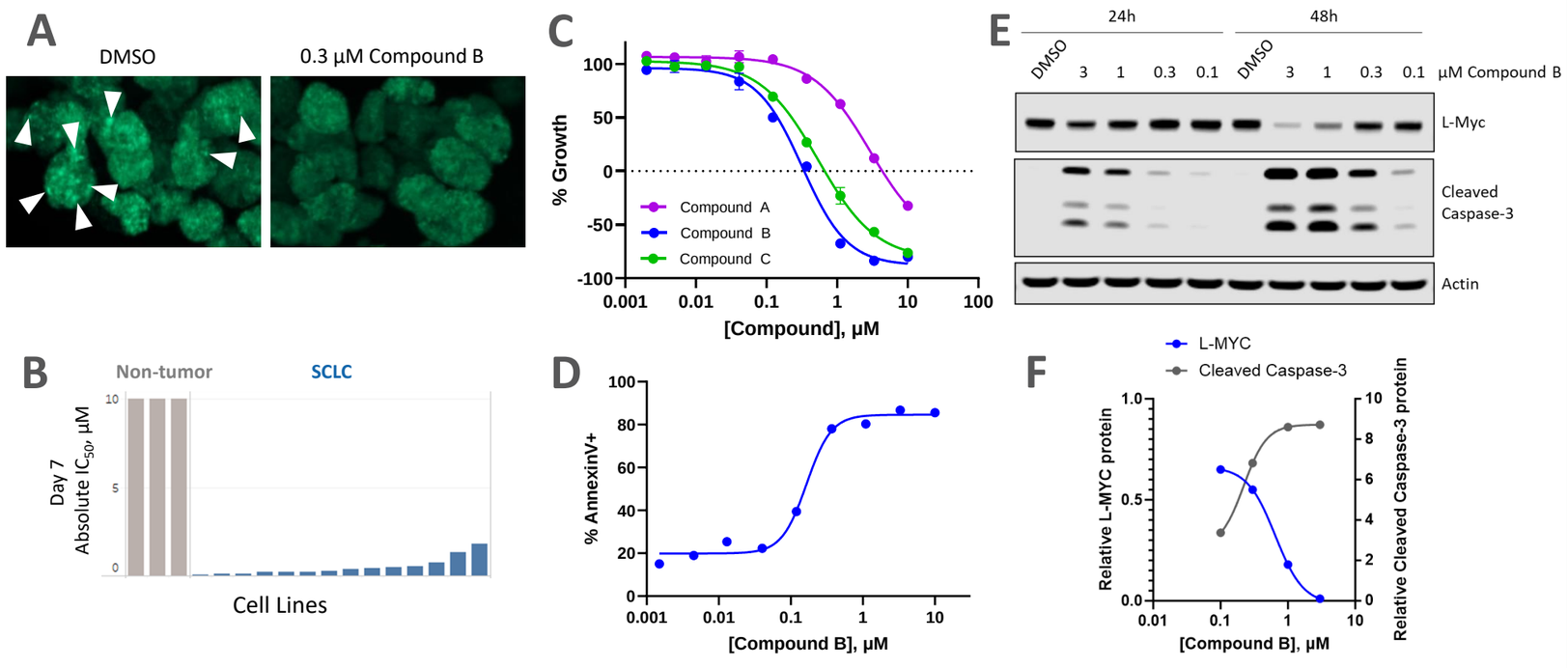


Figure 2. A. Dissolution of YTHDC1 condensates (arrows) by Compound B in SCLC Cell Line "X". **B.** A panel of SCLC and immortalized non cancer cells were treated with Compound B for 7 days and effects on cell proliferation were measured. **C-D.** SCLC Cell Line "X" cells were treated with increasing concentrations of YTHDC1 inhibitors and proliferation (**C**) or apoptosis (**D**) was measured after 5 days by CTG or flow cytometric quantification of Annexin V, respectively. **E.** Cell Line "X" cells were treated for 24 or 48 hours with Compound B. Western blot analysis demonstrates dose-dependent reduction in L-MYC, with concomitant induction of the apoptotic marker, Cleaved caspase 3. **F.** Quantification of the Western blot data from (D) at the 48h timepoint demonstrating **inverse correlation between L-MYC expression levels and induction of apoptosis.**

YTHDC1 inhibition with an orally bioavailable small molecule results in SCLC tumor growth inhibition *in vivo*

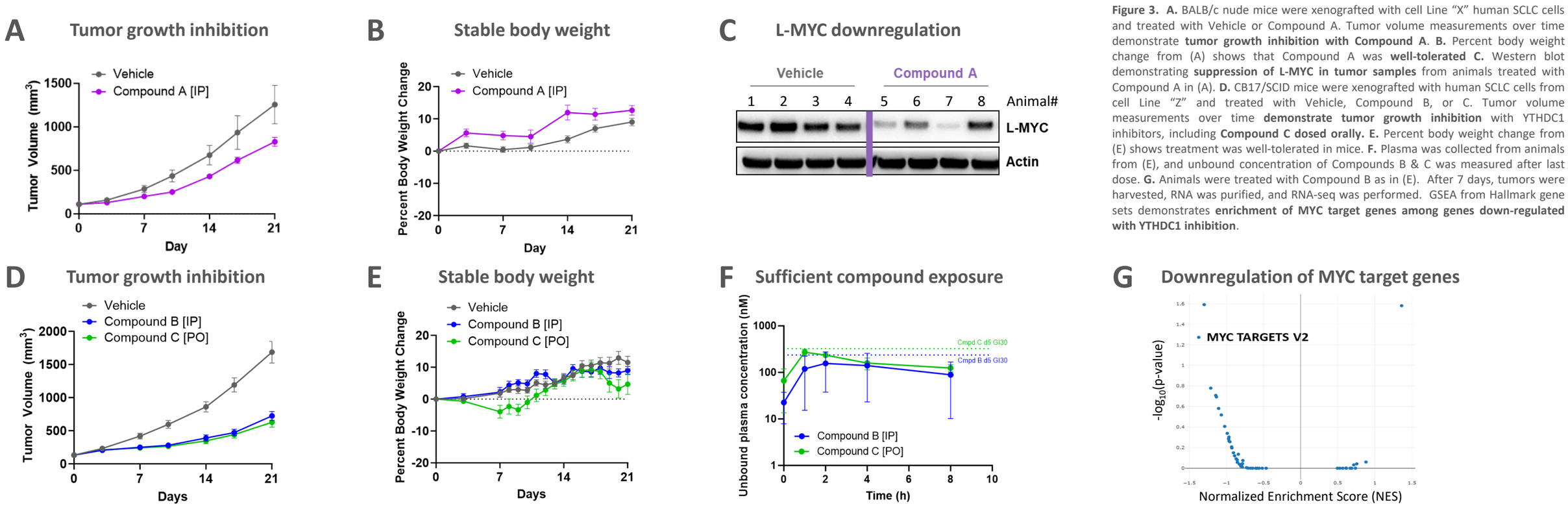


Figure 3. A. BALB/c nude mice were xenografted with cell Line "X" human SCLC cells and treated with Vehicle or Compound A. Tumor volume measurements over time demonstrate **tumor growth inhibition with Compound A**. **B.** Percent body weight change from (A) shows that Compound A was **well-tolerated C.** Western blot demonstrating **suppression of L-MYC in tumor samples** from animals treated with Compound A in (A). **D.** CB17/SCID mice were xenografted with human SCLC cells from cell Line "Z" and treated with Vehicle, Compound B, or C. Tumor volume measurements over time **demonstrate tumor growth inhibition** with YTHDC1 inhibitors, including **Compound C dosed orally. E.** Percent body weight change from (E) shows treatment was well-tolerated in mice. **F.** Plasma was collected from animals from (E), and unbound concentration of Compounds B & C was measured after last dose. **G.** Animals were treated with Compound B as in (E). After 7 days, tumors were harvested, RNA was purified, and RNA-seq was performed. GSEA from Hallmark gene sets demonstrates **enrichment of MYC target genes among genes down-regulated with YTHDC1 inhibition**.