Targeting colorectal cancer by sequestration of beta catenin in nuclear condensates

Kip West[1], Costa Salojin[1], Doug Baumann[1], Adam Talbot[1], Thomas Durand-Reville[1], Ugo Zanelli[2], Sidharth Sirdeshmukh[1], Isaac Klein[1], Ann Boija[1]

[1]*Dewpoint Therapeutics, Boston MA, 02210,* [2]*Dewpoint Therapeutics GMBH, Frankfurt, Germany*

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Abstract

Background: Many malignancies are driven by aberrant activation of the beta catenin signaling pathway, notably colorectal cancer (CRC). Despite extensive biological insights into the underlying mechanism, therapeutic approaches targeting it have been largely unsuccessful, deeming beta catenin 'undruggable'. Recent research has shed light on the essential role of biomolecular condensates in orchestrating numerous cellular processes and biological pathways through membraneless compartmentalization of biomolecules. In this study, we apply emerging insights into condensate biology to discover small molecules, condensate-modifying compounds (c-mods), that inhibit the transcriptionally hyperactive beta catenin in CRC via sequestration within nuclear depot condensates.

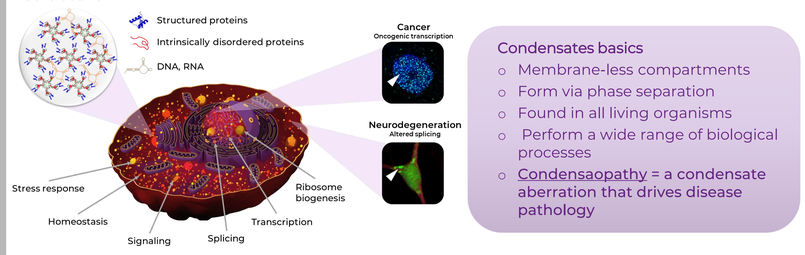
Methods: We employed high content, high throughput phenotypic screening to identify small molecules that drive beta catenin into nuclear puncta. Cancer cell proliferation was quantified using CTG assays and gene expression profiles using qPCR assays. C-mods were profiled in xenograft and PDX models of CRC.

Results: We have identified c-mods that induce beta catenin depot condensates. We demonstrate that the c-mod acts on pathway and selectively kills cancer cells both in vitro and ex vivo. Oral dosing of the c-mod, as a single agent, in xenograft and PDX mouse models of CRC exhibits competitive tumor growth inhibition. In PK/PD studies in CRC xenografts, c-mod tumor exposure correlates with dose-dependent modulation of beta catenin-driven gene transcription and, IHC of tumors from these studies demonstrate beta catenin depot formation. Finally, combination treatment of the c-mod with the standard of care enhanced the anti-tumor activity in the same model, highlighting the potential for first-line therapeutic intervention in CRC.

Conclusion: These findings underscore the potential of condensate biology in targeting challenging high-value oncology targets, historically deemed 'undruggable', and pioneering novel medicines for cancer patients with critical unmet medical needs.

Background

Cellular contents organize in time and space into biomolecular condensates to coordinate a wide diversity of physiological processes. Condensates serve as reaction crucibles, signaling hubs, or storage compartments that specifically concentrate molecular communities of proteins and RNA involved in shared processes while excluding other. **In many cases, condensate abnormalities can explain the pathophysiology of poorly understood or complex multifactorial diseases.** Diseases such as neurodegeneration and cancer, for example, are often the result of dysregulation of multiple pathways and biomolecules. Condensates can function as central nodes of dysfunction in disease, where they integrate and regulate multiple pathogenic biomolecules and pathways in a single structure.

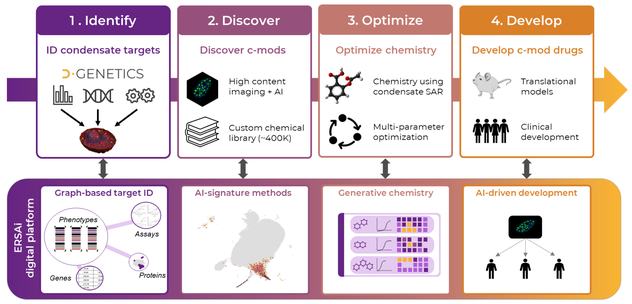


Condensates basics

- Membrane-less compartments
- Form via phase separation
- Found in all living organisms
- Perform a wide range of biological processes
- Condensaopathy = a condensate aberration that drives disease pathology

Condensatopathies are often the shared link between patients bearing diverse genetic abnormalities suffering from the same disease, and between seemingly diverse and complex genetics underlying a uniform disease phenotype. Colorectal cancer is a disease where various mutations of Wnt-pathway components result in constitutive activation of beta catenin and uncontrolled proliferation. The shared central node of Wnt-signaling dysregulation is the formation of aberrant beta catenin condensates that drive oncogenic expression. Our therapeutic approach is to entrap beta catenin into depot condensates and thus prevent its malignant activity.

Dewpoint's end-to-end platform for condensate-targeted drug discovery



Results

Beta catenin c-mods stimulate beta catenin depot formation, changes in gene transcription and are highly potent in CRC and other GI-related cancer types

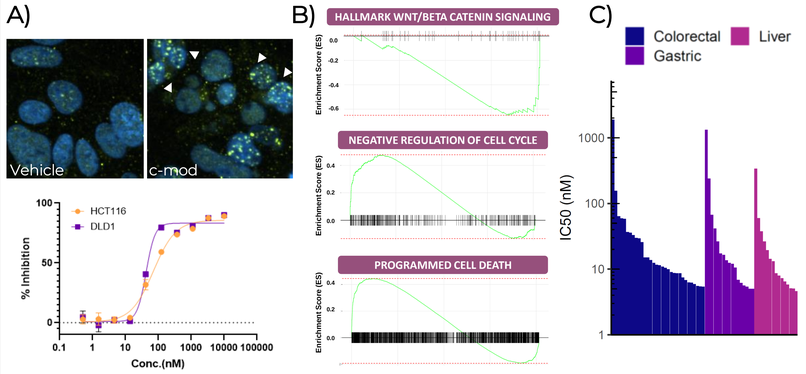


Figure 1: A) DLD-1 and HCT-116 CRC cells were treated with beta catenin c-mods (0.5nM – 10mM). After 24h (IF) or 72h (CTG) cells were processed for beta catenin depot formation (DLD-1 - arrowheads) or cell viability (DLD-1 and HCT-116) respectively; **B)** DLD-1 cells were incubated with 0.3mM c-mod or DMSO control for 24h and then cells were processed for RNAseq analysis and GSEA; **C)** CRC, Gastric and liver cancer cell lines were incubated in the presence or absence of beta catenin c-mod (15nM – 10mM), incubated for 5 days and at the end of the 5 days cell viability was assessed.

Beta catenin c-mods demonstrate significant cytotoxic activity against a panel of CRC patient-derived/patient derived xenograft organoids

Model	IC50 (μM)	AUC	Mutational Status
CRC PDXO-1	0.73	289.2	APC^{mut}; KRAS
CRC PDXO-2	0.05	185.2	APC^{mut}; KRAS; RNF43
CRC PDXO-3	0.09	330.8	APC^{mut}; KRAS; AXIN2
CRC PDXO-4	0.29	256.2	APC^{mut}; RNF43; AXIN1
CRC PDXO-5	0.04	219.7	APC^{mut}; TCF7L2
CRC PDXO-6	0.20	312.1	APC^{mut}; LRP1B; FBXW7
CRC PDXO-7	0.03	213.9	APC^{mut}; KRAS; WNT5A
CRC PDXO-8	0.09	244.9	APC^{mut}; IPP4B
CRC PDXO-9	0.21	329.1	APC^{mut}; KRAS; IPP4B
CRC PDXO-10	0.15	233.5	APC^{mut}

Figure 2: CRC Patient-derived/patient derived xenograft organoids (PDO/PDXO) were seeded in low percentage hydrogel and exposed for 5 days to ½ log doses of c-mod with a starting concentration of 10mM. After the incubation period the cultures were assessed for cell viability by CellTiterGlo. IC50 and AUC were calculated using GraphPad Prism™.

Beta catenin c-mod administration induces beta catenin depots and dose dependent changes in beta catenin gene transcription in vivo

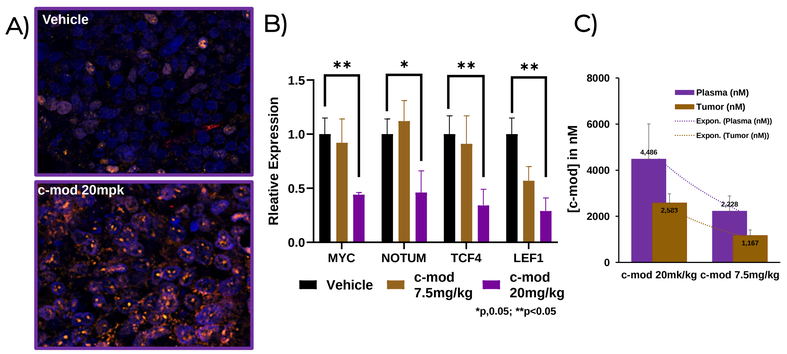


Figure 3: DLD-1 CRC xenografts were established in nude mice and when tumors reached ~300-400mm³ treated with beta catenin c-mods (20 or 7.5 mg/kg BID). After 5 days of treatment plasma and tumor tissue were collected and analyzed for **A)** beta catenin depots using immunofluorescent IHC (arrowheads), **B)** changes in gene transcription using qPCR and **C)** plasma and tumor drug exposures using LC/MS/MS.

Results

Beta catenin c-mods demonstrate robust anti-tumor activity in both cell line and patient-derived xenograft models of CRC

HCT-116 (β-Cat^{GoF}, $KRAS^{GoF}$, $AXIN2^{LoF}$) | **CRC PDX (β-Cat^{hi}, APC^{mut}, $AXIN2^{hi}$)**

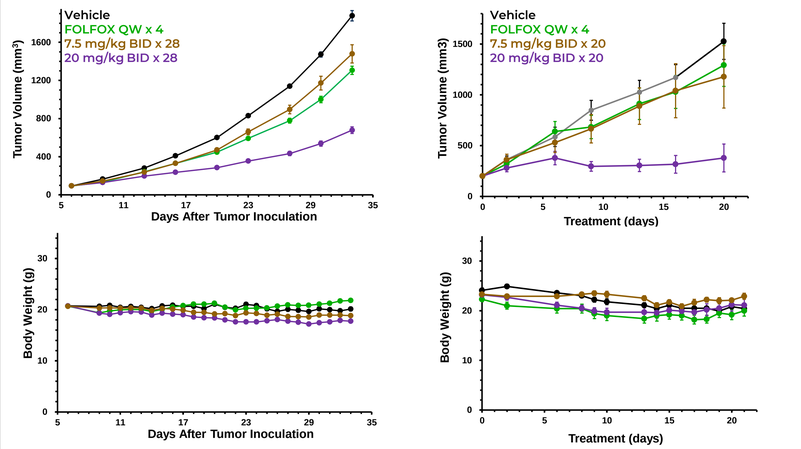


Figure 4: HCT-116 and PDX CRC xenografts were established in nude mice and when tumors reached ~100-200mm³ treated with beta catenin c-mods (20 or 7.5 mg/kg BID x 28 or 20) or FOLFOX (Oxaliplatin 6 mg/kg; levofolinate calcium 90 mg/kg; 5-Fluorouracil 50 mg/kg – QW x 4 or 3). Body weights were assessed daily; tumors volumes were measured twice weekly.

Beta catenin c-mods show enhanced anti-tumor activity when combined with standard of care (SoC)

DLD-1 (APC^{mut}, $KRAS^{GoF}$, MSI+)

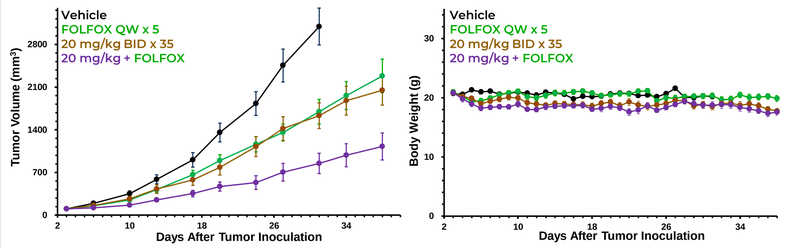


Figure 5: DLD-1 CRC xenografts were established in nude mice and when tumors reached ~100mm³ treated with beta catenin c-mods (20mg/kg BID x 35) or FOLFOX (Oxaliplatin 6 mg/kg; levofolinate calcium 90 mg/kg; 5-Fluorouracil 50 mg/kg – QW x 5) as single agents or in combination. Body weights were assessed daily and tumors volumes were measured twice weekly.

Conclusions

Condensates have been shown to regulate numerous key biological processes and aberrant activity of these condensates have been implicated in driving diseases such as cancer. Here we demonstrate that condensate modifying drugs (c-mods) directed against dysregulated beta catenin transcriptional condensate activity in CRC

- induce beta catenin depot condensates in cancer cells which correlates with cell killing in vitro
- demonstrate robust cytotoxic activity across GI-derived cancers including CRC
- the cytotoxic activity observed in CRC in vitro translates to CRC PDO/PDXO models ex-vivo
- in vivo c-mod administration induces beta catenin depots in tumor cells and dose-dependent down regulation of beta catenin-driven gene transcription which correlates with substantial tumoral drug levels
- finally chronic administration of c-mods leads to significant anti-tumor activity in both cell line and PDX-derived CRC xenograft models and combination with SoC enhances this activity

Taken together, these results show that beta catenin c-mods elicit robust anti-tumor activity against CRC in vitro, ex vivo and in vivo, which correlates with changes in beta catenin localization and transcriptional activity. These findings highlight the potential for targeting aberrant beta catenin signaling through condensate modulation in the treatment of colorectal cancer to address the high unmet medical need of this disease.