Thesis

A Review of Animal Models in Colorectal Cancer Research

submitted by

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Zusammenfassung

Das Kolorektales Karzinom (CRC) ist eine der häufigsten bösartigen Erkrankungen weltweit, deren komplexe Pathophysiologie therapeutische Ansätze erschwert. Trotz Fortschritten bei den chirurgischen und chemotherapeutischen Therapien erfordern Herausforderungen wie Metastasierung, Arzneimittelresistenz und unerwünschte Nebenwirkungen innovative Therapien. Neue Lösungen wie siRNA-basierte Interventionen sind vielversprechend, erfordern aber eine strenge präklinische Validierung, um Hürden bei der Stabilität und Verabreichung zu überwinden. Tiermodelle spielen in diesem Prozess eine zentrale Rolle, da sie eine kontrollierte Plattform zur Erprobung neuer Behandlungen bieten. In dieser systematischen Übersichtsarbeit haben wir 26 Studien aus den letzten 15 Jahren analysiert, die sich mit der injektionsbasierten Karzinogenese in Kleintiermodellen befassten. Unser Ziel war es, standardisierte Praktiken bei der Auswahl von Tieren und Zelllinien, Injektionsmethoden, Volumina und Zellkonzentrationen zu identifizieren. Die Ergebnisse verdeutlichen die Variabilität der Methoden und unterstreichen die Notwendigkeit einer einheitlichen Vorgehensweise, um die zukünftige Forschung zu rationalisieren und die translationale Relevanz zu erhöhen. Unsere Übersichtsarbeit kommt zu dem Schluss, dass präzise und minimalinvasive Ansätze wie die endoskopische Injektion in anatomisch skalierbaren Modellen wie der Ratte die Kluft zwischen experimentellen Designs und der menschlichen Darmkrebs-Pathologie überbrücken könnten. Diese Erkenntnisse sollen die Entwicklung robuster und reproduzierbarer Darmkrebs-Tiermodelle unterstützen, um therapeutische Innovationen voranzutreiben.

Abstract

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide, with complex pathophysiology that complicates therapeutic approaches. Despite advancements in surgical and chemotherapeutic options, challenges such as metastasis, drug resistance, and adverse side effects necessitate innovative therapies. Emerging solutions like siRNA-based interventions demonstrate promise but require rigorous preclinical validation to overcome hurdles in stability and delivery. Animal models play a pivotal role in this process, offering a controlled platform to test new treatments. In this systematic review, we analyzed 26 studies from the past 15 years focused on injection-based carcinogenesis in small animal models. Our aim was to identify standardized practices in animal and cell line selection, injection methods, volumes and cell concentrations. The findings highlight the variability in methodologies, underscoring the need for consistency to streamline future research and enhance translational relevance. Our review concludes that adopting precise and minimally invasive approaches, such as endoscopic injection, in anatomically scalable models like rats, could bridge the gap between experimental designs and human CRC pathology. These insights are intended to guide the development of robust and reproducible CRC animal models for advancing therapeutic innovations.

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Abbreviations

Abbreviation	Description
CRC	colorectal cancer
siRNA	small interfering RNA
HDI	Human Development Index
ASIR	age-standardized incidence rates
UICC	Union for International Cancer Control
ASMR	age-standardized mortality rate
FAP	familial adenomatous polyposis
HNPCC	hereditary non-polyposis colorectal cancer
FIT	fecal immunochemical test
FS	flexible sigmoidoscopy
EMR	endoscopic mucosal resection
CME	complete mesocolic excision
TME	total mesorectal excision
FOLFOX	chemotherapy regimen made up of the drugs folinic acid, 5-fluorouracil, and oxaliplatin
CAPOX	chemotherapy regimen made up of the drugs oxaliplatin and cabecitabine
FOLFIRI	chemotherapy regimen made up of the drugs folinic acid, 5-fluorouracil and irinotecan
FOLFOXFIRI	chemotherapy regimen made up of the drugs folinic acid, 5-fluorouracil, oxaliplatin and irinotecan
EFGR	epidermal growth factor receptor
RNAi	RNA interference
RISC	RNA-induced silencing complex
mRNA	messenger RNA
LNP	lipid-based nanoparticle
PNP	polymer-based nanoparticle
PLGA	polymeric nanoparticles
mCRC	metastasized CRC
AOM	azoxymethane

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1 Introduction

Colorectal cancer (CRC) ranks among the most frequently diagnosed malignancies worldwide, presenting as the third most common cancer in countries with high or very high Human Development Index (HDI), following lung and prostate cancers in men, and breast and lung cancers in women.(1,2) It accounts for a significant global health burden, with approximately 65% of new CRC cases occurring in Europe, North America, and China. These regions exhibit age-standardized incidence rates (ASIR) significantly higher than the global average, highlighting disparities linked to socioeconomic factors.(2,3) CRC's prevalence underscores the importance of primary prevention strategies targeting modifiable risk factors, alongside the implementation of comprehensive, population-based screening programs to reduce incidence and mortality.(3,4)

The anatomical distribution of CRC reveals a predominance in the rectum (60%) and sigmoid colon (20%), with fewer cases in the caecum, ascending colon, and other sections of the large intestine. Notably, approximately half of CRC patients develop distant metastases, either due to tumor recurrence or as a presenting feature of advanced disease, most commonly involving the liver and lungs. Such patterns complicate prognosis and treatment, necessitating robust therapeutic approaches.(5,6)

CRC treatment integrates four main pillars: curative surgery, neoadjuvant radiochemotherapy, adjuvant chemotherapy, and palliative care. Surgical intervention remains the cornerstone for localized disease (UICC stages I–III), while advanced cases often require multimodal strategies, including targeted and systemic therapies.(7) Innovations like small interfering RNA (siRNA) therapies are emerging, offering molecular precision in targeting oncogenes.(8) Despite their promise, siRNA therapies face challenges in delivery and stability, demanding further research and refinement to optimize their clinical application.(9–11)

The use of animal models is instrumental in CRC research, enabling the study of tumor progression, metastasis, and treatment responses.(12) Traditional models, including subcutaneously induced colorectal cancers for transplantation and other injection models, provide foundational insights but often fall short in replicating the complexities of human

CRC. Novel orthotopic models, using endoscopic injection of tumor cells into the intestinal mucosa, have shown promise in addressing some of these limitations. This technique minimizes invasiveness and enhances the anatomical relevance of experimental CRC, paving the way for more accurate preclinical studies.(13) Through a systematic review of CRC small animal models spanning 15 years, this thesis aims to consolidate knowledge and identify best practices. By addressing critical methodological questions, the study seeks to advance the development of an improved CRC animal model, bridging gaps in research and enhancing translational relevance to human disease.

1.1 Colorectal Cancer

Colorectal Cancer is among the most diagnosed types of malignancies worldwide. After lung cancer (1^{st}), prostate cancer (2^{nd}) in men and breast cancer (1^{st}), lung cancer (2^{nd}) in women colorectal cancer amounts for the third highest age-standardized incidence rate (ASIR) of malignancies in countries with a high/very high HDI. In comparison to low HDI countries the ASIR of colorectal cancer in high HDI countries is four times as much. This difference is significantly smaller for the age-standardized mortality rate (ASMR) of CRC due to a higher fatality rate in low HDI countries.(1,2) Colorectal cancer is regarded as a marker of socioeconomic development as countries in transition usually show a simultaneous rise in incidence of CRC. Following this trend, the incidence rate in many Eastern European, Asian and South American countries has been increasing steadily.(2)

A look at the absolute number of CRC cases Worldwide shows, that approximately 65% (1,255,876/1,931,590) of newly diagnosed cases occur in Europe, North America and China. Worldwide the ASIR of colorectal cancer is estimated to be about 19.5 per 100,000 inhabitants. This is far below the average ASIR of Europe (30.4 per 100,000), North America (26.2 per 100,000) and slightly lower than the incidence rate of China (23.9 per 100,000). Looking at country specific ASIRs of the aforementioned regions, Hungary and Slovakia (45.3 and 43.9 per 100,000 inhabitants respectively) top the chart. France and United Kingdom both record a incidence rate above 30.0 per 100,000 and the European country with the lowest ASIR still above the world average is Austria (21.0 per 100,000).(2,3) The age-standardized mortality rate (ASMR) for CRC also shows variation globally. China and Europe (12.0 and 12.3 per 100,000) record a higher ASMR than the world average (9.0 per

100,000) and the average in North America (8.2 per 100,000) is slightly lower than the global average ASMR.(2)

With this in mind the main idea to controlling the high incidence of CRC is primary prevention targeting risk factors and thorough population-based screening programs.(3) There are numerous relative risk factors that can be targeted in efforts of primary prevention of the CRC. These include but are not limited to Obesity, Diabetes mellitus, consumption of red and processed meat as well as smoking of tobacco and drinking of alcohol.(4) Among the risk factors are hereditary diseases and genetic predisposition like FAP (familial adenomatous polyposis) & Lynch Syndrome/HNPCC (hereditary non-polyposis colorectal cancer) including the patients personal and family disease history of cancer, inflammatory bowel disease and numerous bacterial infections of the bowel.(4,14) While these genetic predispositions only account for approximately 5% of CRC incidence, patients with FAP have a lifetime risk of 100% to develop colorectal cancer and in patients with HNPCC the risk of developing colorectal cancer is up to 70% and 60% for male and female patients respectively.(5,14)

In most cases these risk factors cannot be modified or controlled, however they strongly influence screening recommendations as a form of secondary prevention.(3,4) Several studies highlight the relevancy of protective factors in the prevention of CRC. The two most important beneficiary factors are physical activity, which has been supported by numerous studies to reduce the risk of colorectal cancer by as much as 25%.(15,16) As well as dietary factors such as the consumption of dietary fibers and whole grains, an increased intake of Calcium and dairy products and intake of Vitamins D & B6. (3,4)

While the reduction of risk factors and the increase of protective factors are shown to have a significant effect on the incidence of CRC, the importance of thorough screening procedures in the population cannot be ignored.(3) As colorectal cancer has a long preclinical stage, in most cases developing through a benign adenoma to a malignant cancer over a span of approximately 10 years, patients have a long period in which an early detection of colon adenomas or early stage cancer can prove beneficial to the outcome of treatment.(17) The most long standing screening procedure is the digital palpation of the rectum, with which almost 10% of CRC can be palpated.(5) In addition two further methods of screening have been implemented. First, patients are screened using a non-invasive test

called fecal immunochemical test (FIT). It uses specific antibodies to detect small amounts of hemoglobin in a stool sample of the patient, which can be an early sign of cancer development.(3,17) The second method of screening for CRC comprises of several different endoscopic examinations. The most implemented types are the colonoscopy and the flexible sigmoidoscopy (FS). Several studies examining the efficacy of colonoscopy and FS have reported that a reduction of incidence by a range of 18-69% and mortality by a range of 28-68% can be achieved.(3)

In comparison to immunochemical tests and other endoscopic examinations, colonoscopy has the highest sensitivity and specificity in detecting non bleeding neoplasia as well as providing the possibility of gathering histologic material and simultaneous preventive polypectomy.(6) This preventive polypectomy can be performed with various methods depending on the size of a polyp. Small polyps (≤5 mm) can be removed with biopsy forceps, while medium sized polyps (≤10 mm) should be removed with a cold snare. Removal of large polyps (≥10 mm) requires the use of a high-frequency hot snare. For pedicled polyps the use of snare polypectomy is standard, while flat and shallow lesions are removed via endoscopic mucosal resection, EMR.(6)

The localization of CRC is mainly focused in the rectum (60%) and the sigma (20%). Only 10% of CRC are found in the caecum and ascending colon and another 10% are found in the remaining colon.(5) While there is clearly a higher abundance of CRC in the aboral sections of the large intestine, in recent years, a shift from the rectosigmoid to higher colon sections has been observed. This can be explained through better detection of early rectosigmoid cancers as well as the rise in life expectancy of patients.(5,6)

The most common type of colorectal cancer is the adenocarcinoma, it makes up about 95% of all CRC. Other, more rare, types of colorectal cancers include: mucinous, signet-ring cell, adenosquamous, medullary and undifferentiated carcinoma.(6,14) The histology of CRC is divided into four groups of grading. G1 and G2 tumors have a high percentage of glandular structures (from 95% down to 50%), they are often also be referred to as “low-grade”. In G3 tumors glandular structures only make up 5% to 50% of their area and in G4 tumors less than 5% of their area are made up of glandular cell types. G3 and G4 CRC can also be categorized as “high-grade” tumors meaning that they are undifferentiated carcinomas.(6) Additionally, the TNM classification and UICC (Union International Contre

le Cancer) classification are used for staging of the carcinoma, in which “T” stands for primary tumor size and infiltration, “N” describes the infiltration of regional lymph nodes and “M” refers to if and how many peripheral metastases can be found. The UICC code forms through the individual components of the TNM classification and differentiates four main stages of disease (UICC I-IV).(6)

Around 50% of all patients suffering from CRC develop distant metastases during the course of their disease. This can occur either as a result of tumor recurrence, amounting to 80% of patients, or already at the time of diagnosis of the primary tumor, in the case of 20% of patients. The findings of metastases at the time of diagnosis is described as prognostically unfavorable. The metastasis of CRC can occur hematogenous, via the lymphatic system or per continuitatem to adjacent and near organs.(5,6) Distant metastases of CRC occur primarily in the liver or the lungs and can rarely occur in other organ systems like the skeletal system.(6) For hematogenous metastasis of CRC two pathways are distinguished, portal pathway and the cava pathway. This is dependent on the location of the primary tumor and its venous vascularization. CRC of the lower third of the rectum metastasize via the cava pathway directly to the lungs, while CRC above the lower third of the rectum and in the large intestine metastasize via the portal pathway to the liver. (5,6) A similar pattern can be observed for metastases via the lymphatic system. In this case three pathways can be described which are also dependent on the location of the primary tumor. For CRC in the upper rectal third (12-16 cm) lymphatic spread occurs via the paraaortic lymph nodes. Colorectal cancers of the middle rectal third (6-12 cm) spread to paraaortic and lymph nodes of the pelvic wall. CRC of the lower rectal third (< 6 cm) show a lymphatic spread via the paraaortic, pelvic wall and additionally the inguinal lymph nodes. The prognosis of disease is worse the lower in the rectum the CRC sits as additional lymphatic metastasis pathways are added.(5)

1.2 Therapy

The therapy of CRC consists of four main pillars: curative surgery, neoadjuvant radio-chemotherapy, adjuvant chemotherapy and palliative therapy.(5) Prognosis as well as therapy goals are defined by the stage of disease at first diagnosis with respect to individual risk factors. For locally limited stages (UICC I-III) curative surgical therapy is first line. For stages III and subgroups of stages II the addition of adjuvant chemotherapy can lower the

risk of local recurrence.(6,7) For a majority of patients with a stage IV CRC at first diagnosis, the primary goal is palliative therapy. However, in a subgroup of stage IV patients, a curative approach is possible through thorough resection of metastasis in liver or lungs. The adjuvant chemotherapy for stage IV patients consists of several different modalities. Among these are cytostatic drugs, monoclonal antibodies as well as targeted therapies.(6) Neoadjuvant radiotherapy is reserved for deep rectal cancers of stages II-III to reduce local recurrences and improve operability.(5,18) With a combination of different modalities a 5-year-survival-rate of 95% for UICC I can be achieved. CRC of UICC stages II-III have a 5-year-survival-rate of up to 85% and up to 65% respectively. Colorectal cancers of UICC stage IV have a very slim 5-year-survival-rate of 5%.(5)

1.2.1 Surgical Therapy

The indication of surgical therapy for patients with CRC is either curative in stages I-III or performed as an emergency operation in the case of cancer associated perforation or acute stenosis in the form of a manifested ileus. The primary goal of curative surgery is the radical removal of the tumor bearing colon segment *in sanu*. Simultaneously, lymph nodes and vessels as well as the blood supply of the colon segment are removed surgically. The extent of how much segment is removed depends mainly on anatomical cancer localization, lymphatic drainage and vascular supply.(6,18) Standard practice in surgical therapy of CRC is the complete mesocolic excision (CME), referring to the removal of all mesocolic lymphatic tissue and central ligature of supplying blood vessels. This procedure is shown to improve patient outcomes and does not affect the rate of complications.(6)

If the CRC is located in the ascending colon or at the right colon flexure a right hemicolectomy or expanded right hemicolectomy respectively is performed. A ligature is placed at the origin of the ileocolic artery and right/middle colic artery. The two remaining ends of intestine are reconnected via ileotransversostomy.(6,18) A transverse colon resection is performed on patients with a colon cancer in the middle of the transverse colon. Both the right and left flexure is removed in this operation and the blood supply is capped at the origin of the middle colic artery. The two ends are joint by a ascend-descendostomy to restore continuity.(18) In the more common case of a left sided CRC, a left hemicolectomy or expanded right hemicolectomy is performed. The descending and sigma colon are detached from the transverse colon and rectum and the blood supply is clipped at the inferior

mesenteric artery. In this case either a terminal colostomy or a continuation between the transverse or descending colon with the remaining rectum is performed.(6)

Indications for surgery for rectal cancers in particular depend on the distance of the cancer from the anocutaneous line. For CRC within 12cm of the anocutaneous line, a total mesorectal excision (TME) has to be performed. Similar to the complete mesocolic excision the TME removes lymphatic tissue in the surrounding area but the procedure aims to protect the autonomous nervous system and therefore functions of the anus, bladder and sexual organs.(6) In this case continuity can be restored via direct connection of the remaining colon segments with the anal sphincter or through the creation of an artificial rectal pouch with remaining segments of intestine.(18) If the rectal cancer lies deep, within 4cm of the anocutaneous line, a rectal extirpation has to be performed. The defect is consequently covered by a gluteal flap plastic and a definitive colostomy is created.(6)

A colostomy is a, in most cases, permanent artificial orifice of the intestine at the abdominal wall. Usually ostomies are left to empty into an adhesive pouch covering the opening. After an extensive operation and removal of colon segments or the rectum, this procedure secures safe passage of foods and fluids. However, it comes with major impairments to the quality of life of patients. A studies by Sun et at. (19) and Silva et al. (20) discuss these impairments in great detail and propose solutions to potential complications and their arise. Up to 60% of patients with an ostomy experience some form of complications. This incidence is highest in the first five years following the ostomy formation. The complications include negative effects on the daily life such as lifestyle adjustments, clothing restrictions, functional issues as well as odor and hygiene concerns. Moreover the formation of an ostomy may have an impact on the body image manifesting with psychological distress and social stigma.(19,20) Multiple strategies have been formulated by Sun et al. to reduce the negative effects of ostomy complications for patients. Prevention and management of complications, like regular check-ups and use of improved ostomy supplies, in the first five years after ostomy formation play a very important role in these strategies. More key factors for patient satisfaction and complication prevention are patient education and long-term psychological as well as social support.(19,20)

1.2.2 Chemotherapy

For UICC stage I colorectal cancer the first line therapy is surgical resection, adjuvant chemotherapy is not indicated. In cases with stage II or III CRC adjuvant chemotherapy is dependent on clinical and histopathological risk factors. First line therapy for stage II colorectal cancers next to surgical resection is the addition of a fluoropyrimidine-based medication for 6 months.(6,7) Chemotherapy is especially indicated for patients with a high clinical risk of recurrences like: T4 stage, tumor perforation, intraoperative tumor rupture.(7) In patients with stage III CRC the adjuvant chemotherapy is expanded with a combination of medications called FOLFOX. In addition to the fluoropyrimidine-base an oxaliplatin as well as folinic acid are prescribed for 3 to 6 months. Alternatively a CAPOX scheme (oxaliplatin and cabecitabine) can be prescribed, with equipotent outcome. This intensification in therapy plan leads to an increase in relapse-free survival and overall survival.(6,7)

The therapy scheme for stage IV CRC is divided into two pathways. For patients with primary operable metastases a neoadjuvant 3 month cycle of FOLFOX chemotherapy can be started, after which surgical resection of metastasis follows. Postoperative, FOLFOX therapy is continued for another 3 month cycle or started for patients not receiving it prior.(6,7) This branch describes the curative approach of stage IV therapy, however in the majority of patients the primary therapeutic goal is palliative and symptom oriented. In addition to the standard chemotherapy (FOLFOX, FOLFIRI, FOLFOXFIRI) targeted therapies can be inducted depending on the mutation status, localization of the CRC and the overall fitness of the patient.(7) Overall, few different signal pathways can be intercepted with a targeted therapy. The epidermal growth factor receptor (EGFR) can be blocked by the monoclonal antibodies Cetuximab and Panitumumab. The vascular endothelial growth factor receptor can be directly blocked by Bevacizumab. Additionally, the use of checkpoint inhibitors like Pembrolizumab and Nivolumab is also indicated.(6,7)

While the use of chemotherapy and targeted therapies deliver great disease modifying effects, the side effects cannot be overlooked. Common side effects of first line chemotherapy include: gastrointestinal symptoms (nausea, vomiting, diarrhea), changes in blood count (neutropenia, thrombopenia, bone marrow toxicity), polyneuropathy and mucositis.(6) The most common side effect of targeted therapies are allergic reactions and dermal toxicity which can lead to dermal atrophy and loss of hair. Anti angiogenic

substances can lead to proteinuria, cardiovascular hypertension, thromboembolic events and in rare cases to tumor perforation.(6)

1.2.3 Novel Therapy Idea – siRNA

While the efficacy of chemotherapeutics and targeted therapies is long established, it does not come without adverse side effects and impact on patient perceived quality of life.(6,21) This problem gave rise to a new approach in cancer medication. In the past two decades RNA-based therapy, especially small interfering ribonucleic acid (siRNA), has reshaped cancer treatment research and clinical trials through the use of siRNA to regulate the expression of oncogenes on a molecular scale.(8) Through knowledge of the human genome and research on exact nucleotide combinations for oncogenes, synthetic siRNA molecules can be produced targeting these specific genes.(9)

Small interfering RNA interacts with an innate cellular regulatory mechanism called RNA interference (RNAi), in which our bodily cells directly regulate the expression of certain genes and in certain cases cause a targeted gene suppression.(9,10) This novel therapy uses the innate inhibitory function to regulate production of previously undruggable pathogenic proteins and in turn expand the druggable human genome, gaining access to new levers in cancer therapy.(8,22) Synthetic siRNA is a double strand RNA consisting of 21-23 nucleotide long complimentary "sense" and "antisense" strands. Following its entry into the cell, the double strand siRNA can bind to a RNA-induced silencing complex (RISC), it is subsequently unwound through removal of the "sense" pair and the bound "antisense" strand activates the RISC compound.(9,22) After activation, the mRNA of the target gene pairs to the "antisense" strand on the RISC molecule and is then catalyzed and cleaved, leading to a suppression of the target gene.(8,9)

Therapeutic potential for siRNA is high but their effective incorporation in clinical trials pose some difficulties and bottlenecks current research on siRNA medication.(9,22) Their susceptibility to degradation by serum endonucleases and subsequent rapid clearance by the kidneys also brings the serum half-life of siRNA down to minutes.(11,22) Due to the large molecular size, hydrophilic nature and anionic charge, naked siRNA have troubles spontaneously passing the cellular membrane on their own, further limiting bioavailability and cellular internalization.(8,9) Furthermore, the structure of siRNA may trigger unwanted

immunogenic response due to their similarities to viral RNA, potentially leading adverse side effects of treatment and ultimately termination of clinical trials.(9–11)

With the aforementioned difficulties, a proper delivery system for siRNA to the target cells needs to be implemented to secure efficacy of the treatment.(9,10) Throughout the past two decades there have been several different "vectors" used to deliver siRNA to its final destination. These included both viral and nonviral delivery systems like: lenti- and adenoviruses, lipid-based nanoparticles (LNPs), polymer-based nanoparticles (PNPs) and inorganic nanoparticles (INPs).(10,11) LNPs are usually composed of four different lipids: ionizable lipids, phospholipids, cholesterol and PEGylated lipids. These parts all play an important role in the structure, prolonged circulation and uptake of LNPs. The lipids presenting on the surface of LNPs allow for easier endocytosis processes by target cells further improving their bioavailability, efficiency and versatility.(8) The modality of delivery that dominates colorectal cancer research are polymeric nanoparticles (PLGA). They provide a low level of toxicity, high bioavailability and sustained release while also securing long and short-term storage in circulation.(23)

Current use of FDA/EMA approved siRNA therapy is limited to treatment of hepatic disease and hypercholesterolemia.(22) However many more therapeutics are in early and late stage clinical trials.(9,22) The primary way of application of these therapeutics and in trials is by intravenous injection.(10) The liver is a good target for LNP siRNA therapy due to the fenestrated endothelial lining of its blood vessels. In many cases the vascular architecture of tumors is similarly fenestrated and leaky, also making cancerous diseases good targets for siRNA therapy.(9) Focusing on colorectal cancer, several oncogenes like KRAS, BRAF, NRAS, EGFR or MET can be isolated as targets of siRNA therapy. Current research for metastasized CRC (mCRC) show successful siRNA delivery and cellular internalization via PLGA nanoparticles. The PLGA are coated with certain high affinity molecules that target atypically expressed receptors of the target mCRC cells.(23)

Preliminary studies and trials of siRNA therapy for colorectal cancer show promising results of significantly suppressed tumor growth and metastasis in *vivo*.(24) While they are not completely without adverse side effects and limitations, siRNA therapies are starting a revolution in disease and cancer treatment.(11,22) With many more levers to improve safety,

efficacy and economic viability of treatment outcomes(23), the time for further research on siRNA therapy is now.

2 Animal Models

Animal models are a widely used and established method of cancer research. The aim of these models is to properly mimic induction, growth, infiltration and spreading of metastases of tumor cells. Further, animal models can help us understand disease progression, adverse effects on general health as well as efficacy of past, present and future therapeutics in cancer treatment. A paper by Bürtin et al. (12) lists the established use of small animal models in research on colorectal cancer. These murine models include carcinogen-induced models, genetically engineered mouse models as well as transplant models. The latter of which describes the transplanting of alive tumor cells into the mouse to establish a solid tumor model for research. The cell lines or tumor tissue transplanted can be categorized as either syngeneic or xenogeneic, referring to tumor cell lines implanted within the same mouse strain or tissue which can be derived from different murine species and even human colorectal cancers respectively.(12)

These colorectal cancer models can further be distinguished between heterotopic and orthotopic transplantation. In heterotopic tumor models cell lines are typically injected or engrafted into sub cutaneous pouches in the flanks of mice.(25–28) After implantation and growth of the tumor, these types of models are used in subsequent orthotopic engraftment into the cecal or colonic wall via abdominal access.(25,26) While the initial sub cutaneous injection or engraftment of tumor cells is a rather uncomplicated and effective procedure to grow heterotopic tumors, the efficacy of drug administration and clinical similarity to the human colorectal cancer model is very limited. As such the heterotopic model in mice is merely an intermediate step for later orthotopic transplantation.(12) Orthotopic models, in turn, overcome the aforementioned limitations and allow for the establishment of a precise model of colorectal cancer in animals. The intestinal structure of mice and rats is very similar to that of humans additionally featuring a relative large caecum. With their close proximity to the abdominal wall, the caecum and colonic sections can easily be accessed via small incisions or midline laparotomy of the abdominal wall.(12) Many studies use this advantage of fast and uncomplicated abdominal access to the peritoneum as well as caecum or cecal

wall in murine models to establish peritoneal carcinosis and solid tumor models via sub serosal injection for further research.(29–32)

These studies however are still far from a model precisely mimicking growth and infiltration of the primary tumor or modelling dissemination of metastases of colorectal cancer. Intraperitoneal or sub serosal injection of tumor cells show extensive development of peritoneal carcinomatosis but limitations in providing typical ways of metastasis of CRC to liver and lung.(32) Another limitation of injection via abdominal access is the spillage of and contact of tumor cells with organs in the abdominal cavity and the unwanted result of peritoneal carcinomatosis and atypical metastases not present in classic CRC cases.(12) On top of this, gaining access to the intestinal structures requires the use of deep anesthesia and surgical procedures which may hamper the tumor immunogenicity and growth as well as causing stress, inflammation and increased morbidity of the animals.(12,33)

A novel method of establishing an orthotopic animal model of CRC is endoscopic injection of tumor cells directly into the intestinal mucosa. This method provides a much safer injection without the aforementioned risks of cell spillage and invasiveness into the abdominal cavity.(13) Following a proper bowel preparation, a mini endoscope can be inserted into the rectum of the test subject. Via the working channel of the endoscope, Zigmond et al. (13) have shown that, a hypodermic needle can be channeled into the lumen of the colon. After a suitable location is found, the needle is gently inserted into the mucosa of the colonic wall for injection of tumor cells. Through the direct view on and magnification of the injection site a successful introduction of cells can be observed and judged instantly by a “mucosal lifting” sign.(33) A major limitation of this method is the risk of colon perforation by the inserted needle and subsequent spillage into the abdominal cavity. However, in the study by Zigmond et al. with over 200 mice the complication was quite rare occurring in less than 5% of procedures.(13) This proves that minimal invasive endoscopic injection in small animal models is a highly reproducible and well-tolerated method to initiate a orthotopic model of CRC. While the establishment of this method is a large step forward in the field of cancer research, the small animal model of mice still has certain limitations. In the mentioned studies the endoscopic injection sites are limited to the distal colon because of the rigid structure of the scope and lumen size of the colon.(13) The animals used in the mentioned studies were immunodeficient mice (12,13,33) further compromising the validity of current animal models in mimicking solid tumor growths in humans.(34,35)

A study by Haughn et al.(35) and Karas et al.(34) shows the feasibility of endoscopic injection of tumor cells into the cecal wall of immunocompetent rats. With the use of a flexible endoscope the oral sections of colon and the caecum can be reached for injection.(35) Therefore, and to further reduce the expected morbidity and mortality of test subjects, the caecum was selected as the site of injection. The caecum including the formed tumor can be resected without having to perform anastomosis of colon segments.(34) In both studies over 100 procedures were successful in forming a solid tumor in the cecal wall of immunocompetent rats with a very low complication rate of 2-4%.(34,35) The results of both studies show that the cecal injection via flexible endoscope in rats is a feasible novel method to research CRC.

We established a review of research in the field of small animal models of CRC over a timeframe of 15 years from 2009 to 2024. The review includes 26 papers of animal models of CRC including of mice, rats and rabbits. The literature search was conducted on PubMed, screened for eligibility and relevant data was plugged into the table below (see Tables 1-3) for better visualization. We further analyzed the data collected to answer various questions we see as important for the implementation of a small animal model of colorectal cancer at our institute. These questions include: *"What animals were used in the studies?"*, *"Were the animals used in the study immunocompromised?"*, *"Which CRC cell lines were used for injection?"*, *"What was the injection model? sub cutaneous, sub mucosal, directly into the organ?"*, *"What injection volume was chosen?"*, *"What injection concentration was chosen?"* and finally *"What needle size was chosen?"*. With the data collected and the answers to the questions above we aim to establish an advanced small animal model of colorectal cancer for further research in the field as well as to improve the planning and understanding of future small animal models of CRC.

2.1 Methods

Literature Search Strategy

No ethics approval was required for this type of study. Literature research was performed in PubMed database. The following keywords were used to search for valid papers: "colon cancer animal model", "colon cancer injection animal model". The search

was restricted to English language only with a limitation of papers published, in a timeframe of 15 years, from 2009 to 2024.

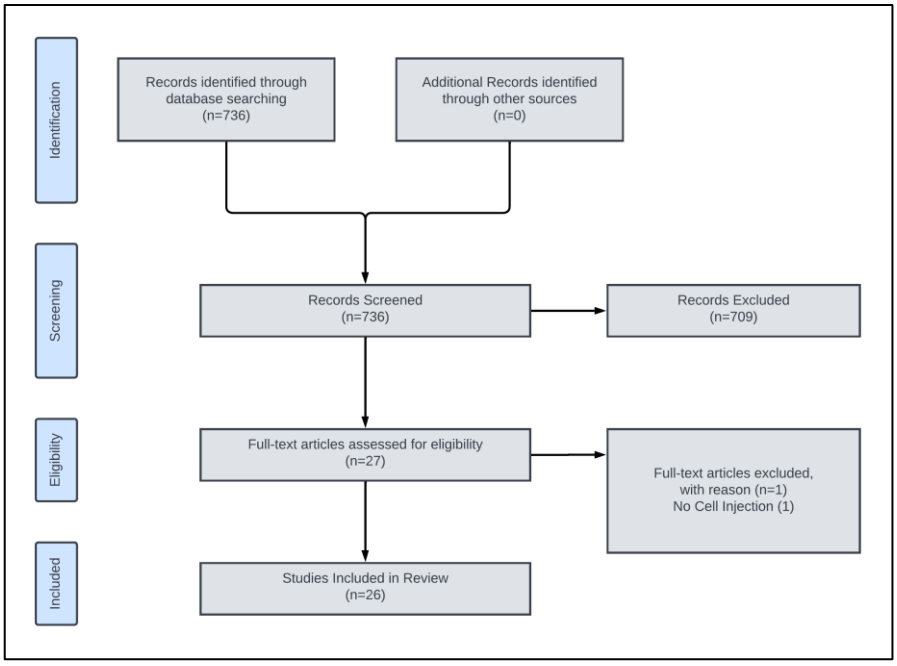

Figure 1: PRISMA flowchart of study selection process.

Eligibility Criteria

In our literature search we included papers that contained a small animal model and the injection or transplantation of colorectal tumor cells of syngeneic cancer cells or a derivative from human colorectal cancer. Due to the primary point of interest being the methodology of the studies and the limited amount of total papers, we included both papers with and without control groups as well as multiple cohorts. These will be listed individually in the provided table (see Tables 1-3). Exclusions were made if the paper did not include the injection of tumor cells. In the case of Souris et al. (36), the subjected mice were injected with azoxymethane (AOM) to create a *sporadic* colon cancer model. As our aim is to primarily study the injection/transplantation of alive tumor cells we decided to exclude this study from our review. There was no cutoff for number of animals in the study groups.

Study Selection and Data Extraction

At first, the studies were screened based on their title and abstract. Full text was obtained for potentially eligible studies. The following data was extracted from all included studies: year of publication, animal model, animal species, immunocompetency, tumor cell

line, origin of cells, injection/transplantation, needle size, cell concentration, injection volume, type of injection and location of injection. If it was possible to extract we also noted the for us relevant endpoint, in most cases euthanasia and organ extraction, as well as the results of injection and tumor growth.

Table 1: Studies and their respective data

Author	Year	Animal	Species	Immunocompromised	Cell Line	Origin of Cells	Injection Transplant	Needle Size	Cell C	Injection V	Type of Injection	Location	Findings
Taniura, T et al. (37)	2020	Mouse	BALB/c	No	CT26	Syngeneic	Injection	-	5,0x10^5	-	s.c.	right flank	Successful tumor growth
		Mouse	C57BL/6	No	MC38	Syngeneic	Injection		5,0x10^5		s.c.	right flank	
Chang, S et al. (38)	2021	Mouse	C57BL/6	No	CT26 (a) HCT116 (b)	mouse derived (a) human derived (b)	Injection	30G	3,0x10^5	0,05ml	submucosal	rectum	-
Terracina, K et al. (29)	2015	Mouse	BALB/c	No	CT26-luc1	Syngeneic	Injection	28G	5,0x10^4	0,1ml	submucosal	caecum	-
Lwin, M et al. (25)	2021	Mouse	nude nu/nu	Yes	LS-174T	Human crc	Transplant	-	1,0x10^6	-	-	-	Tumor >5mm -> Transplant to Caecum
Basirat, E et al. (39)	2022	Mouse	BALB/c, C57BL/6	No	CT26	Syngeneic	Injection	-	7x10^5	0,1ml	s.c.	dorsal flank	Successful tumor growth
Bae, Ki Beom et al. (26)	2020	Mouse	BALB/c athymic	Yes	HCT116	Human crc	Injection	30G	2x10^6	0,1ml	in organ	Spleen	10/13 Tumor implantation
				Yes	HCT116	Human crc	Inj&Trans	-	5x10^6	0,2ml	s.c.	flank	13/13 Tumor implantation LiverM+ and PeritM+ in both groups
		Mouse	BALB/c athymic										
Thalheimer, A et al. (40)	2009	Mouse	BALB/c nu/nu	Yes	HT29 & SW620	Human crc	Injection	32G	1x10^5, 1x10^6, 5x10^6	0,2ml	i.v.	portal vein	High # of Liver and Lung M+ Mortality high in 5x10^6 concentration group
Thalheimer, A et al. (27)	2009	Mouse	BALB/c nu/nu	Yes	HT29 & SW620	Human crc	Transplant	-	-	-	-	-	if prim. tumor >15mm, transplant to caecum of healthy animal this happened up to 9x
Caetano-Oliveira, R et al. (41)	2018	Rat	RNU Rat	Yes	WiDr (a)		Injection	30G	1x10^7		Submucosal	cecostomy, distal colostomy	60 days, 59% Tumor development
		Rat	RNU Rat	Yes	C2BBe1 (b)	Human crc	Injection	30G	1x10^7	-	Submucosal		60 days, 38% Tumor development
		Rat	RNU Rat	Yes	LS1042 (c)		Injection	30G	1x10^7		submucosal		60 days, 0% Tumor

Table 2: Studies and their respective data

Author	Year	Animal	Species	Immunocompromised	Cell Line	Origin of Cells	Injection Transplant	Needle Size	Cell C	Injection V	Type of Injection	Location	Findings
Wang, H et al. (42)	2017	Mouse	athymic mice	Yes	SW620-luc	Human crc	Injection	-	2,5x10^6	-	in organ	Spleen	5 Weeks post Inj: 58% Tumor+, 38% LiverM+
		Mouse	athymic mice	Yes	SW620-luc	Human crc	Injection		2,5x10^6		in organ	Spleen	71% Tumor+, 50% LiverM+
Uccello, T et al. (30)	2022	Mouse	C57BL/6J	No	MC38-luc	Syngeneic	Injection	32G	2,5x10^4	0,05ml	submucosal	rectum	100% Tumor+
Prieto, V et al. (43)	2017	Rabbit	New Zealand white rabbit	Yes	HCT116, HT29	Human crc	Injection	25G	1,8x10^5	0,2ml	in organ	liver	75% Tumor+ in HT29 Inj. No tumor in HCT116 Inj
Spyridopoulou, K et al. (44)	2018	Mouse	BALB/c	No	CT26	Syngeneic	Injection	-	5x10^6	-	s.c.	flank	No specific result for implantation success
Helderman, R et al. (45)	2022	Rat	WAG/Rij	Yes	CC531	Syngeneic	Injection	-	2x10^6	1ml	i.p.	Peritoneum	Metastatic model successful
Höhn, P et al. (31)	2020	Rat	WAG/Rij	Yes	CC531	Syngeneic	Injection	-	1x10^5	1ml	i.p.	Peritoneum	s.c. and i.p. tumor weight were determined
		Rat	WAG/Rij	Yes	CC531	Syngeneic	Injection	-	1x10^6	1ml	s.c.	Not specified	Tumor+ successful
Silva, E et al. (46)	2024	Mouse	C57BL/6	No	MC38	Syngeneic	Injection	-	1x10^5	-	s.c.	right flank	95% Tumor+
Kim, H et al. (47)	2022	Mouse	NSG	Yes	HCT116	Human crc	Injection	-	3x10^6	-	s.c.	not specified	No specific result for implantation success
		Mouse	NSG	Yes	HCT116	Human crc	Injection		1,25x10^5		in organ	Spleen	
		Mouse	CD2F1	No	CT26	Syngeneic	Injection		1x10^6		s.c.	not specified	
		Mouse	CD2F1	No	CT26	Syngeneic	Injection		2,5x10^5		in organ	Spleen	
Huot, J et al. (48)	2020	Mouse	NSG	Yes	HCT116	Human crc	Injection	-	3x10^6	-	s.c.	not specified	No specific result for implantation success
		Mouse	NSG	Yes	HCT116	Human crc	Injection		1,25x10^5		in organ	Spleen	
Paulson, B et al. (49)	2019	Mouse	BALB/c nude	Yes	HCT116	Human crc	Injection	33G	3x10^5	0,1ml	submucosal	distal colon	Sacrificed after 3 Weeks, Tumor induction rate of 85%

Table 3: Studies and their respective data

Author	Year	Animal	Species	Immunocompromised	Cell Line	Origin of Cells	Injection Transplant	Needle Size	Cell C	Injection V	Type of Injection	Location	Findings
Taibi, A et al. (32)	2019	Mouse	BALB/cByJ	No	CT26-luc	Syngeneic	Injection	23G	3x10^4	0,2ml	i.v.	Tail Vein	no PeritonealM+
		Mouse	BALB/cByJ	No	CT26-luc	Syngeneic	Injection	12G	3x10^4	0,2ml	s.c.	Abdominal wall	single PeritonealM+
		Mouse	BALB/cByJ	No	CT26-luc	Syngeneic	Injection	12G	3x10^4	0,2ml	i.p.	Peritoneum	limited PeritonealM+
		Mouse	BALB/cByJ	No	CT26-luc	Syngeneic	Injection	12G	3x10^4	0,2ml	i.p.	Peritoneum post irritation	extensive PeritonealM+
Chen, C et al. (50)	2018	Mouse	BALB/c	No	CT26	Syngeneic	Injection	-	2x10^5	0,2ml	i.p.	Lower Abdominal Cavity	Tumor induction model successful
Bettenworth, D et al. (33)	2016	Mouse	Athym. nu CD1	Yes	HT29	Human crc	Injection	30G	1x10^7	0,1ml	s.c.	L & R shoulder	Tumor growth +
		Mouse	Athym.nu CD1	Yes	Caco2	Human crc	Injection	30G	2x10^6	0,1ml	s.c.	L & R shoulder	No tumor growth
		Mouse	CD1 nu	Yes	Caco2	Human crc	Injection	30G	2x10^5	0,05ml	submucosal	Colon	No tumor growth
		Mouse	CD1 nu	Yes	HT29	Human crc	Injection	30G	2x10^5	0,05ml		Colon	No tumor growth
		Mouse	NOD/SCID	Yes	HT29	Human crc	Injection	30G	2x10^5	0,05ml	submucosal	Colon	Extensive tumor growth
Okazawa, Y et al. (28)	2018	Mouse	NOD/Shi-scid IL2Rγnull	Yes	not specified	Human crc	Transplant	-	Fragments	-	s.c.	L & R flank	PDX Development
		Mouse			PDX of Group 1	Human crc	Injection	22G	5x10^5	0,05ml	submucosal	Rectal mucosa	100% Tumor+
		Mouse			PDX of Group 1	Human crc	Injection	-	4x10^4	0,05ml	in organ	Spleen	LiverM+
Kusuoka, O et al. (51)	2018	Mouse	BALB/c	No	CT26	Syngeneic	Injection	-	1x10^7	-	s.c.	Scapular s.c. tissuer	Tumor induction model successful
Najah, H et al. (52)	2017	Mouse	BALB/c	No	CT26	Syngeneic	Injection	-	1x10^4	-	i.p.	Peritoneum	100% Peritoneal carcinosis +
Karas, J et al. (34)	2011	Rat	BD-IX rats	No	DHD/K12TRb	Rat colon cancer	Injection	23G	1x10^7	0,1ml	submucosal	Caecum	>90% Tumor+

2.2 Results and Discussion

In our research we have included 26 studies on small animal models of colorectal cancer from 2015 to 2024. We collected all possible data regarding methods and results and compiled three tables (see Tables 1–3) to visualize the information gathered. The following will be a review of this data, focusing on answering the aforementioned self-imposed questions.

"What animals were used in the studies?"

In a total of 26 reviewed and included studies, mice were used in 21 studies (80.7%). Of which 17 studies included only one mouse strain and 4 studies researched multiple strains. Mouse strains included BALB/c (n=12), C57BL/6 (n=5), athymic nu/nu mice (n=2), NOD/SCID (n=2), NSG (n=2) and CD1 nu (n=1). Rats were used in 4 studies (15.4%). Specifically rat strains included WAG/Rij (n=2), BD-IX (n=1) and RNU (n=1). One study included (3,8%), conducted research on New Zealand white rabbits (n=1). The data indicates that mice are the preferred animal model for colorectal cancer research, accounting for the majority of the reviewed studies. BALB/c and C57BL/6, which are immunocompetent strains, were mainly used in syngeneic models and allowed for the application of murine-derived cell lines (CT26 & MC38). These models could be used to study tumor-immune interactions and testing immunotherapy. In the reviewed studies immunocompromised strains like the athymic nu/nu, NOD/SCID and NSG mice were used in xenograft models due to their lack of immune response. This allowed for more successful engraftment of human CRC cells to observe tumor growth and metastasis without the possibility of rejection of foreign cells. Rats were less commonly used in the collected studies but they served a more important role in metastasis and surgical studies, where their larger size is advantageous. The single use of rabbits highlights their limited application in CRC research in comparison to mice and rats.

"Were the animals used in the study immunocompromised?"

The data from the review shows an even spread of the use of immunocompromised and immunocompetent animals. In 13 of the 26 included studies (50%) researchers used immunocompromised animals. Immunocompetent animals were used in 12 studies (46,2%) while 1 study (3,8%) conducted research on both models in separate cohorts.

Immunocompromised models, using strains such as athymic nu/nu, NOD/SCID and NSG mice allowed for the use of human CRC cell lines or patient-derived xenografts. These models lack functional immune systems, which prevents the rejection of human cells and allows for the study of tumor growth and metastasis in a controlled manner. Furthermore, these models allow the growth of a primary tumor subcutaneously and subsequent transplantation into the colon to achieve a more humanized cancer environment on which further research can be conducted. In studies using immunocompetent animals, mostly BALB/c, C57BL/6 mice or BD-IX rats, researchers introduced only syngeneic tumor cell lines, meaning that the injected tumor cells were derived of a colonic tumor from the same species of animal. This method can provide insight into interactions of tumor and immune system like immune evasion, tumor progression and response to inhibitor molecules from immunotherapies. The use of immunocompetent animals in cancer research provides a more similar tumor environment to that of human colon cancer than studies of immunocompromised animals. While the tumor induction rates in the reviewed studies for both modalities (immunocompetent and/or immunocompromised) were overall highly successful, in CRC research using animal models it is important to carefully select the appropriate model based on specific research goals.

"Which CRC cell lines were used for injection?"

In the reviewed studies wide variety of both human derived and syngeneic mouse or rat cancer cell lines were used. Human derived CRC cell lines (HCT116, LS-174T, HT29, SW620, WiDr, C2BBe1, LS1042 and Caco2) were used in 11 studies (42.3%) and mainly in studies including immunocompromised animals as explained above. Syngeneic mouse or rat derived CRC cell lines (CT26 or MC38 and CC531 respectively) were used in 13 studies (50%), all of which used immunocompetent animals for the testing. Two studies (7,7%) used both human derived and syngeneic CRC cell lines in separate cohorts. The most common syngeneic cell lines used in the reviewed studies were CT26 and MC38. CT26 is a undifferentiated colon carcinoma cell line derived from BALB/c mice. It exhibits rapid growth and readily metastasizes and further shares several features with aggressive human colorectal carcinomas. In CRC studies CT26 cell lines are used in syngeneic models to study tumor-immune interactions.(48) The second more common syngeneic cell line is MC38. It originates from C57BL/6 mice and forms a moderately differentiated adenocarcinoma cell line. MC38 has a high potential for mutations, showing similarities to hypermutated human CRC and is therefore sensitive to immune checkpoint inhibitors, providing a valuable model

for immunotherapy studies.(49) In the syngeneic rat models in the review the most common cell line was CC531. This cell line is derived from WAG/Rij rats and is commonly used to model colorectal cancer metastasis, particularly in studies focusing on peritoneal dissemination like in our reviewed studies.

In the studies inducing human derived cell lines there was a more diverse spread of cell lines than in the studies of syngeneic animal models. This could be seen as an expression of the general cancer treatment and research trend of more personalized medicine. The most common human derived cell line in our studies was HCT116. They originated from the colon of a 48-year-old Caucasian male with colorectal cancer and are highly relevant in cancer research due to a mutation of the KRAS gene and its signaling pathway. The cell line shows an epithelial-like morphology and is characterized by a high oncogenic aggressiveness which therefore makes it a potential model to mimic aggressive tumor phenotypes.(50) Another important human derived cell line in colorectal cancer research is HT29. The HT29 cell line was derived from the primary tumor of a 44-year-old Caucasian female with a colorectal adenocarcinoma. This cell line is sensitive to chemotherapeutics like 5-fluorouracil and oxaliplatin, which are part of standard treatment regimen for colorectal cancer.(51) In comparison to HCT116, the HT29 cell line have the ability to differentiate to different cell lines of colorectal cancer and can therefore mimic the diverse aspects of colorectal cancer in vivo.(50)

"What was the injection model?"

Injection models are crucial in this type of tumor research. They allow researchers to study tumor growth, immunologic reactions and metastasis depending on the type of injection model chosen. In the reviewed studies six different injection models where utilized depending on the primary research goal. A subcutaneous injection model was used in 12 studies, some of which had either multiple cohorts with the same injection model or a different model depending on the goals of the study. Subcutaneous injection is the most commonly used method in this review. Its simplicity and limited invasiveness makes it a highly reproducible method for animal research. Additionally, tumor growth can be easily observed or even measured and monitored externally which makes it suitable for studying general tumor progression and possible therapeutic responses. For the research on CRC, sub cutaneous injection and growth of tumor is primarily used to judge the success of introducing tumor cells into the animals without rejection (52) or to provide a primary tumor of which

fragments are subsequently transplanted to other test subjects (26). A glaring limitation of these types of studies in the CRC field is the lack of accurate replication of the natural tumor environment, as the most common injection site was a *subcutaneous pouch in the flank* of the animals nowhere near the actual occurrence site of colorectal cancer or its metastases.

Submucosal injection is the second most commonly used injection model in the 26 reviewed studies. In seven studies a submucosal injection of tumor cells was chosen, with most injections sites being in the submucosa of the rectum or the distal colon. This type of injection is especially useful for replicating tumors in their natural tumor environment, in the case of CRC being the distal colon and rectum. This can provide a more physiologically relevant model for studying tumor growth in the colon and its interactions with surrounding tissues as well as patterns of metastasis. While sub mucosal injection provides the most humanized environment in CRC research it is technically complex and can require specialized equipment. The injection of tumor cells into the submucosa of the colon also requires either direct access to the organ via the creation of a colostomy at the abdominal wall (28), a complex procedure with many possible side effects, or the access via the colonic lumen using a proper endoscope with a working channel. The latter of these procedures may show limited success in small animal models such as mice (33) and therefore primarily used in rat models. Additionally, tumor monitoring may be difficult, requiring advanced imaging techniques, bioluminescent augmented tumor cells, endoscopic or ultrasound observation.(29,37,40,44)

The use of intraperitoneal injection of tumor cells was used in five of the reviewed studies. This mode of injection aims to primarily establish a metastasis model of CRC. The direct injection into the peritoneum allows for easy spread of tumor cells within the abdominal cavity and to various organs. This model shows high potential of tumor induction in the reviewed studies, especially in the study of Taibi et al. (32) in which one cohort of BALB/c mice received an intraperitoneal injection of tumor cells post irritation of the injection site with a sterile cotton swab. This lead to “extensive peritoneal metastasis” providing a potential late stage CRC model. Another injection model for the establishment of a metastasis model of CRC is intravenous injection. In two of the reviewed studies tumor cells were injected intravenously. Thalheimer et al. (53) injected BALB/c nu/nu mice with human CRC derived tumor cells via the portal vein to establish a direct metastasis model in the liver and lungs with high success. However models like intraperitoneal or intravenous

injection require an operational access to the abdominal cavity as well as preparation of the peritoneum which increases strain on the animals and adds further possible complications to the procedure. Additionally, models like these do not replicate the natural localization of early stage CRC and therefore have limited use for studies targeting drug delivery or efficacy for CRC.

In several studies, six to be exact, researchers used a intra organ injection model. These models can be implemented to produce a metastasis model of CRC to study the tumor growth, organ specific metastasis characteristics and monitor the effectiveness of therapy. In the reviewed studies the most common locations for intra organ injections were the spleen and the liver of the animals. In almost all cases this type of model was implemented in immunocompromised animals. Wang et al. (37), Prieto et al. (38), Bae et al. (26) and Okazawa et al. (28) successfully developed a metastasis model of advanced metastasized CRC in immunocompromised cohorts using human derived CRC cell lines. The results of these studies show a high percentage of tumor induction in the injected organ as well as additional hepatic metastasis in models with intra splenic injection. Similar to the intraperitoneal and intravenous injection models, this type of model does not perfectly replicate CRC in all its stages. While it provides a successful way to induce CRC tumor metastasis in specific organs and therefore mimics a late stage CRC setting, a surgical access to the abdominal organs is required further complicating the procedure and study.

"What injection volume was chosen?"

In the reviewed studies, the choice of injection volume was an important parameter that influenced the success rate of tumor establishment, the accuracy as well as potential problems of the procedure and animal health. Injection volumes ranged from 0.05 mL to 0.2 mL with two outliers of 1 mL (31,40) depending on the injection model, concentration of cells and animal species. In our studies, a small volume of injection solution, 0,05 mL, was used for submucosal and intra organ injection models. These models had a high variation in tumor cell concentrations of the injected volume. Smaller volumes were usually used in cases where tissue damage could detrimental to the outcome of the study, like in submucosal models to prevent bursting the cecal wall, causing leakage of tumor cells or perforation. A volume of 0.05 mL could minimize the risk of over tensions and damage to the tissue especially in sensitive organs like the colon. Nevertheless, smaller volumes may be a limiting

factor to tumor induction and may thus necessitate higher cell concentrations to ensure induction and usability of the animal model.

Other models in the review chose an injection volume of 0.1 mL, particularly studies in which subcutaneous and intraperitoneal injection was exercised. In sub cutaneous models, this volume may provide a sufficient amount of cells to ensure a successful tumor induction at the site but at the same time may exacerbate the risk of tissue damage through overloading the injection site. A higher injection volume in intraperitoneal injection may facilitate better spreading of tumor cells and a higher potential for the formation of metastases. Volumes of 0.2 mL were occasionally used in models with sub cutaneous, intraperitoneal or intravenous injection. This volume was likely chosen when the dangers of tissue damage through over tension was negligible, an peritoneal metastasis model or a systemic metastasis model via the venous system was desired.(32,38,45,53) Helderman et al. (40) and Höhn et al. (31) used an injection volume of 1 mL both for subcutaneous and intraperitoneal injection in a rat model with successful results. This drastically larger volume is likely used due to the anatomically larger size of rats compared to the mice in the review.

"What injection concentration was chosen?"

The cell concentrations used for injection in the reviewed studies varied widely ranging from $1x10^4$ cells/mL to $1x10^7$ cells/mL of suspension. These differences in concentration are determined by a number of factors including the animal model, injection method and location, tumor model and the objective of the study. A number of studies have utilized lower concentrations, particularly for submucosal and intra organ injection models or when the aim of the study is a more gradual tumor growth or more localized tumor formation like a single metastasis. These concentrations ranged from $1x10^4$ cells/mL to $5x10^5$ cells/mL. Chang et al. (54) utilized a concentration of $3x10^4$ for their submucosal injections into C57BL/6 mice and Okazawa et al. (28) used $5x10^5$ cells/ml suspension for submucosal injection at the rectal mucosa in NOD/shi-scid mice.

Seven of the reviewed studies favored cell concentrations of $1x10^6$ cells/mL to $5x10^6$ cells/mL suspension, particularly for subcutaneous injection but also in models of intra organ, intravenous or intraperitoneal injection. Höhn et al. (31) utilized an subcutaneous injection model with a cell concentration of $1x10^6$ cells/mL in a WAG/Rij rat model to successfully induce tumors. In a study by Wang et al. two metastatic models of CRC were

developed in immunocompromised mice through the injection of the spleen with cell concentrations of 2,5x10^6 cells/mL, showing positive results of tumor induction in both cohorts. In another cohort study, published by Thalheimer et al. (53), three groups of BALBc nu/nu mice were injected intravenously in the portal vein with a solution concentration of 1x10^5, 1x10^6 and 5x10^6 cells/mL respectively. While the study produced successful metastatic models of CRC in liver and lungs, there was a significantly high mortality rate in the group with the highest injection concentration, 5x10^6 cells/mL.

Few studies used high cell concentrations of 1x10^7 cells/mL suspension in their research. Kusuoka et al. (46) established a syngeneic injection model in BALB/c mice with the aforementioned cell concentration and Bettenworth et al. (33) included a group of athymic nu CD1 mice in a human derived CRC model also with an injection concentration of 1x10^7 cells/mL suspension. Both studies resulted in a positive subcutaneous tumor induction. In a study by Karas et al. (34), BD-IX rats were injected with a syngeneic CRC cell line of a concentration of 1x10^7 cells/mL in a submucosal model with high success. In a study by Caetano-Oliveira et al. (55) on the other hand, RNU rats were injected with human derived CRC cells lines via a submucosal injection site at the colonic mucosa, producing only limited success of tumor induction in all three groups.

"What needle size was chosen?"

The needle size is closely tied to the type of injection model, cell volume and concentration being used in the research on animal models of CRC. While the range of needle size was large, from 12 Gauge to 33 Gauge, the majority of studies in the review utilized smaller needle sizes. Needle sizes of 25G-33G were used primarily in submucosal injection models. Paulson et al. (44) developed a submucosal injection model of a mouse with a 33G syringe and a injected volume of 0.1 mL with a successful tumor induction rate. A study by Uccello et al. (30) shared similar methodology and success rate with slight differences in the injected volume, 0.05 mL, and a syringe size of 32G. Okazawa et al. (28) on the other hand utilized a needle size of 22G for their submucosal injection model into the rectal mucosa of mice. While the outer needle diameter of 22G syringes is over three times as large as the outer diameter of 33G needles their success rate in tumor induction was similar to the aforementioned studies by Uccello et al. and Paulson et al.. In a different injection model, Thalheimer et al. (53) injected 0.2 mL of tumor cells into the portal vein of mice using a 32G needle, likely to minimize trauma of the central vein. Smaller needles are

advantageous for precise and localized injection models such as submucosal and intravenous injection. They reduce the risk of damaging sensitive tissue and allow for a more precise placement of tumor cells, especially in models where anatomical relevance is important for the goal of the study. However, the injection with such small needles in precise locations like the submucosa requires more experienced personnel. In one case a comparatively large needle size was chosen. Taibi et al. (32) utilized a 12G needle for their 0.2 mL injections in two cohorts of intraperitoneal injections and one cohort of subcutaneous injection. These larger needles can be used in cases where larger volumes of tumor cells or tissue fragments need to be injected to prevent clogging or if the location of injection does not need to be as precise like for a metastasis model. However the use of larger needles can increase the risk for tissue trauma and therefore should not be used in precise injection techniques such as submucosal injection.

2.3 Conclusion

In the field of colorectal cancer (CRC) research, the development and use of small animal models have been pivotal for advancing our understanding of tumor biology, metastasis, and therapeutic interventions. This review underscores the substantial variability in methodological approaches across studies, reflecting the diversity of research objectives and the complexity of CRC modeling. It highlights that there is no universally optimal method for implementing an injection model for CRC. Common practices identified include the use of immunocompetent animals with syngeneic tumor cells, immunocompromised animals with human-derived CRC cells, submucosal and low-volume injections, and larger-volume intraperitoneal or intravenous injections.

The review also identifies several limitations of currently implemented methodologies. These include a lack of standardization in tumor cell concentrations, significant variation in cell lines and needle sizes used, and incomplete reporting on critical methodological parameters such as injection volume and needle dimensions. Furthermore, there is a notable dominance of mouse models over rat models, likely influenced by differences in cost, ease of handling, and infrastructure requirements. This disparity, however, underscores the need to expand research into other animal models like rat models, which offer advantages such as anatomical scalability and the feasibility of applying more sophisticated techniques.

Mouse models, although well-established and successful, have seen little innovation in the last 15 years, particularly in CRC research. A paradigm shift toward exploring rat models may yield new insights and methodologies. For instance, Helderman et al. (40) and Höhn et al. (31) successfully developed immunocompromised rat models to study late-stage metastatic CRC through intraperitoneal syngeneic tumor cell injections. Similarly, Karas et al. (34) demonstrated the efficacy of submucosal injections into the rat caecum via laparotomy, achieving high tumor induction success rates.

An additional avenue for enhancing CRC modeling lies in refining the anatomical relevance of tumor induction. Most reviewed studies utilized subcutaneous injections at the animal's flank, a site that fails to mimic the microenvironment and tissue interactions of human CRC. While submucosal or intraperitoneal injections provide a closer approximation, they are associated with significant procedural challenges, including the need for surgical intervention, extended anesthesia, and the ethical concerns of increased physical and psychological stress on the animals. A promising alternative to such invasive approaches is the endoscopic injection model, which allows for precise and localized tumor induction while minimizing tissue damage. Paulson et al. (44) established an endoscopic mouse model with an 85% success rate in tumor induction via submucosal injection to the distal colon. However, the anatomical constraints of mice may pose challenges for expanding this approach, suggesting that rats, with their larger anatomical features, are better suited for such procedures. The feasibility of this approach has been demonstrated by Caetano-Oliveira et al. (55), who achieved positive tumor induction through submucosal injection of human CRC cells into the colonic wall of immunocompromised RNU rats.

The reviewed studies emphasize that endoscopic injection models, when refined, could provide unparalleled benefits, including the ability to monitor tumor growth non-invasively through video endoscopy. To ensure successful implementation of this technique, considerations include the use of small injection volumes (0.05 to 0.1 mL) to reduce tissue damage, standardized cell concentrations and fine needle gauges (30G to 33G) to minimize perforation risks. Furthermore, when utilizing human CRC cell lines (HCT116 or HT29), immunocompromised animal strains should be employed to avoid immune rejection, whereas syngeneic models would benefit from immunocompetent strains to explore immune-tumor interactions comprehensively.

In conclusion, advancing CRC research necessitates a strategic evolution in small animal modeling. This includes embracing rat models for their anatomical advantages, focusing on submucosal injection techniques for enhanced anatomical relevance, and leveraging endoscopic approaches to minimize invasiveness while maintaining precision. Standardization of methodological parameters across studies will further enhance reproducibility and translatability, ultimately bridging the gap between experimental CRC models and human disease.

References

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. 2021 Aug 15;127(16):3029–30.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May 4;71(3):209–49.
3. Li N, Lu B, Luo C, Cai J, Lu M, Zhang Y, et al. Incidence, mortality, survival, risk factor and screening of colorectal cancer: A comparison among China, Europe, and northern America. Cancer Lett. 2021 Dec;522:255–68.
4. Finlay A Macrae M. WebPage "https://www.uptodate.com/contents/colorectal-cancer-epidemiology-risk-factors-and-protective-factors." [cited 2023 Nov 14]. Colorectal cancer: Epidemiology, risk factors, and protective factors - UpToDate. Available from: https://www.uptodate.com/contents/colorectal-cancer-epidemiology-risk-factors-and-protective-factors
5. Herold G. Innere Medizin. Dr. med. Gerd Herold; 2024. 491–496 p.
6. Messmann H, Schnoy E. Klinische Gastroenterologie. Messmann H, editor. Stuttgart: Georg Thieme Verlag KG; 2021. 509–555 p.
7. Hofheinz RD, Arnold D, Borner M, Faber G, Folprecht G. Kolonkarzinom - Leitlinie. In: Onkopedia - https://www.onkopedia.com/de/onkopedia/guidelines/kolonkarzinom/@@guideline/html/index.html [Internet]. DGHO Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie e.V.; 2024 [cited 2024 Jun 13]. p. 1–48. Available from: https://www.onkopedia.com/de/onkopedia/guidelines/kolonkarzinom/@@guideline/html/index.html
8. El Moukhtari SH, Garbayo E, Amundarain A, Pascual-Gil S, Carrasco-León A, Prosper F, et al. Lipid nanoparticles for siRNA delivery in cancer treatment. Journal of Controlled Release. 2023 Sep;361:130–46.
9. Zhang MM, Bahal R, Rasmussen TP, Manautou JE, Zhong X bo. The growth of siRNA-based therapeutics: Updated clinical studies. Biochem Pharmacol. 2021 Jul;189:114432.
10. Alshaer W, Zureigat H, Al Karaki A, Al-Kadash A, Gharaibeh L, Hatmal MM, et al. siRNA: Mechanism of action, challenges, and therapeutic approaches. Eur J Pharmacol. 2021 Aug;905.
11. Isazadeh H, Oruji F, Shabani S, Behroozi J, Nasiri H, Isazadeh A, et al. Advances in siRNA delivery approaches in cancer therapy: challenges and opportunities. Mol Biol Rep. 2023 Nov 23;50(11):9529–43.
12. Bürtin F, Mullins CS, Linnebacher M. Mouse models of colorectal cancer: Past, present and future perspectives. World J Gastroenterol. 2020 Apr 7;26(13):1394–426.
13. Zigmond E, Halpern Z, Elinav E, Brazowski E, Jung S, Varol C. Utilization of murine colonoscopy for orthotopic implantation of colorectal cancer. PLoS One. 2011;6(12).
14. Duale Reihe: Innere Medizin. 4th ed. Stuttgart: Georg Thieme Verlag; 2018. 575–584 p.

15. Wolin KY, Yan Y, Colditz GA, Lee IM. Physical activity and colon cancer prevention: a meta-analysis. Br J Cancer. 2009 Feb 24;100(4):611–6.
16. Boyle T, Keegel T, Bull F, Heyworth J, Fritschi L. Physical Activity and Risks of Proximal and Distal Colon Cancers: A Systematic Review and Meta-analysis. JNCI: Journal of the National Cancer Institute. 2012 Oct 17;104(20):1548–61.
17. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. COLORECTAL CANCER. Nat Rev Dis Primers [Internet]. 2015 Nov 5 [cited 2023 Nov 14];1:15065. Available from: /pmc/articles/PMC4874655/
18. Henne-Bruns D. Duale Reihe: Chirurgie. 4th ed. Stuttgart: Thieme Verlag; 2012. 376–399 p.
19. Sun V, Grant M, McMullen CK, Altschuler A, Mohler MJ, Hornbrook MC, et al. Surviving Colorectal Cancer. Journal of Wound, Ostomy & Continence Nursing. 2013 Jan;40(1):61–72.
20. Silva K de A, Duarte AX, Cruz AR, de Araújo LB, Pena G das G. Time after ostomy surgery and type of treatment are associated with quality of life changes in colorectal cancer patients with colostomy. PLoS One. 2020 Dec 3;15(12):e0239201.
21. Layos L, Martínez-Balibrea E, Ruiz de Porras V. Curcumin: A Novel Way to Improve Quality of Life for Colorectal Cancer Patients? Int J Mol Sci. 2022 Nov 14;23(22).
22. Friedrich M, Aigner A. Therapeutic siRNA: State-of-the-Art and Future Perspectives. BioDrugs. 2022 Sep 23;36(5):549–71.
23. Sousa AR, Oliveira AV, Oliveira MJ, Sarmento B. Nanotechnology-based siRNA delivery strategies for metastatic colorectal cancer therapy. Int J Pharm. 2019 Sep;568:118530.
24. Wang S, Gao S, Zeng Y, Zhu L, Mo Y, Wong CC, et al. N6-Methyladenosine Reader YTHDF1 Promotes ARHGEF2 Translation and RhoA Signaling in Colorectal Cancer. Gastroenterology. 2022 Apr;162(4):1183–96.
25. Lwin TM, Turner MA, Amirfakhri S, Nishino H, Debie P, Cosman BC, et al. Rapid tumor-labeling kinetics with a site-specific near-infrared anti-CEA nanobody in a patient-derived orthotopic xenograft mouse model of colon cancer. J Surg Oncol. 2021 Dec 26;124(7):1121–7.
26. BAE KB, KIM SH, KANG MS, KIM DH. An Animal Model of Colorectal Cancer Liver Metastasis With a High Metastasis Rate and Clonal Dynamics. Anticancer Res. 2020 Jun 2;40(6):3297–306.
27. Thalheimer A, Otto C, Bueter M, Illert B, Gattenlohner S, Gasser M, et al. Tumor Cell Dissemination in a Human Colon Cancer Animal Model: Orthotopic Implantation or Intraportal Injection? European Surgical Research. 2009;42(3):195–200.
28. Okazawa Y, Mizukoshi K, Koyama Y, Okubo S, Komiyama H, Kojima Y, et al. High-sensitivity detection of micrometastases generated by GFP lentivirus-transduced organoids cultured from a patient-derived colon tumor. Journal of Visualized Experiments. 2018 Jun 14;2018(136).
29. Terracina KP, Aoyagi T, Huang WC, Nagahashi M, Yamada A, Aoki K, et al. Development of a metastatic murine colon cancer model. Journal of Surgical Research. 2015 Nov;199(1):106–14.
30. Uccello TP, Kintzel SA, Mills BN, Murphy JD, Garrett-Larsen J, Battaglia NG, et al. Development of an Orthotopic Murine Model of Rectal Cancer in Conjunction With Targeted Short-Course Radiation Therapy. Adv Radiat Oncol. 2022 Mar;7(2):100867.

31. Höhn P, Braumann C, Freiburger M, Koplin G, Dubiel W, Luu AM. Anti-tumorigenic Effects of Emodin and Its' Homologue BTB14431 on Vascularized Colonic Cancer in a Rat Model. Asian Pacific Journal of Cancer Prevention. 2020 Jan 1;21(1):205–10.
32. Taibi A, Albouys J, Jacques J, Perrin ML, Yardin C, Durand Fontanier S, et al. Comparison of implantation sites for the development of peritoneal metastasis in a colorectal cancer mouse model using non-invasive bioluminescence imaging. PLoS One. 2019 Jul 31;14(7):e0220360.
33. Bettenworth D, Mücke MM, Schwegmann K, Faust A, Poremba C, Schäfers M, et al. Endoscopy-guided orthotopic implantation of colorectal cancer cells results in metastatic colorectal cancer in mice. Clin Exp Metastasis. 2016 Aug 1;33(6):551–62.
34. Karas JR, Essani R, Haughn C, Uchal M, Bishawi MM, Bergamaschi R. Colonoscopic injection for murine solid cecal cancer model. Surg Endosc. 2011;25(9):2956–9.
35. Haughn C, Uchal M, Raftopoulos Y, Rossi S, Santucci T, Torpey M, et al. Development of a total colonoscopy rat model with endoscopic submucosal injection of the cecal wall. Surgical Endoscopy and Other Interventional Techniques. 2006;20(2):270–3.
36. Souris JS, Zhang HJ, Dougherty U, Chen NT, Waller J V, Lo LW, et al. A novel mouse model of sporadic colon cancer induced by combination of conditional Apc genes and chemical carcinogen in the absence of Cre recombinase. Carcinogenesis. 2019 Nov 25;40(11):1376–86.
37. Taniura T, Iida Y, Kotani H, Ishitobi K, Tajima Y, Harada M. Immunogenic chemotherapy in two mouse colon cancer models. Cancer Sci. 2020 Oct;111(10):3527–39.
38. Chang S. Generation of Colon Cancer Model Based on Colonoscopy Injection. Methods Mol Biol. 2021;2224:147–52.
39. Basirat E, Dehghan D, Abbasi A, Pakravan N. In vitro and in vivo Evidence on Intra-tumor Injection of Allogeneic Serum for Immunotherapy in a Mouse Model of Colon Cancer. Iran J Allergy Asthma Immunol. 2022 Oct 30;
40. Thalheimer A, Otto C, Bueter M, Illert B, Gattenlohner S, Gasser M, et al. The intraportal injection model: A practical animal model for hepatic metastases and tumor cell dissemination in human colon cancer. BMC Cancer. 2009 Dec 24;9(1):29.
41. Caetano-Oliveira R, Gomes MA, Abrantes AM, Tavares-Silva E, Oliveira MC, Laranjo M, et al. Revisiting colorectal cancer animal model – An improved metastatic model for distal rectosigmoid colon carcinoma. Pathophysiology. 2018 Jun;25(2):89–99.
42. Wang H, Wu X, Lezmi S, Li Q, Helferich WG, Xu Y, et al. Extract of Ginkgo biloba exacerbates liver metastasis in a mouse colon cancer Xenograft model. BMC Complement Altern Med. 2017 Dec 2;17(1):516.
43. Prieto V, Ludwig JM, Farris AB, Nagaraju GP, Lawal TO, El-Rayes B, et al. Establishment of human metastatic colorectal cancer model in rabbit liver: A pilot study. PLoS One. 2017 May 5;12(5):e0177212.
44. Spyridopoulou K, Aindelis G, Lampri E, Giorgalli M, Lamprianidou E, Kotsianidis I, et al. Improving the Subcutaneous Mouse Tumor Model by Effective Manipulation of Magnetic Nanoparticles-Treated Implanted Cancer Cells. Ann Biomed Eng. 2018 Dec 3;46(12):1975–87.

45. Helderman RFCPA, Restrepo MT, Rodermond HM, van Bochove GGW, Löke DR, Franken NAP, et al. Non-Invasive Imaging and Scoring of Peritoneal Metastases in Small Preclinical Animal Models Using Ultrasound: A Preliminary Trial. Biomedicines. 2022 Jul 1;10(7).
46. SILVA EF, FERNANDES BN, MARINELLO P, DEMINICE R, JUNIOR JCF, SOARES-LIMA SC, et al. Pre and Post-high-intensity Interval Training Delays Colon Tumor Onset in a Syngeneic Mouse Model. Anticancer Res. 2024 Mar;44(3):1209–17.
47. Kim HG, Huot JR, Pin F, Belcher DJ, Bonetto A, Nader GA. Metastatic or xenograft colorectal cancer models induce divergent anabolic deficits and expression of pro-inflammatory effectors of muscle wasting in a tumor-type-dependent manner. J Appl Physiol. 2022 Dec 1;133(6):1273–83.
48. Huot JR, Novinger LJ, Pin F, Bonetto A. HCT116 colorectal liver metastases exacerbate muscle wasting in a mouse model for the study of colorectal cancer cachexia. Dis Model Mech. 2020 Jan 1;13(1).
49. Paulson B, Kim IH, Namgoong JM, Kim YG, Lee S, Moon Y, et al. Longitudinal micro-endoscopic monitoring of high-success intramucosal xenografts for mouse models of colorectal cancer. Int J Med Sci. 2019;16(11):1453–60.
50. Chen CH, Kuo CY, Chen SH, Mao SH, Chang CY, Shalumon KT, et al. Thermosensitive injectable hydrogel for simultaneous intraperitoneal delivery of doxorubicin and prevention of peritoneal adhesion. Int J Mol Sci. 2018 May 4;19(5).
51. Kusuoka O, Fujiwara-Tani R, Nakashima C, Fujii K, Ohmori H, Mori T, et al. Intermittent calorie restriction enhances epithelial-mesenchymal transition through the alteration of energy metabolism in a mouse tumor model. Int J Oncol. 2018 Feb 1;52(2):413–23.
52. Najah H, Jouvin I, Besbes S, Cifuentes D, Eveno C, Pocard M. Specific computed virtual chromoendoscopy for detection of peritoneal carcinomatosis: an animal study. Surg Endosc. 2017 Oct 1;31(10):4034–43.
53. CT26.WT - CRL-2638 | ATCC [Internet]. [cited 2025 Jan 10]. Available from: https://www.atcc.org/products/crl-2638
54. Shields NJ, Peyroux EM, Ferguson AL, Steain M, Neumann S, Young SL. Late-stage MC38 tumours recapitulate features of human colorectal cancer – implications for appropriate timepoint selection in preclinical studies. Front Immunol. 2023 Apr 21;14.
55. HCT116 Cell Line - A Comprehensive Guide to Colorectal Cancer Research [Internet]. [cited 2025 Jan 10]. Available from: https://www.cytion.com/Knowledge-Hub/Cell-Line-Insights/HCT116-Cell-Line-A-Comprehensive-Guide-to-Colorectal-Cancer-Research/
56. HT-29: Human Colorectal Adenocarcinoma Cell Line (ATCC HTB-38) | Memorial Sloan Kettering Cancer Center [Internet]. [cited 2025 Jan 10]. Available from: https://www.mskcc.org/research-advantage/support/technology/tangible-material/human-colorectal-adenocarcinoma-cell-line-ht-29