Validated DLD-1 Xenograft Model:
Subcutaneous And Orthotopic Xenograft Tumor Model

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Advancing Colon Cancer Research Through Xenograft Modeling

Colorectal cancer is a leading cause of cancer-related mortality globally, characterized by a heterogeneous array of genetic and epigenetic alterations that complicate treatment and contribute to therapeutic resistance. Common mutations in genes such as KRAS, APC, TP53, and PIK3CA play central roles in tumor initiation and progression, yet despite advances in targeted therapies and combination chemotherapeutics, many patients experience relapse and limited long-term response. Xenograft models, particularly those derived from human colorectal cancer cell lines, have emerged as indispensable tools for investigating tumor biology and evaluating drug efficacy *in vivo*. These models allow for controlled, reproducible studies of oncogenic signaling and therapeutic response, although they are limited by the absence of an intact immune system and the inability to fully replicate the complexity of the tumor microenvironment. Within the broader academic discourse, there is increasing recognition of the need to incorporate regulatory elements such as long non-coding RNAs into preclinical models, as these molecules are now understood to influence key signaling pathways and contribute to chemoresistance. Integrating such molecular layers into xenograft-based research holds promise for uncovering novel mechanisms of drug response and improving the translational relevance of colorectal cancer studies.

DLD-1 Cell Line

The DLD-1 cell line, established from a human colorectal adenocarcinoma, serves as a widely utilized model in oncology research due to its well-characterized genetic profile and relevance to the chromosomal instability subtype of colorectal cancer. It harbors oncogenic KRAS (G13D) and TP53 (R273H) mutations, along with a loss-of-function mutation in the APC gene, contributing to dysregulation of Wnt/β-catenin signaling and uncontrolled proliferation. DLD-1 is microsatellite stable and has been employed extensively to investigate mechanisms of chemoresistance, particularly in response to 5-fluorouracil and oxaliplatin, and to evaluate the efficacy of targeted therapies such as MEK and PARP inhibitors. It has also served as a platform for studying synthetic lethality in KRAS-mutant contexts and for developing isogenic models via CRISPR/Cas9-mediated gene editing. Despite its widespread use, existing studies have largely overlooked the epigenetic and non-coding RNA regulatory mechanisms that may underlie drug resistance and tumor progression. Moreover, limited attention has been given to the role of the tumor microenvironment and the lack of immune system interactions in DLD-1-based research, leaving key gaps in understanding the broader determinants of treatment response.

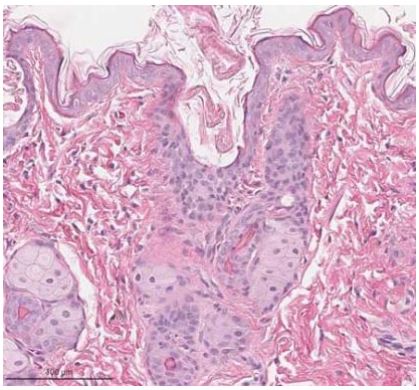

Figure 1. Tumor Histology. H&E stained section of a subcutaneously-implanted DLD-1 tumor (Altogen Labs).

Altogen Labs Validated DLD-1 Xenograft Model

All *in vivo* studies conducted at Altogen Labs are performed under strict adherence to IACUC regulations and GLP compliance standards, ensuring the highest levels of animal welfare and scientific integrity. Upon arrival, animals are given a period of acclimatization within the controlled vivarium environment, allowing them to physiologically adjust before study initiation. Following this period, mice are sorted according to body mass to ensure balanced cohort assignment and reduce variability in tumor growth kinetics. Daily health checks are performed by trained personnel to monitor for tumor appearance, progression, and clinical signs of distress, providing ongoing assessments of animal well-being and experimental status.

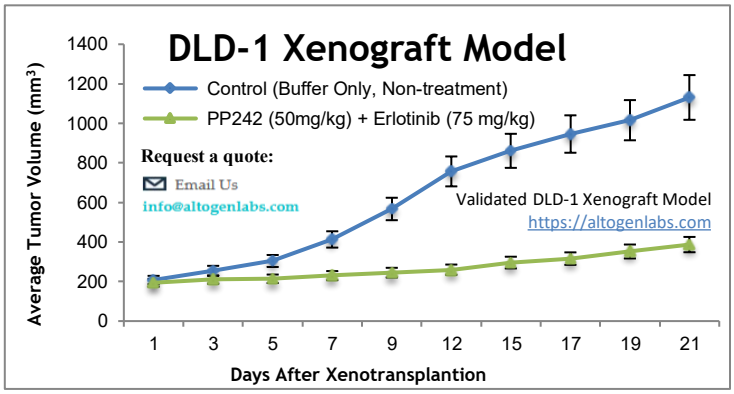

Figure 2. Tumor growth of DLD-1 colon cancer xenografts in immunocompromised mice. Values represent mean tumor volume ± SEM. Study performed by Altogen Labs.

Altogen Labs also offers a suite of downstream molecular and pathological services, including the collection of tumor and normal tissues, paraffin embedding and sectioning for histological analysis, total RNA and protein isolation, and quantitative assessment of gene and protein expression using RT-qPCR and Western blotting. For studies utilizing the DLD-1 colorectal cancer xenograft model, Altogen Labs provides a comprehensive selection of experimental endpoints and analytical capabilities tailored to the research objectives. Standard efficacy measurements include tumor growth delay (TGD), which captures latency of tumor initiation, and tumor growth inhibition (TGI), which evaluates the percentage reduction in tumor volume in treated versus control groups. Investigators can customize dosing frequency, administration routes, and treatment duration to model acute or chronic exposure. To accommodate diverse research aims, the DLD-1 model can be adapted to alternative engraftment sites, such as orthotopic implantation in the cecum for localized disease modeling, tail vein or left ventricular injections for hematogenous metastasis, mammary fat pad injection for ectopic tumor growth, and intraperitoneal injection for peritoneal carcinomatosis studies. Additional services include blood chemistry panels for systemic toxicity assessment, survival analysis with optional longitudinal health monitoring programs, gross necropsies, and histopathological examination of major organs. Metabolic profiling and lipid distribution assays are also available for studies focused on systemic drug effects or cancer metabolism. As an internal efficacy control, cyclophosphamide can be administered to a positive control group at 50 mg/kg via daily intramuscular injection for the duration of the study, providing a benchmark for therapeutic response comparisons.

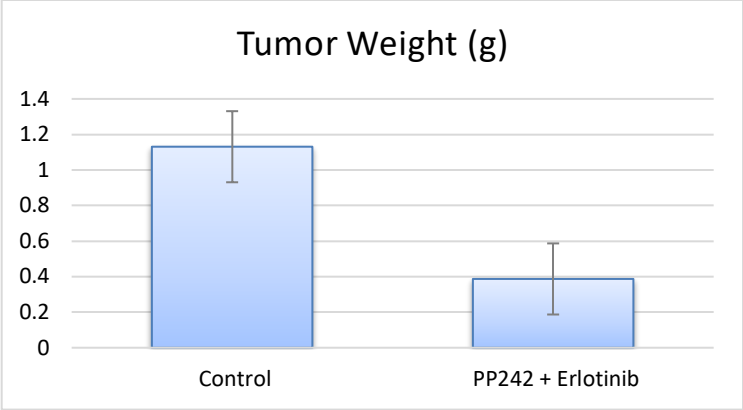

Figure 3. Final tumor weight of DLD-1 colorectal cancer xenografts in immunocompromised mice treated with PP242 (50 mg/kg) and erlotinib (75 mg/kg) compared to untreated control mice (buffer only). Data represent mean values ± SEM at study termination (Altogen Labs).

Subcutaneous Xenografts of DLD-1 in Colorectal Cancer Research

Subcutaneous xenograft transplantation is one of the most widely utilized methodologies in preclinical oncology for evaluating tumor growth kinetics, therapeutic efficacy, and molecular responses *in vivo*. This approach involves the implantation of human cancer cells into the subcutaneous tissue of immunocompromised mice, providing a reproducible and accessible platform for monitoring tumor development and response to treatment. The DLD-1 colorectal adenocarcinoma cell line, derived from a Dukes' type C colon tumor, is particularly well-suited for subcutaneous xenograft studies due to its stable tumorigenicity, well-defined genetic profile, and responsiveness to chemotherapeutic and targeted agents. As a microsatellite stable (MSS) cell line harboring oncogenic KRAS (G13D), TP53 (R273H), and APC mutations, DLD-1 provides a clinically relevant model for investigating tumor biology within the chromosomal instability (CIN) subtype of colorectal cancer.

Numerous studies have employed subcutaneous DLD-1 xenografts to investigate resistance mechanisms to standard-of-care therapies such as 5-fluorouracil and oxaliplatin, as well as to evaluate novel targeted therapies including MEK, PARP, and ATR inhibitors. The model has also been instrumental in validating findings from CRISPR/Cas9-edited isogenic cell lines, particularly in delineating the contributions of oncogenic KRAS to drug sensitivity and tumor progression. The ease of tumor monitoring through external caliper measurements allows for high-throughput comparisons of treatment arms and longitudinal assessment of tumor growth inhibition. While subcutaneous models do not fully recapitulate the tumor microenvironment or immune interactions present in orthotopic settings, they remain a cornerstone of drug development pipelines due to their scalability and experimental tractability. Recent studies have begun to explore the integration of molecular endpoints such as transcriptomics, proteomics, and epigenetic profiling to enhance the translational relevance of subcutaneous xenografts. Emerging interest in the regulatory functions of long non-coding RNAs and their roles in chemoresistance underscore the utility of the DLD-1 model in dissecting post-transcriptional regulatory networks within an *in vivo* context. When implemented with robust experimental design and complemented by orthogonal molecular analyses, subcutaneous transplantation of DLD-1 cells offers a powerful and versatile approach for advancing colorectal cancer research.

Orthotopic Modeling of DLD-1 Colorectal Tumors

Orthotopic xenograft transplantation represents a critical advancement in preclinical modeling by enabling the implantation of human tumor cells into the organ of origin, thereby preserving essential aspects of the native tumor microenvironment, including stromal architecture, vascularization, and organ-specific interactions. In colorectal cancer research, orthotopic models have proven particularly valuable for studying tumor growth dynamics, local invasion, and the early steps of metastasis in a biologically relevant context. The DLD-1 colorectal adenocarcinoma cell line, originally derived from a Dukes' type C tumor, has been successfully utilized in orthotopic implantation studies, despite its more common use in subcutaneous models. These orthotopic applications involve surgical implantation of DLD-1 cells into the cecal or colonic wall of immunocompromised mice, allowing for anatomically accurate tumor development and more predictive evaluations of therapeutic efficacy. Bioluminescent variants of DLD-1, such as DLD-1-luc, have facilitated longitudinal, non-invasive tracking of tumor growth and progression, enhancing the utility of this model in therapeutic studies. However, it is noteworthy that DLD-1 cells exhibit limited spontaneous distant metastatic potential in orthotopic settings, likely due to their low expression of pro-metastatic factors such as Fascin1. This characteristic, while limiting for metastasis-focused investigations, makes the model particularly suitable for evaluating localized tumor growth, stromal interactions, and responses to locoregional therapies. Incorporating orthotopic DLD-1 models into experimental pipelines allows researchers to interrogate drug efficacy within a more physiologically relevant microenvironment, offering a refined preclinical platform that bridges the gap between *in vitro* assays and clinical application.

Case Study: Targeting Feedback Resistance in DLD-1 Colorectal Carcinoma

In a study published in *PLoS One* journal by Wang et al., the therapeutic potential of combining the mTOR kinase inhibitor PP242 with the EGFR inhibitor erlotinib was evaluated in colorectal carcinoma, with a particular focus on the DLD-1 cell line. The authors demonstrated that PP242 alone produces only transient inhibition of mTORC1 and mTORC2, as evidenced by temporary suppression of S6 and AKT (Ser473) phosphorylation followed by a rebound in activity. This recovery was linked to increased EGFR phosphorylation, occurring through a PI3K-independent pathway. When erlotinib was added, the combination sustained suppression of both mTOR complexes, reduced DLD-1 cell viability, inhibited colony formation, and triggered apoptosis, as shown by caspase-3, DFF45, and PARP cleavage. *In vivo*, subcutaneous DLD-1 xenografts treated with the combination exhibited significantly reduced tumor growth compared to those treated with PP242 alone or vehicle, reinforcing the synergistic effects of dual pathway inhibition.

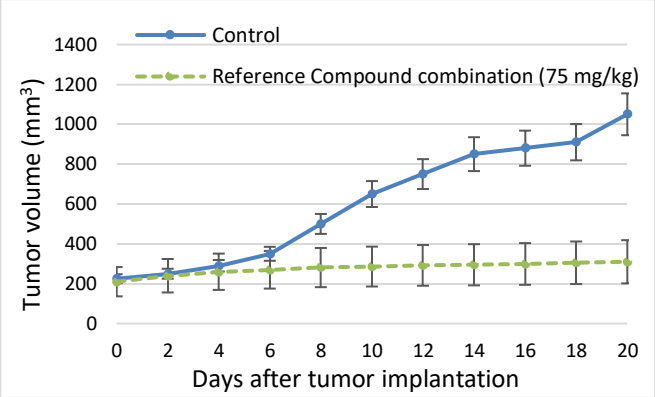

Figure 4. Tumor growth of DLD-1 xenografts in immunocompromised mice treated with a reference compound in combination with erlotinib (75 mg/kg) compared to untreated controls. (Altogen Labs).

The data presented by Wang et al. support the conclusion that EGFR activation undermines the efficacy of second-generation mTOR inhibitors when used as monotherapy, and that co-targeting EGFR and mTOR enhances antitumor effects. The experimental design was comprehensive, employing Western blotting, RTK phosphorylation arrays, apoptosis assays, and xenograft models, although the small sample size of the animal study (n=4 per group) and omission of an erlotinib-only control group *in vivo* limit interpretive breadth. Nevertheless, the study offers valuable insights into the resistance mechanisms that compromise mTOR-targeted therapies. These findings position DLD-1 as a relevant preclinical model for evaluating combination strategies in KRAS-mutant, EGFR-expressing colorectal cancers. Future investigations should focus on identifying the downstream mediators of EGFR-driven mTORC2 reactivation and testing combination therapies in immune-competent or humanized models to further bridge the gap between preclinical and clinical research.

Additional Case Study: Transcriptional Repression of β-Catenin by KLF4 in DLD-1 Cells

In the study published by Zhang et al. in *Molecular and Cellular Biology* journal, and under the auspices of the American Society for Microbiology, the authors investigate the molecular interaction between Kruppel-like factor 4 (KLF4) and β-catenin, focusing on their functional antagonism in colorectal cancer. Utilizing the DLD-1 colorectal cancer cell line, which harbors an *APC* mutation that leads to constitutive activation of the Wnt/β-catenin pathway, the research demonstrates that inducible expression of KLF4 significantly inhibits β-catenin-mediated transcriptional activity. Specifically, KLF4 represses the expression of canonical Wnt target genes such as *c-MYC* and *AXIN2*, reduces cell proliferation, and induces

expression of differentiation markers like intestinal alkaline phosphatase. In xenograft models using DLD-1-KLF4 cells, tumor growth was markedly reduced in mice administered doxycycline, correlating with suppressed Ki67 staining and increased mucin production, suggesting enhanced differentiation and reduced tumor cell proliferation.

Zhang et al. further demonstrate that KLF4 physically interacts with the C-terminal transactivation domain of β-catenin, inhibiting its transcriptional activity without altering β-catenin stability or nuclear localization. This suppression is not observed in KLF4 mutants lacking zinc finger domains, which retain the ability to bind β-catenin but fail to repress transcription, indicating the necessity of intact DNA-binding domains for KLF4's tumor suppressor function. The methodologies used are comprehensive and include luciferase reporter assays, immunoprecipitation, RNA interference, *in vivo* tumor xenografts, and immunohistochemistry. However, the reliance on immunocompromised mice limits the assessment of immune interactions, and further clarification is needed on the downstream transcriptional programs mediating KLF4's suppressive effects. Collectively, the findings presented by Zhang et al. position KLF4 as a potent antagonist of β-catenin in colorectal cancer. They underscore the potential of targeting this axis in Wnt-driven malignancies, with the DLD-1 model providing a relevant system to study differentiation therapy and transcriptional reprogramming in colorectal tumorigenesis.

Epigenetic Silencing of WNT-TCF Signaling in DLD-1 Cells

Withanolide F, a steroidal lactone compound, demonstrates potent and durable suppression of WNT-TCF signaling in colorectal cancer cells, particularly in the DLD-1 cell line. This pathway, commonly hyperactivated due to mutations in *APC*, is a critical driver of tumor initiation, progression, and maintenance of cancer stem cell properties. In DLD-1 cells, treatment with Withanolide F leads to robust repression of canonical WNT target genes, including *AXIN2*, *LGR5*, and *cMYC*, with effects that persist long after drug withdrawal. Tumor xenografts derived from DLD-1 cells show marked regression following systemic administration of the compound, and sustained silencing of WNT-TCF responses is observed in surviving tumor-derived cells. Notably, the compound also significantly reduces the formation of secondary and tertiary clonogenic spheroids, indicating long-term inhibition of cancer stem cell renewal. These persistent effects are accompanied by downregulation of key chromatin remodeling genes and a measurable decrease in global H3K4me1 levels, suggesting an epigenetic basis for the observed transcriptional silencing.

The pattern of gene regulation induced by Withanolide F is both selective and consistent across multiple assays. In DLD-1 cells, more than two-thirds of a validated WNT-TCF gene signature is repressed, surpassing the effect of comparator agents such as ivermectin, which share some WNT-inhibitory activity but lack epigenetic potency. These findings are supported by molecular rescue experiments demonstrating that constitutively active TCF can restore gene expression, confirming the specificity of WNT-TCF blockade. Importantly, the inhibition of histone modifiers such as *EED*, *ZMYND8*, and *SMARCAL1*, alongside histone mark changes, implies that the compound not only interferes with transcription factor activity but also imposes broader chromatin-based regulation. This mode of action distinguishes Withanolide F from conventional inhibitors that require continuous exposure to maintain effect. The implications for cancer therapy are significant: by reprogramming oncogenic transcriptional networks and depleting self-renewing cell populations, a short course of treatment could yield sustained therapeutic outcomes. Further investigation is warranted to determine the full scope of epigenetic remodeling and to explore its utility in immunocompetent models or clinical translation.

MM-129 Induces Apoptosis in DLD-1 Colorectal Cancer Cells

A novel heterofused 1,2,4-triazine sulphonamide compound, MM-129, demonstrates potent anticancer activity in colorectal carcinoma models, with efficacy in the DLD-1 cell line. *In vitro* assays show that MM-129 exhibits strong cytotoxicity with an IC50 of 3.1 μM in DLD-1 cells, significantly lower than the concentrations required for comparable effects by standard agents such as 5-fluorouracil and roscovitine. The compound effectively suppresses DNA synthesis, disrupts mitochondrial membrane potential, and activates both intrinsic and extrinsic apoptotic pathways. Apoptosis is confirmed by elevated activity of caspases 3/7, 8, 9, and 10, along with externalization of phosphatidylserine and marked mitochondrial depolarization. *In vivo*, zebrafish xenograft models implanted with DLD-1 cells exhibit a 56 percent reduction in tumor fluorescence following MM-129 treatment, with even greater efficacy observed when combined with 5-fluorouracil, indicating a synergistic antitumor effect.

These findings reveal a consistent pattern of apoptosis induction and tumor growth inhibition that correlates with a reduction in phosphorylated Bruton's tyrosine kinase, a survival-associated kinase implicated in colorectal cancer progression. MM-129 induces the highest apoptotic response among the tested agents, reflecting its capacity to simultaneously disrupt cell survival pathways and activate programmed cell death mechanisms. The methodology integrates molecular and imaging techniques, including flow cytometry, Western blotting, and live imaging in zebrafish.

However, the use of embryonic zebrafish precludes assessment of long-term toxicity and immune interactions, and short-duration exposure models do not capture chronic treatment dynamics. The DLD-1 model offers a well-characterized genetic background, including mutations in APC and KRAS, making it an ideal system for preclinical evaluation of apoptosis-targeting therapies. Future work should validate these effects in mammalian models, explore the broader kinase inhibition profile of MM-129, and assess its pharmacokinetics and toxicity under physiologically relevant conditions.

Dual Suppression of WNT and AKT Oncogenic Signaling in DLD-1 Cells

The combination of MM-129, a 1,2,4-triazine derivative, and indoximod, a kynurenine pathway inhibitor, exhibits significant antitumor activity in colorectal cancer models, with pronounced effects in DLD-1 cells. These cells, which carry activating mutations in *KRAS* and inactivating mutations in *APC*, display constitutive activation of the PI3K/AKT and WNT signaling pathways. Co-treatment disrupts these oncogenic cascades by downregulating AKT expression, reducing mitochondrial membrane potential, promoting phosphatidylserine externalization, and activating apoptotic markers including caspase-3/7, -8, and -10. Zebrafish xenograft models confirm these effects, with DLD-1-derived tumors showing a greater reduction in tumor burden and apoptotic signaling than HT-29. MM-129 drives the bulk of antiproliferative activity, while indoximod enhances cell death, likely through modulation of immunoregulatory and metabolic pathways. Notably, indoximod downregulates IDO1, a key enzyme in immune tolerance and epithelial-to-mesenchymal transition, although MM-129 alone does not affect IDO1 levels.

A consistent pattern of synergistic action emerges, where MM-129 targets survival and proliferation, and indoximod complements these effects by interfering with immune evasion mechanisms. DLD-1 cells respond more robustly than HT-29, likely due to their distinct oncogene expression profile, including elevated COX-2, GRP78, and IDO1. The study employs a comprehensive methodology including flow cytometry, gene expression analysis, and zebrafish xenograft validation, though limitations remain in terms of long-term toxicity and immune system modeling in non-mammalian hosts. The findings underscore the therapeutic potential of dual-pathway targeting in colorectal cancer and validate the DLD-1 cell line as a relevant preclinical model for tumors with activated AKT and WNT signaling. Future research should prioritize mammalian validation, immune profiling, and exploration of pharmacodynamic interactions, with a focus on translating this drug combination into therapeutic strategies for colorectal tumors exhibiting the DLD-1 molecular phenotype.

Altogen Labs offers comprehensive preclinical services designed to support oncology drug development, including pharmacology and toxicology testing, ELISA and cell-based assay development, and IC50 profiling across over 100 cancer cell lines. The laboratory operates over 80 validated xenograft models for *in vivo* anti-tumor efficacy testing, including well-established models for brain, lung, breast, pancreatic, prostate, ovarian, gastric, colon, and melanoma cancers. Altogen also provides advanced encapsulation services for mRNA, siRNA, and DNA using liposome formulations, and specializes in the generation of stable cell lines, including RNAi-based gene knockdown and inducible systems. Additional services include *in vivo* toxicology (mouse and rat LD50 studies), teratoma formation analysis, GLP-compliant cryopreservation and master cell banking, and a wide range of RNA interference capabilities such as *in vivo* siRNA delivery, RNAi-based library screening, and gene expression profiling via RT-PCR.

Figure 5. Routes of drug administration available for DLD-1 *in vivo* xenograft studies at Altogen Labs. Administration options include intratumoral, intramuscular, oral gavage, intravenous, intratracheal, subcutaneous, intraperitoneal, continuous infusion, intranasal, and advanced micro-injection techniques.

Among the most widely used colon cancer models available at Altogen Labs is the DLD-1 xenograft model, derived from a human colorectal adenocarcinoma. The DLD-1 cell line exhibits key oncogenic alterations, including mutations in *KRAS*, expression of *MYC*, *MYB*, *FOS*, *SIS*, and *P53*, and loss of functional *APC*, a tumor suppressor gene. These cells display classic epithelial morphology and express carcinoembryonic antigen (CEA) and colon antigen 3. DLD-1 cells are frequently used in preclinical studies due to their reproducible tumor formation in immunocompromised mice. Xenograft studies with DLD-1 have been employed to evaluate signaling pathways such as RTK-KIT, TLR5-mediated immunity, and non-invasive fluorescence-based imaging of tumor response to chemotherapy. Altogen Labs' validated DLD-1 study design includes standardized cell preparation, subcutaneous implantation in mice, treatment group randomization, tumor monitoring, and endpoint histopathological and molecular analysis. Available endpoints include tumor growth inhibition, tumor growth delay, toxicity, survival analysis, blood chemistry, and lipid metabolism profiling. Researchers can also choose alternative cell injection sites for metastasis or orthotopic studies and may employ a cyclophosphamide-treated control group as a reference standard.

References:

Hermanowicz JM, Szymanowska A, Sieklucka B, Czarnomysy R, Pawlak K, Bielawska A, Bielawski K, Kalafut J, Przybyszewska A, Surazynski A, Rivero-Muller A, Mojzych M, Pawlak D. Exploration of novel heterofused 1,2,4-triazine derivative in colorectal cancer. *J Enzyme Inhib Med Chem.* 2021 Dec;36(1):535-548. doi: 10.1080/14756366.2021.1879803. PMID: 33522320; PMCID: PMC7850456.

Kwiatkowska I, Hermanowicz JM, Czarnomysy R, Surażyński A, Kowalczuk K, Kałafut J, Przybyszewska-Podstawka A, Bielawski K, Rivero-Müller A, Mojzych M, Pawlak D. Assessment of an Anticancer Effect of the Simultaneous Administration of MM-129 and Indoximod in the Colorectal Cancer Model. *Cancers (Basel).* 2023 Dec 26;16(1):122. doi: 10.3390/cancers16010122. PMID: 38201550; PMCID: PMC10778160.

Seth C, Mas C, Conod A, Mueller J, Siems K, Kuciak M, Borges I, Ruiz i Altaba A. Long-Lasting WNT-TCF Response Blocking and Epigenetic Modifying Activities of Withanolide F in Human Cancer Cells. *PLoS One*. 2016 Dec 14;11(12):e0168170. doi: 10.1371/journal.pone.0168170. PMID: 27973612; PMCID: PMC5156407.

Wang Q, Wei F, Li C, Lv G, Wang G, Liu T, Bellail AC, Hao C. Combination of mTOR and EGFR kinase inhibitors blocks mTORC1 and mTORC2 kinase activity and suppresses the progression of colorectal carcinoma. *PLoS One*. 2013 Aug 22;8(8):e73175. doi: 10.1371/journal.pone.0073175. PMID: 23991179; PMCID: PMC3750018.

Zhang W, Chen X, Kato Y, Evans PM, Yuan S, Yang J, Rychahou PG, Yang VW, He X, Evers BM, Liu C. Novel cross talk of Kruppel-like factor 4 and beta-catenin regulates normal intestinal homeostasis and tumor repression. *Mol Cell Biol*. 2006 Mar;26(6):2055-64. doi: 10.1128/MCB.26.6.2055-2064.2006. PMID: 16507986; PMCID: PMC1430292.

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