Activation Lethality through the WNT/β-catenin pathway drives efficacy in colorectal cancer

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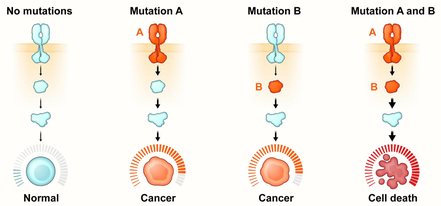
Background

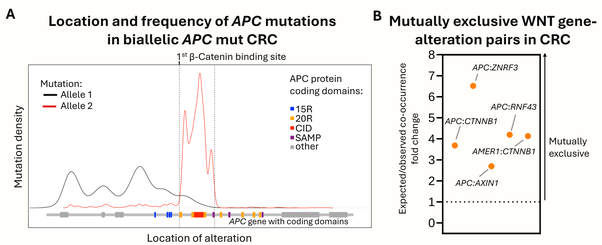
- WNT/βeta-catenin pathway is an early driver of CRC with >70% of patients harboring *APC* damaging mutations
- *APC*-mutant FAP patients display an unusual pattern, in which 1 allele of APC always retains partial β-catenin binding and functionality[1]
- Recent genetic and functional genomic studies have indicated that *APC* mutant cancers are sensitive to further WNT pathway activation[2]
- This phenomenon is consistent with Activation Lethality, in which cancers are vulnerable to further hyperactivation of oncogenic drivers

Model of Activation Lethality



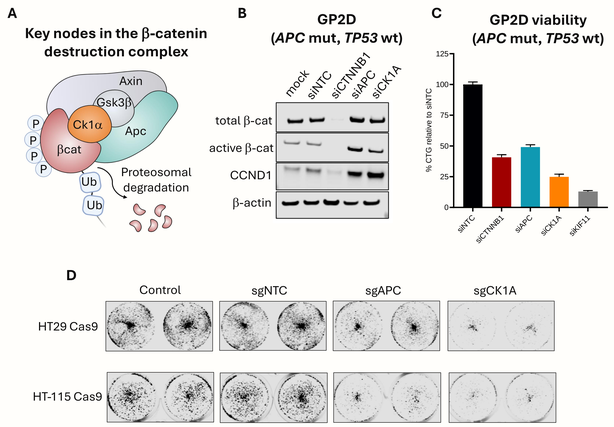
Results

1. CRC genetics suggest selective pressure against WNT hyperactivation



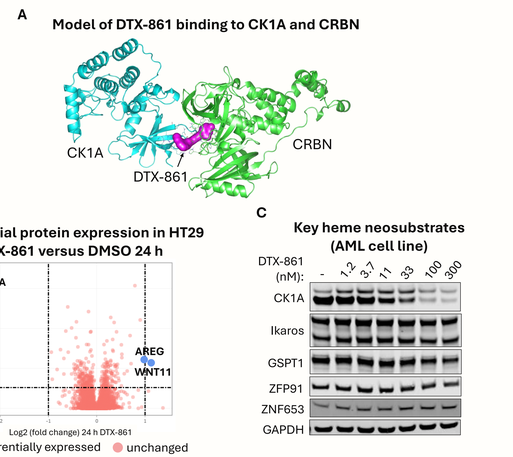
A. CRC tumors retain one partially functional APC allele, consistent with a "just right" level of β-catenin regulation. Lines: Mutation density distribution of 5,054 CRC samples with two or more *APC* stopgain mutations colored by allele and mapped to *APC* gene location (samples from TCGA and AACR-GENIE). **B**. Each point indicates a WNT gene-alteration pair whose co-occurrence is observed significantly less than expected by chance (analysis of TCGA and AACR-GENIE samples; FDR < 10%, after having accounted for tumor subtype)

2. Genetic experiments reveal APC and CK1A are essential in *APC* mutant cancers



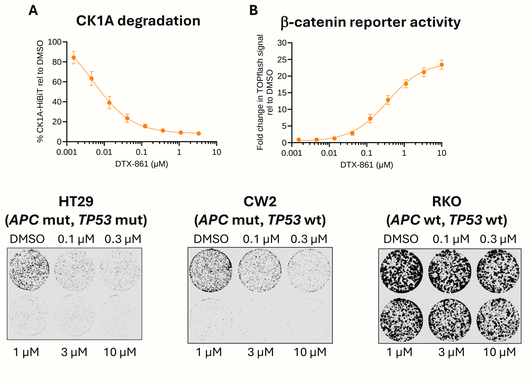
A. Key proteins in the β-catenin destruction complex. **B**. Levels of β-catenin, active β-catenin and downstream target cyclin D1 (CCND1) following 72 h knockdown with indicated siRNAs. **C**. Cell viability (CTG signal) 5 days post knockdown of indicated genes (KIF11 included as positive control. **D**. Colony formation following sgRNA knockout of indicated targets

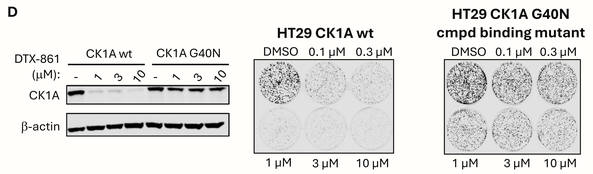
3. DTX-861 is a novel CK1A selective CRBN molecular glue degrader



A. Predicted model of DTX-861 binding to CK1A and CRBN. **B**. Total proteomics comparing differentially expressed proteins following 24 h DTX-861 treatment (1μM) versus DMSO control in HT29 CRC cells. Blue: differentially changed proteins, Red: unchanged. **C**. Protein levels of known cereblon molecular glue hematopoietic neosubstrates following 24 h DTX-861 treatment at indicated concentrations in an AML cell line

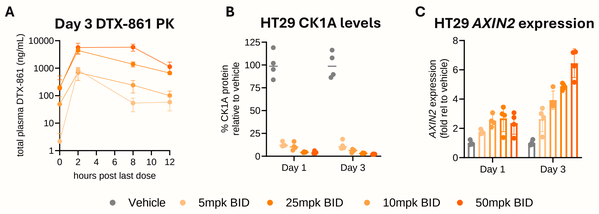
4. DTX-861 hyperactivates β-catenin and is efficacious in *APC* mutant CRC cell lines





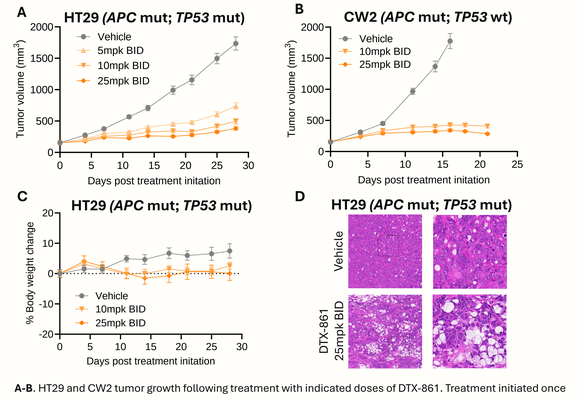
A. HT29 CK1A-HiBiT signal following 24 h dose-response treatment with DTX-861 (mean relative to DMSO control +/- SD, n=8). **B**. TOPflash (TCF reporter) activity in HT29 cells following 24 h dose-response treatment with DTX-861 (mean relative to DMSO control +/- SD, n=14). **C**. Colony forming assay in *APC* wt and *APC* mutant cell lines with DTX-861 at indicated concentrations. **D**. Left: CK1A protein levels and Right: Colony formation in wild-type HT29 (HT29 CK1A wt) and HT29 pool harboring a CK1A G40N mutation to prevent DTX-861 binding (HT29 CK1A G40N)

5. DTX-861 shows dose dependent PK, PD and WNT activation following oral dosing



A. Day 3 DTX-861 plasma levels at timepoints indicated (BID, n=4, mean +/- SD) . **B**. CK1A protein levels in HT29 xenografts on day 1 and day 3. Tumors collected 8 h post-last dose (BID, n=4, individual points and median line shown). **C**. *AXIN2* mRNA expression levels in HT29 xenografts on day 1 and day 3. Tumors collected 8 h post last dose (BID, n=4, individual points and bars showing mean +/- SD shown)

6. DTX-861 is well tolerated and efficacious in *APC* mutant CRC *in vivo*



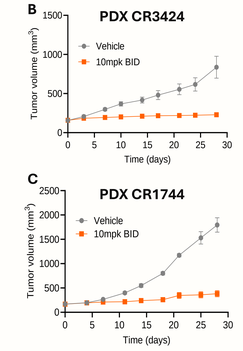
A-B. HT29 and CW2 tumor growth following treatment with indicated doses of DTX-861. Treatment initiated once tumors reached 100mm^3-300mm^3 (n=10, mean +/- SEM) . **C.** Body weight change from baseline of mice bearing HT29 tumors treated with DTX-861 (n=10, mean +/- SEM). **D.** H&E staining of representative vehicle and DTX-861 treated HT29 tumors on day 28

7. DTX-861 shows activity across multiple *APC* mut CRC PDX models

A CRC PDX panel: response and mutation status

Model	% TGI	*APC* mut	*TP53* mut	*KRAS* mut	MSI status
CR1634	91%	■		■	MSS
CR3424	90%	■			MSS
CR1744	87%	■	■		MSI-H
CR9515*	72%	■	■		MSS
CR1554	70%	■	■	■	MSS
CR9511	62%	■	■	■	MSS
CR1245	58%	■	■	■	MSS
CR2501*	56%	■	■	■	MSS
CR3151	55%	■	■	■	MSS
CR2508	46%	■	■	■	MSS
CR6242	22%	■	■	■	MSS
CR6235	10%	■	■	■	MSS

- DTX-861 dosing: 10mg/kg, BID, API; *CRC9515 and CRC2501: 25mg/kg, BID (ASD formulation)



A. Response (average %TGI, n=3) following DTX-861 treatment and mutational status of key drivers in CRC PDX panel. **B-C.** CR3424 and CR1744 growth response following treatment with indicated dose of DTX-861. Treatment initiated once tumors reached 100mm^3-300mm^3 (n=3, mean +/- SEM)

Conclusions

- Human genetics and pharmacological or genetic inhibition of β-catenin destruction complex members validate WNT hyperactivation as a target in *APC* mutant CRC
- DTX-861 is a selective molecular glue CK1A degrader
- CK1A degradation with DTX-861 hyperactivates β-catenin and is efficacious in CRC CDX and PDX models
- Thus DTX-861 mediated CK1A degradation validates WNT as a pathway for Activation Lethality in colorectal cancer

References: 1. Albuquerque C, et al (2002) The 'just-right' signaling model: APC somatic mutations are selected based on a specific level of activation of the β-catenin signaling cascade. Hum Mol Genet 11:1549–1560; **2**. Chang L, et al (2023) Systematic profiling of conditional pathway activation identifies context-dependent synthetic lethalities. Nat Genet 1–12.

Abbreviations: CRC: colorectal cancer, FAP: Familial adenomatous polyposis, Mut: mutant, WT: wild-type, CK1A: casein kinase 1 alpha, PK: pharmacokinetics, BID: twice a day, PDX: patient-derived xenograft, TGI: tumor growth inhibition

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