Condensate modulator sequesters β-Catenin into depot condensates and demonstrates competitive anti-tumor activity in animal models of colorectal cancer

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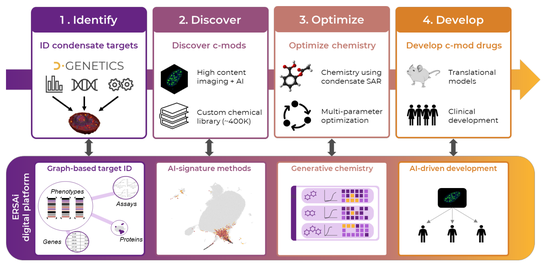
Introduction

Constitutive activation of β-Catenin is a well-known driver of malignancy. However, traditional drug discovery approaches have proven challenging and largely unsuccessful in identifying therapeutic agents to modulate the function of β-Catenin. Biomolecular condensates have recently been demonstrated to play key roles in regulating most cellular processes and biological pathways by compartmentalizing biomolecules in membrane-less organelles, thus lending novel opportunities to drug previously "undruggable" targets (1).

Here, we leverage condensate biology to discover small molecules that reverse the hyperactive function of β-Catenin in colorectal cancer by entrapping it into depot condensates (2). We developed a high-throughput phenotypic assay that identifies β-Catenin condensate-modifying compounds (c-mods). Through condensate screening and functional secondary assays, we identified and optimized c-mods that: sequester of β-Catenin into depots, induce selective cancer cell killing, and reverse oncogenic β-Catenin specific gene expression programs.

C-mods identified in our screen are effective in genetically diverse colorectal cancers and a range of Wnt-associated cancers, providing the opportunity to treat a broad patient population. Furthermore, oral dosing of a lead c-mod demonstrates competitive tumor growth inhibition as a single agent in xenograft and PDX models of colorectal cancer. Taken together, these results highlight the promise of condensate biology in drugging previously intractable high-value targets in oncology and developing novel treatments for patients suffering from diseases of high unmet need.

Dewpoint's state of the art c-mod discovery platform



- Condensate target ID
- Zero-shot hit discovery
- Functional prediction
- Closed-loop SAR
- >300M images
- >370K compounds
- >2B cell phenotypes

AI-powered end-to-end experimental platform.

References

1. Mitrea, D.M., Mittasch, M., Gomes, B.F., Klein, I.A., Murcko, M.A. Modulating biomolecular condensates: a novel approach to drug discovery. Nat Rev Drug Discov 2022;21:841–862.
2. Bernkopf D. B., Daum G., Brückner M., Behrens J. Sulforaphane inhibits growth and blocks Wnt/β-catenin signaling of colorectal cancer cells. Oncotarget 2018;9:33982-33994.
3. Zamudio, A.V., Dall'Agnese, A., Henninger, J.E., Manteiga, J.C., Afeyan, L.K., Hannett, N.M., et al., Mediator Condensates Localize Signaling Factors to Key Cell Identity Genes. Mol Cell 2019;5:753-766.e6

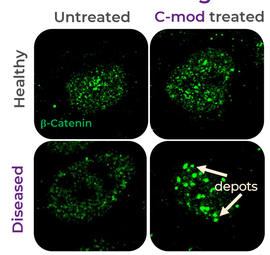
Therapeutic approach

Exploit principles of condensate biology to treat colorectal cancer

- Constitutive activation of β-Catenin in colorectal cancer causes transcriptional reprogramming and uncontrolled cell proliferation
- C-mods modulate β-Catenin activation by inducing nuclear depot formation
- Immunofluorescent (IF) of β-Catenin is used to monitor depot formation
- The quantifiable phenotype enables high throughput, high content imaging screening and structure-activity relationship studies to drive drug discovery

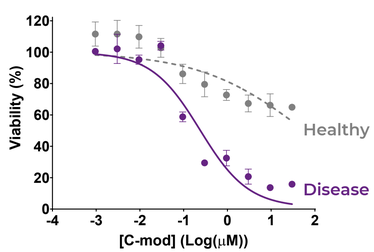
Selective inhibition of β-Catenin in colorectal cancer cells

Sequestration of β-Catenin into condensates in malignant cells



IF images of malignant vs. healthy colon cells upon c-mod treatment.

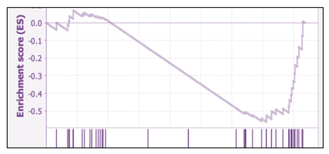
Selective cancer cell killing



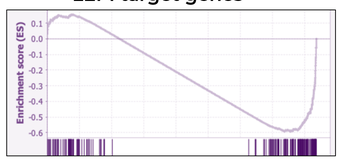
Cell viability of malignant vs. healthy colon cells upon c-mod treatment.

Specific modulation of β-Catenin driven genes

Wnt/β-Catenin signaling



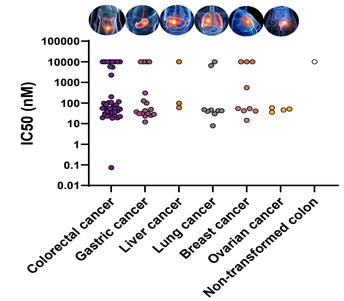
LEF1 target genes



GSEA plots showing selective down-regulation of Wnt/β-Catenin and LEF1 target genes in colorectal cancer cells when treated with depot-inducing c-mod.

Potency across indications and anti-tumor activity in vivo

β-Catenin c-mod is active in a broad-range of Wnt-associated cancers



Cell viability assay of 99 cancer cell lines upon lead series c-mod treatment.

β-Catenin c-mod demonstrates anti-tumor activity *in vivo*

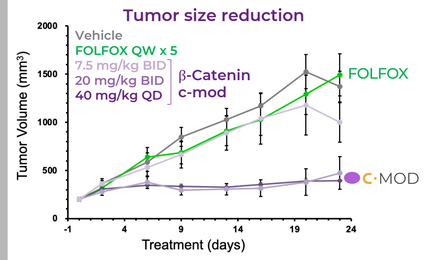
Model	β-Catenin pathway alteration	c-mod response
CRC PDX	High wt β-Catenin; APC mut; Axin2 high	Y
HCT116	β-Catenin/KRAS GoF, Axin2 LoF	Y
COLO320	APC/TP53 LoF	Y
DLD1	APC mut, KRAS GoF	Y
CRC PDX	High wt β-Catenin; APC mut; KRAS GoF	Y
CRC PDX	High wt β-Catenin; APC mut; KRAS GoF	Y
NCI H460	KRAS GoF	Y

*CRC = colorectal; LoF = loss of function; GoF = gain of function; wt = wild type; mut = mutant

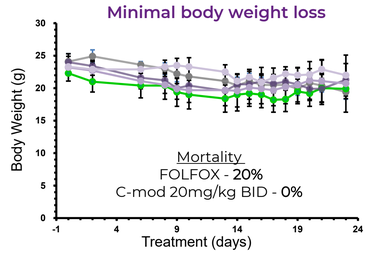
Profound effects in colorectal cancer PDX model

Lead series c-mod induces tumor growth stasis in PDX model derived from heavily pretreated CRC patient with high β-Catenin expression

Tumor size reduction



Minimal body weight loss



Summary

By building on principles of condensate biology and employing high-throughput phenotypic screening we have identified novel β-Catenin-specific small molecule inhibitors (c-mods) that demonstrate:

- Robust modulation of Wnt/β-Catenin regulated signaling
- Potent cancer cell-specific cytotoxicity across a broad range of Wnt-driven cancer types
- *In vivo* anti-tumor activity in multiple PDX and CDX cancer models

Together these data underline the potential of leveraging condensate biology to drive drug discovery and supports the continued development of β-Catenin c-mods for the treatment of Wnt-driven cancers.

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