PRMT5 as a target in CRC interception: synthetic lethal dependency identified in APC-LOF preclinical models of FAP disease

Abstract #2363

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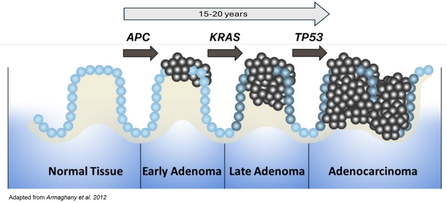
ABSTRACT

Colorectal cancer (CRC) is one of the most common causes of cancer-related deaths in the world and loss of the Adenomatous Polyposis Coli (*APC*) gene represents the disease -initiating event in the majority of CRC cases. Strategies for targeting *APC*-loss of function (*APC*-LOF) may provide an opportunity to intercept tumorigenesis in high-risk CRC patients, such as those with Familial Adenomatous Polyposis (FAP). To discover targets for therapeutic intervention in *APC*-LOF-driven disease, we conducted a high-throughput screen in isogenic *APC* knockout (*APC*^{KO}) colorectal organoids of >6000 compounds with known clinical relevance. From this screen, we uncovered a novel synthetic lethal dependency of *APC*-LOF on protein arginine methyltransferase 5 (PRMT5). JNJ-64619178 (JNJ-9178), a potent clinical PRMT5 inhibitor, demonstrated an >800-fold window in growth inhibition of engineered *APC*^{KO} organoids compared to wild-type (WT). Pharmacodynamic assessments showed a dose-dependent decrease in SmD3, an established biomarker for PRMT5 target engagement, with JNJ-9178 treatment, while no decrease was observed with a weakly active compound, providing evidence that the antiproliferative activity observed in the *APC*^{KO} organoids is a direct consequence of on-target PRMT5 inhibition. We further validated this PRMT5/APC dependency by testing other S-adenosylmethionine (SAM)-competitive PRMT5 inhibitors which show a similar trend in selectively inhibiting growth of the *APC*^{KO} organoids vs WT. Furthermore, we assessed the impact of JNJ-9178 on colon polyp burden in the *Apc*$^{min/+}$-DSS mouse model, an in vivo model of FAP, where we started treatment either before (early interception) or after (late interception) significant polyp burden had been established. A 72% and 66% reduction in polyp burden was observed, respectively, compared to vehicle-treated mice after 3 weeks of dosing (BID). Taken together, these findings showcase the impact of PRMT5 inhibition in both human and mouse models of FAP disease. Orally bio-available PRMT5 inhibitors, including JNJ-9178, present challenges as therapeutics for CRC interception due to on-target cytopenias observed with systemic exposure. To address these dose-limiting toxicities, we have developed a GI-restricted approach and discovered new compounds that only inhibit PRMT5 activity locally in the intestinal tract. This strategy is detailed in a submitted manuscript.

Genetic Hallmarks for CRC Disease Progression[1-4]



Adapted from Armaghany et al. 2012

- **Cancer Interception** attempts to block cancer before the accumulation of multiple mutations allows for advancement to adenocarcinoma: window for treatment opportunity is between the first mutation and oncogenic transformation
- FAP is a hereditary disorder characterized by the development of hundreds to thousands of adenomas in the epithelium of the colon and rectum without non-surgical treatment options[3]
- The discovery of a small molecule inhibitor specifically targeting pathways involved in polyp formation and growth could revolutionize management of this disease

MATERIALS AND METHODS

Chemicals: All compounds used in this study were either synthesized according to previously reported procedures[6] or procured from standard chemical suppliers

Organoid cultures: The *APC*, *KRAS*, and *TP53* mutant isogenic lines were generated by electroporation using previously described sgRNAs and template vectors[3]. Electrotransfer of *APC*, *KRAS*, *TP53* sgRNAs, and reporters was performed following a published protocol[7]

Cell proliferation: Day 0, patient-derived organoids (PDOs) were harvested by incubating with 1 mg/mL Dispase II (Life Technologies Europe B.V.) for 30 minutes at 37°C, washed twice using organoid culturing medium with Rho-kinase inhibitor, and filtered using a 100 and 20 µm mesh filter. 250 PDOs per well were resuspended in organoid screening medium with Rho-kinase inhibitor (5 µM, Abmole Bioscience) with 5% Matrigel. 40 µL of PDO suspension was dispensed in clear-bottomed, black-walled 384-well plates with ultra low-attachment coating (Corning). A ten-point concentration range of the treatments and controls were dispensed in technical triplicates using a

IC_{50} Curve Analysis: IC_{50} curves were generated as follows: The average value for the low control (staurosporine) and the high control (DMSO) were calculated. Subsequently all values were normalized as shown: $Value_{normalized} = (Value_{raw} - Average_{low})/(Average_{high} - Average_{low})$. Percent inhibition was calculated for sample wells based on the equation:

$$\%Activity = \left[\frac{(x-cr)}{(sr-cr)}\right] * 100$$

Where: X = Sample activity; cr = central reference; calculated based on wells containing all assay components and no compound (DMSO only). sr = scale reference; calculated based on wells containing Staurosporine

- For IC_{50} determination, dose response data are processed using the following equation:

$$Y = S0 + \frac{S\,Inf - S0}{1 + \left(\frac{IC50}{10^{C}}\right)^{H}}$$

Where: S_0 = Activity level at zero concentration of test compound, S_{inf} = Activity level at infinite concentration, IC_{50} = Concentration at which activity reaches 50% of maximum level, c = Concentration in logarithmic units corresponding to the values on the x-axis of the dose-response curve plot Hill coefficient n = Measure of the slope at IC_{50}

Flow cytometry: Colon organoids were seeded as 9,000 organoids per well and compounds or controls were added in medium in 4 different concentrations (0.001, 0.01, 0.1, 1µM), with refreshment on day 4. At day 7, colon organoids were harvested by adding Dispase 1 mg/mL to the culture medium and incubating at 37°C up to 1 hour to digest Matrigel™. The organoids were washed and centrifuged for 5 minutes at 450 *g*. Organoids were dissociated to single cells by adding TrypIE for 5-10 minutes while incubating at 37°C. Samples were processed according to the eBioscience™ Annexin V Apoptosis Detection Kit eFluor™ 450 (Catalog number 88-8006-74). The samples were acquired within 4 hours using the MACSQuant Analyzer 10 and analyzed using FlowJo v10.8.

Animals: Female *Apc*$^{min/+}$ (C57BL/6J-*ApcMin/J*, RRID: IMSR_JAX:002020, aged 4-5 weeks) were obtained from the Jackson laboratory (Bar Harbor, ME) and all animal studies described were conducted following the approved protocols by the Institutional Animal Care and Use Committee (IACUC) at Johnson and Johnson Innovative Medicine.

In vivo PD: Mice were exposed to 2% (w/v) dextran sodium sulfate (DSS) in drinking water *ad libitum* for one week. Starting at 2 weeks post-DSS treatment, mice were randomized, grouped, and orally administrated with either test articles or vehicle BID for one week. At the end of each study, mice were euthanized, and colon tissues were removed, cut open, washed and prepared for PD analysis. For studies involving immunohistochemical analysis, whole colons were harvested, Swiss-rolled and then fixed in a 10% neutral buffered formalin solution.

IHC: Tissue samples for IHC were fixed and paraffin embedded. Sections (4um) were subjected to staining using standard IHC protocols for SDMA (Millipore Sigma 07-412 SYM10), PRMT5 (Cell Signaling Technology 79998s) and Ki-67 (Roche Diagnostics 790-4286 IVD)

In vivo distal colon epithelial dissociation: After treatment, distal colons were processed to Isolate the epithelial layer using chemical dissociation as described previously[8] with minor modifications. In brief, distal colon was removed, washed, cut longitudinally, then incubated in Dissociation Reagent 1 (5 mM EDTA, 1.5 mM DTT, 10 uM Y-267632 (Tocris), 0.1% (w/v) HALT Protease/Phosphatase inhibitor) at 4°C for 25 minutes with shaking. Samples were then incubated in Dissociation Reagent 2 (5 mM EDTA, 10 uM Y-267632, 0.1% HALT inhibitor) at 37°C for 20 minutes with shaking. Forceps were used to scrape the epithelial layer into cold DPBS before centrifuging at 1,500 rpm for 5 minutes at 4°C. Supernatant was removed, pellet was resuspended in RIPA buffer with 2x HALT inhibitor, and lysed.

Immunoblot analysis: Organoid lysates or epithelial layer homogenates were collected. Total protein was determined by BCA assay. Lysates were separated on 4-12% Bis Tris gels and transferred onto PVDF membranes using an IBLOT-2 apparatus. Membranes were blocked for 1h at RT, incubated in 1°Ab overnight at 4°C, washed in TBST, incubated in 2°Ab for 1 h at RT, and washed in TBST. Membranes were developed using an Odyssey CLx and analyzed using Image Studio version 5.2.

In vivo efficacy: *Apc*$^{min/+}$ were exposed to 2% (w/v) DSS in drinking water *ad libitum* for a duration of one week. In the early intervention model, mice were randomized and grouped, starting at 2 weeks post-DSS treatment, receiving testing compounds or vehicle orally, either QD or BID for up to three weeks. In the late invention model, mice were grouped for treatment commencing at 3 weeks post-DSS treatment. At the endpoint, animals were euthanized and colon tissues were fixed and imaged for evaluating adenoma size. The area of all the adenomas in each imaged colon was analyzed using the ImageJ software.

RESULTS

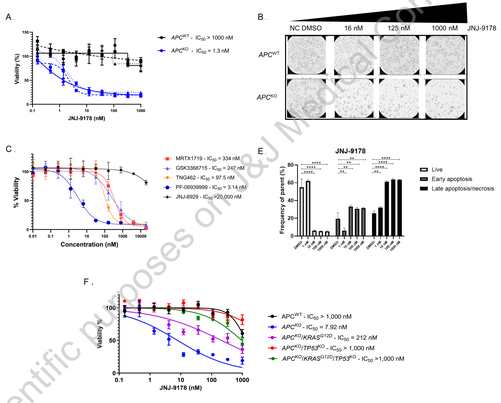
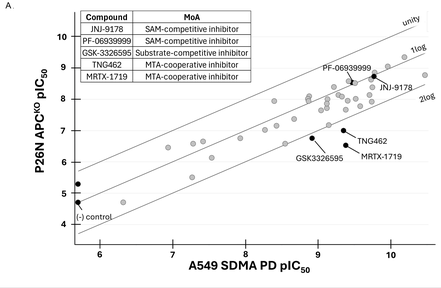


Figure 1: JNJ-9178 inhibits growth and induces cytotoxicity in human *APC*^{KO} organoids. **A** Growth inhibition of JNJ-9178-treated P26N *APC*^{KO}, engineered versus *APC*^{WT} human colon organoids. **B** Bright field images of JNJ-9178-treated P26N *APC*^{KO}-engineered versus *APC*^{WT} human colon organoids. **C, D** Growth inhibition of P26N *APC*^{KO} organoids treated with various compounds. **E** Flow cytometry of JNJ-9178-treated *APC*^{KO} organoids. *F* Cell proliferation assay of JNJ-9178 treatment in a series of P26N APC^{KO} co-mutant engineered organoids.



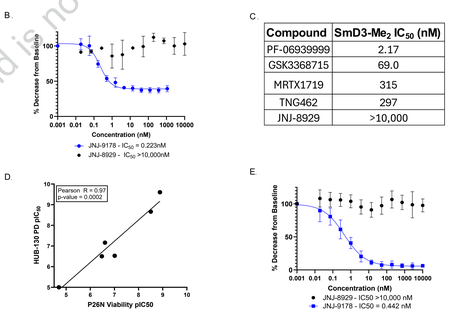


Figure 2: PRMT5 inhibitors demonstrate cross-species functional activity. **A** Correlation plot of P26N *APC*^{KO} organoid growth inhibition IC_{50} values and global SDMA protein expression measured by high content imaging in human A549 cells. **B** IC_{50} curves of SmD3-Me_2 protein expression in the HUB130 human *APC*^{KO} engineered organoids. **C** Table of IC_{50} values of various PRMT5 inhibitors tested in the HUB130 *APC*^{KO} engineered human organoids. **D** Correlation plot of the P26N viability pIC_{50} values versus the HUB130 SmD3-Me_2 PD pIC_{50} values. **E** IC_{50} curves of SmD3-Me_2 protein expression measured by immunoblot in *Apc*$^{min/+}$-DSS mouse colon organoids treated with compounds.

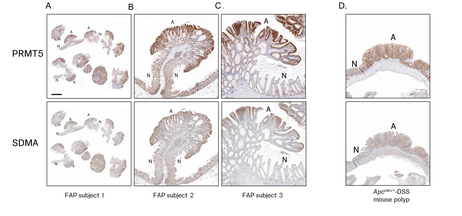
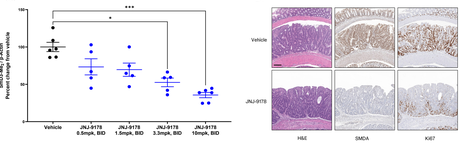


Figure 3. PRMT5 and SDMA are upregulated in colon adenoma tissues. **A** IHC analysis of PRMT5 and SDMA protein expression across multiple adenomas from the same donor. **B,C** IHC analysis of PRMT5 and SDMA protein expression in additional independent human donor FAP adenoma tissues compared to adjacent normal. **D** IHC analysis of PRMT5 and SDMA protein expression in *Apc*$^{min/+}$-DSS mouse colon adenoma tissues versus adjacent normal. A= adenoma, N= normal adjacent. Scale bar: subject 1, 2mm, subject 2, 800 um, subject 3, 200 um, *Apc*$^{min/+}$-DSS, 150 um.



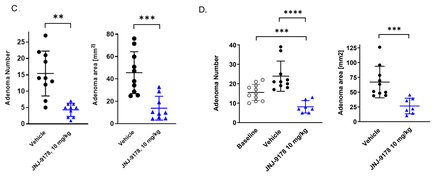


Figure 4. JNJ-9178 demonstrates target engagement and inhibits growth and proliferation of *Apc*$^{min/+}$-DSS mouse colon adenomas. **A** SmD3-Me_2 reduction in JNJ-9178-treated *Apc*$^{min/+}$-DSS colon adenoma epithelial cells measured by immunoblot after 7 days. **B** IHC analysis of SDMA and Ki67 protein expression decrease in *Apc*$^{min/+}$-DSS mouse colon adenoma tissue treated with JNJ-9178. **C** Early interception in vivo efficacy study: Inhibition of colon adenoma count and area in *Apc*$^{min/+}$-DSS mice treated with 10mpk of JNJ-9178, QD, versus vehicle for 19 days. **D** Late interception in vivo efficacy study: Inhibition of colon adenoma count and adenoma area in *Apc*$^{min/+}$-DSS mice treated with 10mpk, QD, of JNJ-9178 after 21 days.

Summary

- PRMT5 was identified as a synthetic lethal target for APC in CRISPR/Cas9-engineered human colon organoids
- PRMT5 inhibitors of various chemotypes inhibit growth of *APC*^{KO} engineered human colon organoids with no impact to wild-type counterparts
- There is a strong correlation between *APC*^{KO} organoid growth inhibition and impact on global SDMA inhibition thus providing evidence that the observed loss of viability is a direct consequence of PRMT5 inhibition
- The PRMT5/APC dependency is diminished in the presence of *KRAS* and *TP53* oncogenic driver mutations
- PRMT5 protein expression and global SDMA levels are upregulated in both human and mouse adenoma tissue compared to normal adjacent
- JNJ-9178 treatment suppresses colon adenoma formation and progression in both early and late interception models of *Apc*$^{min/+}$-DSS mice
- JNJ-9178 treatment was also shown to reduce the number of pre-existing adenomas at the start of treatment, suggesting that PRMT5 inhibition has the potential to eliminate established disease burden in FAP
- Successful clinical development of a PRMT5 inhibitor as a therapeutic strategy for preventing tumorigenesis in patients at a high risk for developing APC-LOF driven CRC could have a profound impact on the management of FAP.

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