*Pharmaceutical Sciences*, 2025, 31(1), 3-21
doi:10.34172/PS.024.40334
https://ps.tbzmed.ac.ir/

*Review Article*

# A DR4, DR5 Targeting Conjugated TRAIL Treatment for Colorectal Carcinogenesis: A Way to Future?

**Parikshit Roychowdhury[1], Indhumathi Thirugnanasambandham[1], Anindita De[2], Mirunalini Gobinath[3], Samanwita Khanra[1], Veera Venkata Satyanarayana Reddy Karri[1], Nihar Ranjan Bhuyan[4*], Gowthamarajan Kuppusamy[1*]**

[1]*Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Ooty, Nilgiris, Tamil Nadu, India.*
[2]*Department of Pharmaceutics, School of Pharmacy, JSS university Noida, India.*
[3]*Department of Pharmacognosy, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Ooty, Nilgiris, Tamil Nadu, India.*
[4]*Department of Pharmaceutical Chemistry. Himalayan Pharmacy Institute, Majitar, East Sikkim, India.*

**Article Info**

*Article History:*
Received: 24 Mar 2024
Accepted: 23 Sep 2024
ePublished: 22 Oct 2024

Keywords:
-Colorectal cancer
-Death receptors
-Drug conjugates
-TNF
-TRAIL

**Abstract**

TRAIL or tumor necrosis factor-related apoptosis-inducing ligand has been one of the major frontiers for the chemotherapeutic approach to treating carcinogenesis. Despite the emergence of TRAIL resistance cancer cell lines, it has been extensively studied for its unique property to induce apoptosis and provide specificity to any other conjugated chemotherapeutic agent. TRIAL highly reduces the dose and increases specific and targeted action against the cancer cells. It is a specific agonist for the death receptors DR4 and DR5 present on the cancer cell surface. Normal cells have more expression of decoy type of death receptors, which makes the use of TRAIL safer for regular cells. The TRAIL-drug conjugate systems have been under the radar due to their possible high synergistic potential and may open the door for the future cancer-specific targeted treatment frontier. This current study was conducted with a particular aim to provide a concise and simple amalgamation of current scenarios of different conjugations of this molecule along with various other molecules, RNAs, ligands, and anticancer drugs. Along with possible delivery systems of TRIAL that can have a significant future and the promise that is held by this particular way of cancer combinational chemotherapy with special interest in colorectal cancer.

## Introduction

Even though there have been numerous attempts to create novel strategies aimed at the therapy of cancer, it still eludes the grasp of researchers all over the world by remaining one of the major causes of death in the world. Present therapies for cancer include surgical removal of tumors and traditional radio and chemotherapy. The basics of these treatment options are either removal of the tumor limiting cancer cell division and/or promoting the death of cancer cells.[1] Despite numerous attempts and successes, cancer can notoriously be reoccurring and can build resistance to therapies. Huge efforts are being made to develop novel paths to increase the specific targeting and leap over tumors' resistance mechanism to current therapies.[2] Colorectal cancer (CRC) is termed the third leading newly diagnosed carcinoma on the planet and the major cause of death in the United States.[3-5] Most CRCs are diagnosed as adenocarcinoma, comprising about 90% of all recorded cases. Some of the other rare diagnoses include adenosquamous carcinoma, spindle cell carcinoma, squamous cell carcinoma, and undifferentiated carcinoma.[6] Genetic mutation and inheritance account for almost 35-40% of CRC whereas the majority, 60-65% cases recorded are sporadic, which is defined as occurring in individuals without any ancestral history of CRC.[7,8]

William Coley noticed that few sarcomas reduced in size with bacterial infections. This led to the first discovery of Tumor necrosis factor (TNF) for the first time.[9,10] The term Tumor necrosis factor was introduced much later on in the mid-20$^{th}$ century when it was found that shrinking of tumor size was observed as the direct result of the recruitment of one protein. This attribute of the protein led to the identifying term.[11] This protein led studies to potentially establish TNF as a target to induce apoptosis and search for similar molecules. Molecules like CD95 were tested for systemic use but this process shut down almost immediately due to severe reported hepatotoxicity. However, as it is said "third times a charm", another TNF superfamily (TNFSF) member, that was identified as Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL, also termed as Apo2L or TNFSF10) showed high selective induction of apoptosis in malignant cells without

*Corresponding Authors: Gowthamarajan Kuppusamy, E-mail: gowthamsang@jssuni.edu.in & Nihar Ranjan Bhuyan, Email: nihar.bhuyan@yahoo.com

causing adverse effects on normal cells like its predecessors namely TNF or CD95 agonists.[12,13] The present work aims to give insights into the pathogenesis, conventional and novel treatment options for CRC, as well as TRAIL and its various delivery techniques, and to have an insight into the future direction it may have.

## Methods

To bring the latest studies and research that have been done on CRC and TRAIL we conducted a thorough literature search, specifically the reputed databases such as Scopus, PubMed, and Google Scholar were used. A clinical study information search was conducted using clinical key by Science Direct and clinicaltrials.gov.in. The focus of the information search was till June 2024. The keywords that were emphasized during the search were 'TRAIL', "targeting' 'TRAIL formulations' 'Colorectal cancer'. Data on epidemiology and colorectal cancer were obtained through the use of WHO's open cancer database GLOBOCAN.

## Colon Cancer Epidemiology

CRC sits in the 3rd position in global incidence numbers as per WHO GLOBOCAN data (Figure 1). Breast and lung are the only other cancers having a higher incidence.[14] CRC is held accountable for almost 10% of total diagnosed carcinomas. According to GLOBOCAN20, CRC is the second most diagnosed cancer only trailed by breast cancer, and the third most diagnosed malignancy after lung and liver cancer in females and males respectively. However, mortality in females is almost 19% lower than in males, where mortality among all carcinomas in both males and females stands at around the 12-13% range.[14] Geographically, if we take numbers per 1,00,000 population CRC incidents are most in developed countries in the EU. The number of incidents reduces with the reduction in the human development index. However, considering the sheer number of cases diagnosed, China tops the board followed by the United States of America, Japan, Russia, and then India. Recent continuous development in countries like India and African nations has started a trend of the increased rate of CRC as well, and it is projected that the number of diagnosed cases of CRC in the world may

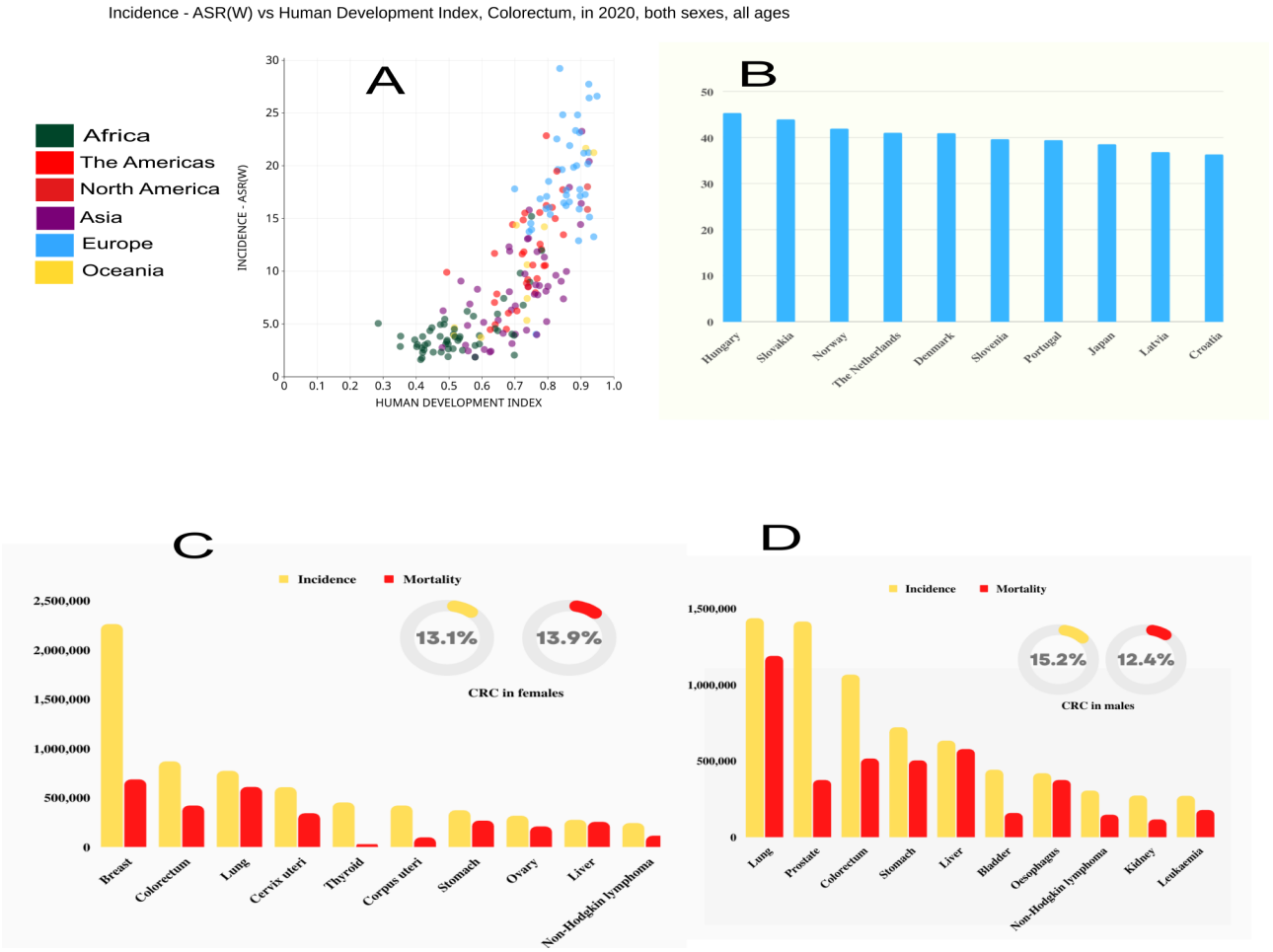


**Figure 1.** GLOBOCAN 20 statistics on cancer. 1A: relationship of human development index with the incidence of CRC, countries with higher development index showed high incidence of CRC, 1B: countries with highest CRC load (data is cases per 100,000 people), 1C: Incidence and mortality due to the leading carcinomas in females worldwide. 1D: incidence and mortality due to the leading carcinomas in males.

rise approximately to 2.5 million per year by 2035.[15]

### *Risk factors of CRC*

Epidemiological studies have shown male sex and advanced age to be two of the major risk factors for the progression and incidence of the disease. The growth and pathogenesis of CRC are influenced by both genetic and socio-environmental risk factors (Figure 2). >10% of all CRC cases reported have been directly linked with varying degrees of hereditary history, risk of developing CRC also changes with factors such as the number of family members affected in the past and the age when it was diagnosed.[16,17] Studies conducted by Czene *et al.*,[18] and Lichtenstein *et al.*[19] based on familial and twin study models it was observed that estimated CRC heritability is spread over a range of 12-35%. Various common single-nucleotide polymorphisms are successfully identified to date, which are held responsible for the higher incidence of CRC, but the majority of the hereditary factors that may influence CRC incidence or progression still require in-depth study as they continue to be a mystery for the researcher.[20] Out of all the reported cases of CRC, 5-7% only have been directly linked to a particular well-defined nucleotide polymorphism.[21] Individuals suffering from chronic inflammatory bowel disease (IBD) and patients who priorly have been diagnosed with either CRC or adenomas are always at higher risk of developing CRC therefore they should be subjected to a higher level of surveillance and monitoring of the problem for early detection[22,23] CRC linked with genes are often branched into two major terms, non-polyposis: which includes Lynch syndrome and familial CRC and polyposis syndromes. Due to the higher number of polyps present in the latter type, it is generally easily diagnosed and monitored by physicians with simple polyp examinations, however, the former type, especially Lynch syndrome is often misdiagnosed and stays hidden for the smaller number of adenomas resembling sporadic lesions. Patients who are diagnosed with Lynch Syndrome, have also been found to be at high risk for endometrial malignancies such as carcinomas occurring in the small intestine, ovaries, stomach, ureter, hepatobiliary system, and renal pelvic region.[24] Apart from the genetic risks and reduced presence of mismatch repair systems, several other factors contribute to the high incidence rate of CRC, though they are highly modifiable they continue to be the larger contributors to the increasing number of CRC identifications in the present scenario. Lifestyle habits such as smoking, chronic consumption of alcohol, sedentary lifestyle leading to higher body weight, dietary habits like consumption of a larger amount of red meat, and continuous processed food intake. Sometimes it is assumed that the association between CRC and type II diabetes is only due to an inactive lifestyle and obesity but factually it was seen that even after corrections of these problems, the

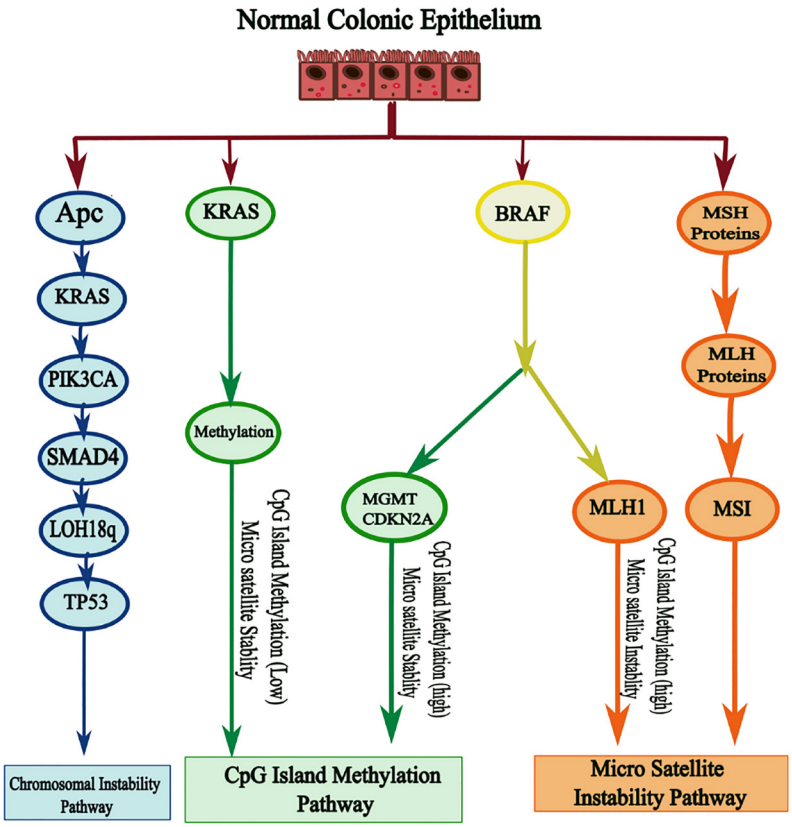


**Figure 2.** Representation of the 3 major pathways for CRC development.

individuals having type II diabetes have always been at high risk of CRC.[25-29] The research focused on intestinal flora revealed that chances of CRC were significantly higher in individuals infected with organisms such as *Fusobacterium nucleatum, Bacteroides fragilis,* etc.[30,31]

### *Pathogenesis of colorectal cancer*

Polyps are generally considered the starting point for most malignancies, polyp can be defined as a neoplastic precursor lesion or wound which is eventually evolved from an aberrant crypt. It is estimated that this polyp eventually progresses to CRC in 10-15 years. The majority of CRCs are assumed to have originated from a stem cell or stem-cell-like cell. Accumulation of chromosomal alterations at a genetic and epigenetic level results in the stem cells of cancer; they are seen at the base of the crypts formed in the lining of the colon and serve as the point of initiation, maintenance, and progress of the tumors[32,33] All of the CRCs in reported progress through one of the two pathways, 70-90% of the CRC develops through an adenoma-carcinoma pathway where the rest 10-20% have shown to have serrated neoplasia pathway containing distinct genetic and epigenetic factors and steps in a sequential pathway.[34] Lynch syndrome, being rather uncommon, consists of 2-7% of total cases, which progress through a microsatellite instability phenomenon.[15] The concise way of progression of CRC by different mechanisms is depicted in Figure 3, and the pathophysiology of CRC is depicted in Figure 4.

### *Subtypes of colorectal cancer*

CRC is subdivided into types based on molecular features; right-sided CRC is fairly distinct from left-sided CRC in patients; these two types are even dissimilar embryologically, biologically, and even by their tendencies of metastasis. The difference between them is increasingly being treated as a point of interest for prediction and as a marker of CRC progress and outcomes of therapeutics by different drugs like anti-EGFR molecules.[35,36] Consensus molecular subtypes or CMS classification of CRC was done in 2014, keeping gene expression as the foundation of the difference. This system divided CRC into 4 groups or subtypes: CMS 1 or MSI immune type, CMS2 or canonical type, CMS3 or metabolic type, and CMS4 or mesenchymal type.[37] CMS1 and CMS3 are the ones often seen in the right-sided CRC. At present, the choice of systemic treatment for CRCs is based on mutation status and sidedness of the tumors but CMS classifications are currently being explored in clinical trials as a novel prediction tool.[3]

### *Diagnosis of colorectal cancer*

#### *Clinical symptoms*

Despite having a limited number of visible symptoms, CRC can be an early detection by physical signs like rectal bleeding (though common for benign and malignancy), For patients aged over 45, any episode of rectal bleeding is a direct indication for colonoscopy examination, for patients within a lesser age limit, the factors are however broader such as blood mixed feces, sudden weight loss, anorexic conditions, and changes in bowel movement and it's the frequency.[38]

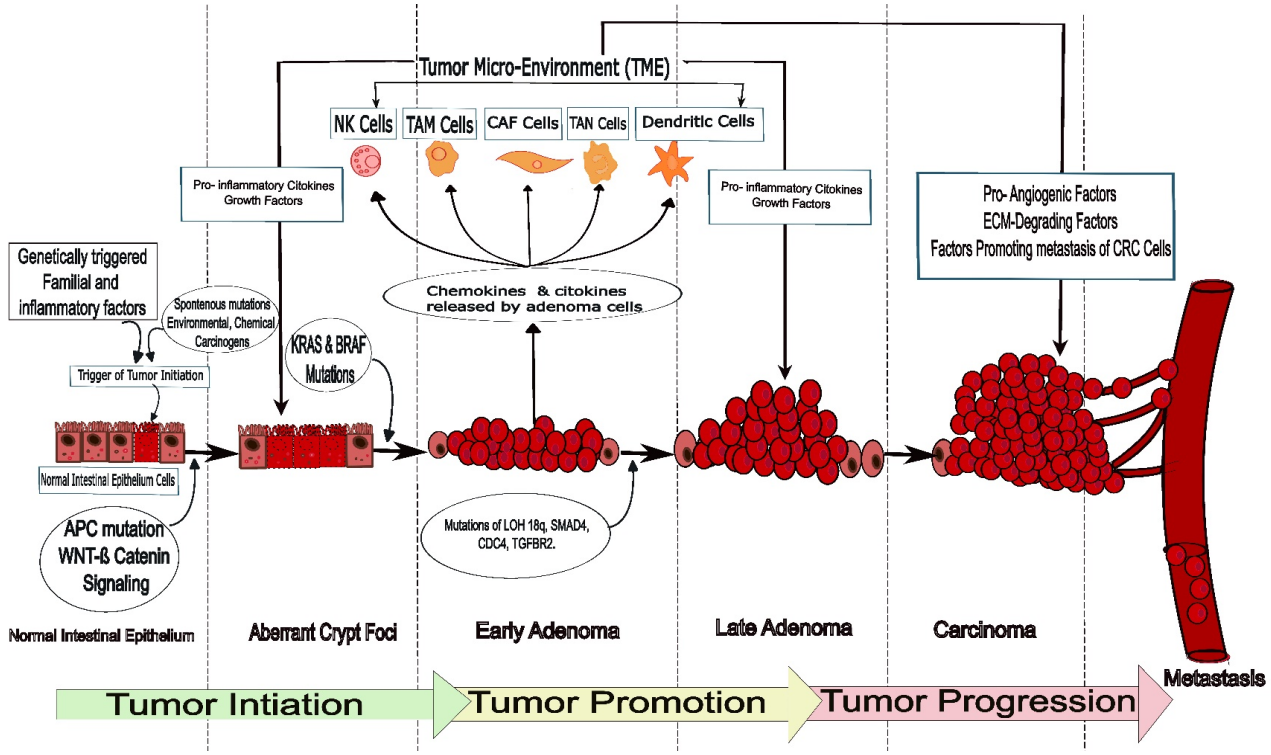


**Figure 3.** Detailed pathophysiology for initiation, promotion, and progression of CRC.

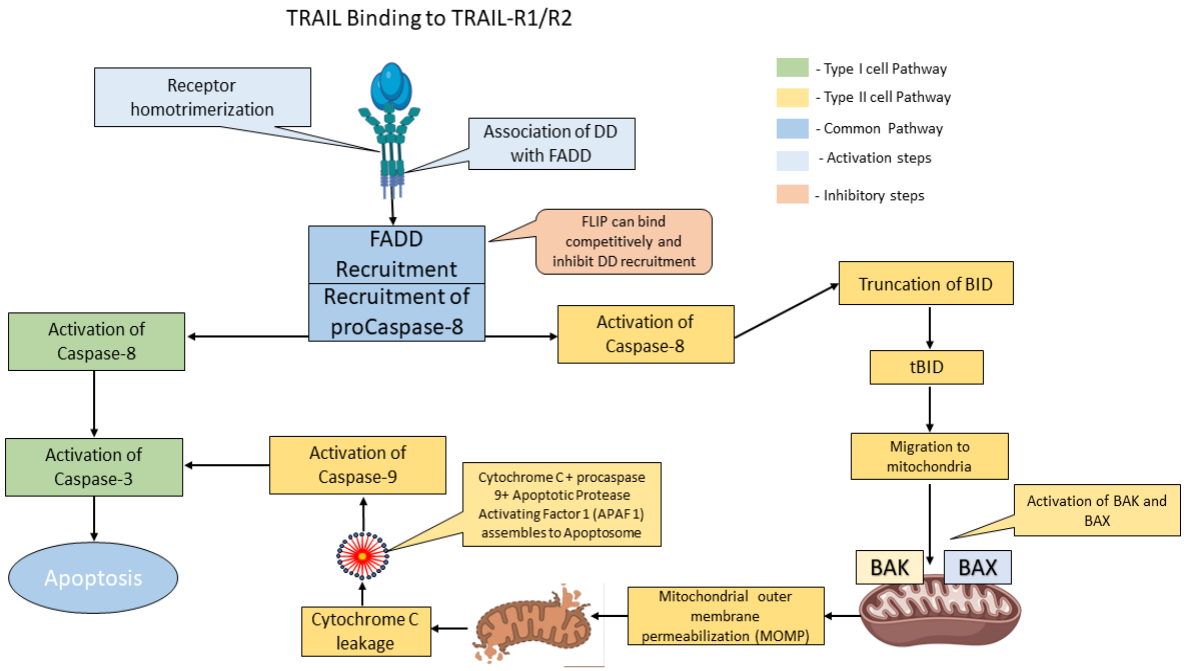


**Figure 4.** Schematic representation of the mechanism of TRAIL in apoptosis.

*Endoscopy technique*

Colonoscopy is the go-to method for diagnosing and detecting tumors and risk factors of CRC. An endoscopist can detect both, the presence of advanced lesions and subtle small lesions in mucosa which need to be subjected to extensive investigation of mucus membrane and in-depth study of bowel preparations.[39]

*Imaging techniques*

After colonoscopy examination, imaging acts as a secondary step to ensure accurate location mapping and distant staging of CRC. This is known as locoregional staging, it is done periodically by means of MRI which in turn helps to modify and change the therapeutic guidelines for individual patients. CT scanning and PET-CT imaging are gaining popularity in staging and evaluating the disease load. Though PET-CT use still faces a lot of debate. CT scanning is usually used for distant staging of the liver and lungs. MRI is used for lesions of the liver.[40,41]

*Laboratory assessment tools*

Laboratory techniques such as CBC (Complete Blood Count), and the concentration of carcinoembryonic Antigen are the generally recommended guidelines for the detection of CRC.[42]

*Histopathological screening*

Since the beginning of carcinoma identification, histopathology has been the major pillar for TNM staging and grading, Subtyping, and evaluation of metastasis. Tumor-based markers are nowadays gaining popularity, and implementation of mismatch-repair testing, and immunoscoring systems is helping in the early detection of Lynch syndrome.[43,44]

***Current available therapeutic strategies and their limitations***

*Endoscopic resection technique*

Due to increased surveillance and higher amount of screening of individuals from vulnerable groups such as people with genetic CRC history with reoccurring inflammation are now can be subjected to treatment by resection of the early malignant polyps in an en-bloc manner, several T1 cancer and even submucosal invasive cancers can be resected using the available techniques which include, a) en-bloc endoscopic mucosal resection b) endoscopic submucosal dissection, c) endoscopic full-thickness resection. The choice of the preferred technique is dependent on the extent of invasion in the submucosal layer and the staging of the malignancy, studies have indicated that this technique can be both affordable and safer than that of surgical removal however still many do not get the chance to discuss the opportunities with their caregivers. The procedure is a demanding technique that requires highly trained and skilled professionals therefore it is only advised to be carried out in well-established centers with proper manpower.[45,46]

***Surgical management***

When it comes to treatment to cure CRC, surgical removal is at the top and the forefront of the list. Different methods are used for different locations of CRC. For colonic malignancies, laparoscopy has established itself as the standard go-to procedure in many countries throughout the world as the technique has shown enhanced short-term

benefits and effectiveness in clinical trials. The second and most common technique is by dissecting sharply along embryological planes using the principles of mesocolic excision.

However these processes possess a potential issue of lack of specialization and adequate training, another locus for controversy around the surgical procedures is the amount of extent of the lymphadenectomy between the two types, that is extensive D3 type and the more limited D2 type excision. The latter might even add to the morbidity risk factors. Carcinomas of the rectum are even hard to remove surgically due to the accessibility problem and complex structure and positioning of the pelvis, the standardized procedure for rectal malignancies is termed total mesorectal excision, the extent of the procedure is generally determined by the involvement of the sphincter in malignancies and the surrounding tissues. There is still debate about using laparoscopic procedures in rectal cancer.[47,48]

### *CRC radiotherapy*

It has been established by several trials that preoperative radiotherapy can be better than postoperative ones, especially in reducing the risk of recurrences. Even though it entirely depends upon the stage of cancer, in recent times, radiotherapy has seen its use being maximized in the cases that are termed as medium to high risk keeping MRI staging as the basis. Where the place of most used therapy is held by chemoradiotherapy consisting of a dose of "45-50 gray in 25-28 fractions", using fluoropyrimidine in the role of radiation sensitizer. For tumors in the rectum, generally short course radiotherapy is implied, especially in the European Union. At present, chemoradiotherapy is followed by close observation of every clinical response of the patient, which gave rise to the new approach of preserving colon treatment. Most of the early detection cases are treated with radiotherapy, if not, total mesorectal excision is preferred singularly. This showed a trend of preservation of the colon for 50-60% of the patients however the rest still need to ultimately go for surgery and overtreatment of radiotherapy causing severe disruption of colonic structure.[49,50]

### *Local treatments for metastatic CRC*

With recent studies, there has been an increase in the number of available local therapies for the treatment of stage IV CRC. They are now applicable to several patient categories. Local therapies are developed keeping long-term treatment and possibly mitigating the disease. High tumor morbidity is reported with advanced technological innovations in localized therapies. When CRC is metastatic, and localized therapies are required for the metastases, for the liver, ablative therapy with systemic treatment is the preferred option most of the time. Mostly chosen is radiofrequency ablation for liver and percutaneous applications. Stereotactic radiotherapy and microwave-assisted ablation are the ones that are preferred in the case of larger lesions and those that are associated with vascular structures. The condition that is most debated is the treatments for the metastases in the lungs, in this regard, stereotactic radiotherapy, surgical resection, and ablation, all are viable choices of therapy. Invasion of tumors in the peritoneum is generally regarded as untreatable but cytoreductive surgery and hyperthermic chemotherapy have been proven to reduce mortality in such cases.[51,52]

### *Systemic chemotherapeutic approach*

A MOSAIC study in 2009 proved that the inclusion of oxaliplatin with fluorouracil or capecitabine improves the survival rate for poorly differentiated and high-risk T4-type carcinomas. This soon became the new standard for chemotherapeutic approach but the addition of oxaliplatin brought the serious problem of cumulative sensory neuropathy. The benefits of adjuvant therapies are ruled out if there is a presence of dMMR (DNA mismatch repair) as a marker in patients with stage II tumors. A similar approach of therapy is followed even for patients with rectal cancer. One more controversy or drawback of adjuvant chemotherapy in rectal cancer is, that drugs like irinotecan and biological agents that work well in a metastatic situation have failed to show efficacy in an adjuvant therapy setting. Earlier, 6 months of chemotherapy as an adjuvant approach was followed as the standard, in the year 2006, the IDEA (International Duration Evaluation of Adjuvant Chemotherapy) collaboration was established conducted a randomized clinical trial with 12,834 participants and concluded that the duration of adjuvant chemotherapy should be 3 months for efficacy and reduced toxicity, especially reduction in chances of cumulative neuropathy.[53-56] For the cases of metastatic CRC, the treatment regime includes a base of a chemotherapeutic drug, such as fluoropyrimidines, oxaliplatin, and irinotecan, on top of the base, a biological agent like anti-VEGF (vascular endothelial growth factor) or anti-EGFR (epidermal growth factor receptor) antibodies are added based on patient and tumor-specific factors. Several lines of similar regimens are likely added to the therapy for patients with metastatic CRC.

### *CRC and miRNA*

MiRNAs usually are heavily protected in every species due to their high importance in protein translation and its regulation, they are involved as versatile modulators in the progression and generation of CRC in inflammation, apoptosis, cell cycle mediators, cellular migration, stress response, as well as in pathogenic processes such as chemoresistance, chemosensitivity, etc. In recent years various miRNAs have been studied and were found to be effective in the treatment of CRC. miRNA-1 was seen to modulate the MAPK and PI3/AKT pathway thus causing suppression of EMT transition. UHRF1 was found to be regulated by the presence of miRNA-9. Downregulation of the BCL2 and SOX2 was seen in the treatment comprising

miRNA-15a when given with 5 fluorouracil (5FU), synergistic action was seen in the case of miR-22, and it was confirmed that this miRNA enhanced the sensitivity of CRC to the treatment regiment of 5 FU. The miRNA-30 group (miRNA 30a, 30a-5p,30b) showed targeting of the insulin receptor, ITGB3, KRAS, PI3CD as well as BCL2. MiRNA 203 delivered anti-CRC action by regulating ZNF217 and EIF5A2.[57]

### *Biologics and other therapy options*

The first approved active biological agent targeting angiogenesis was Bevacizumab, having an anti-VEGF action. It showed significant improvement in survival rate among all types of CRC patients in a clinical trial conducted in 2004.[58] This was followed by agents like aflibercept and ramucirumab. CRC sidedness plays a major role in the determination of biological therapy because of their different origin, for example, anti-EGFR antibodies are practically rendered useless in right-sided CRCs as a first line in the metastatic situation. Apart from that, CRCs are tested for RAF and RAS i.e., NRAF, KRAS, and BRAF mutations before the suggestion of any anti-EGFR therapy. In the current CRC scenario, left-sided metastatic CRC agents such as cetuximab or panitumumab (anti-EGFR), or bevacizumab from the anti-VEGF category are chosen as optimum first-line therapy.[59,60]

Monoclonal antibodies, combined with chemotherapy and MEK inhibitors have emerged as highly effective in many clinical trial settings and therefore are now included in standard guidelines. Newer agents approved for metastatic CRC include a dirty tyrosine kinase inhibitor, regorafenib, and a combination dosage of trifluridine and tipiracil, coded as TAS-102 which acts as an oral anti-metabolite. They are effective specifically for those patients who are non-responsive toward the first-line systemic approach of treatment.[61,62]

## Nanotechnology in the Treatment of CRC

The major drawback that has been hitting the chemotherapeutic approach of treatment of both metastatic and non-metastatic colorectal malignancies has been the excessive toxicity and array of adverse reactions that are associated with them. Therefore, for the past four decades, researchers have been heavily dedicated and directed towards the exploration of pharmaceutical nanotechnology as the basis of diagnosis and therapy of CRCs. Nanotechnology, in general nanoparticles (NPs) in pharmaceuticals, is preferred due to their high level of compatibility and suitability, that is it is very easy to manipulate and diversify them structurally altering their biological properties suiting them for any of the tasks like staging, treating, and diagnosing CRC.[63] When it comes to drug molecules, the application of nanotechnology mostly resonates with the enhancement of solubility, therefore increasing bioavailability and absorption, giving the molecule a higher degree of stability, and enabling it to be target-specific and even attributed to the property of controlled release.[64] The higher ease of modification also enables us to tag and modify the surface of nanoparticles with various organic and chemical agents to design them as multi-targeting formulations, but also to overcome the issue of acquired drug resistance due to their size, they will not require any transport mechanism to enter the cells. With NPs it is easy to target receptors or proteins present in the intracellular domain that is inside the cytoplasm or nucleus.[65] Nanoparticles that have been proven to show actions against CRC majorly fall under 3 classes, polymeric, metallic, and organic NPs. Each class comes with its unique set of advantages and among these 3 classes, 7 different types of NPs are of high interest, namely iron oxide NPs, quantum dots, polylactic-co glycolic acid (PLGA) NPs, dendrimers, silver (Au) NPs, Carbon nanotubes, exosomes, novel bionics or cell bases carriers (red blood cells, stem cells, bacteria, platelets, neutrophils) and liposomes when it comes to colorectal malignancy management.[66,67]

## Origin of TRAIL

TNF-related apoptosis-inducing ligand (TRAIL), also called Apo2 ligand or Apo2L, was first described as a molecule that can induce apoptosis in a Fas (Apo1 receptor of TNF family)-independent manner[68] Later it was revealed that the apoptosis is through Fas-associated proteins. When it was first described, TRAIL was found to be a member of the TNF superfamily (SF). The interest in TRAIL sparked when it was reported that it induces apoptosis through attachment with one of two death receptors (DRs) namely DR4 and DR5,[69] while it spared the normal vital cells in the body. This has led studies to develop TRAIL, TRAIL receptor (TRAIL-R), and TRAIL conjugating antibodies for decades to develop a tumor-specific targeted therapy. As far as the physiological effects of TRAIL are concerned, it's associated with cytotoxic effector cells and helps in homeostasis as a mediator in effector immune cells for the "activation-induced cell death" (AICD) pathway.[70,71]

## Chemistry of TRAIL

Structurally TRAIL can be described as a type 2 transmembrane protein consisting of 281 amino acids and a predicted molecular weight of 32.5 kDa, but when the moiety is matured and fully glycosylated the molecular weight is predicted as 41 kDa.[72] A TRAIL monomer is said to contain two antiparallel β-pleated sheets which form a β sandwich that is situated as a core scaffold, and it interacts with the nearby subunits in a head-to-tail manner to make a bell-shaped homo-trimer, that is protein unit containing three identical polypeptide chains. The two ends of this, named "bottom" and "top" are wide and narrow respectively. Highly disordered loops make the top unit, β strands A", A, H, C, and F are the ones that form the β inner sheet responsible for the intersubunit contacts, and the strands B', B, G, D, and E form the outer β sheet. The arrangement of the TRAIL homotrimer is such that one end of the β sandwich in each subunit is packed against the inner sheet of the adjacent subunit. TRAIL exhibits

a relatively high content of aromatic residues (17% of total residues). Arene remnants, being eight in number namely, Histidine-125, Phenylalanine-163, Tyrosin-183, Tyrosin-185, Tyrosin-189, Tyrosin-243, Phenylalanine-274, and Phenylalanine-278 are the ones that are present on the surface of the inner sheet and furnish a hydrophobic stage for substantial extremity interactions between adjoining subunits.[73]

## Receptors of TRAIL

TRAIL is unique among all the TNFSF members, out of which 4 are membrane-bound receptors and one is a soluble receptor. Their names are DR4/TRAIL-R1, DR5/TRAIL-R2,[74] DcR1/TRAIL-R3,[69,75] and TRAIL-R4/DcR2.[76] Apart from these four, another soluble receptor for TRAIL has been described in the late 1990s, called osteoprotegerin, interestingly, this was first believed to be a receptor for RANKL/OPGL. Later, it was shown to have binding with TRAIL,[77,78] Among these receptors, only the DR4, and DR5 are the ones which are known to have cytoplasmic death domain (DD) which is responsible for the action of evocation of apoptosis, the other receptors, however, do not possess any such function on programmed cellular death. These receptors have also been mentioned as decoy receptors. The reason for their non-functionality is that DcR1 lacks an active cytosolic death domain region, and DcR2 has a truncated, cytoplasmic Death Domain that is also nonfunctional. It remains a turbid illusion among researchers of the possible physiological role of osteoprotegerin.[79] TRAIL, as a Receptor Target for Cancer, so many researchers revealed the various mechanisms that aid or control cell death. Dulanermin and SCB-313 have recently been investigated as an agonist for TRAIL.

## Mechanism of TRAIL in the Induction of Apoptosis

Details have been unveiled in the past decade regarding the mechanism of TRAIL-induced apoptosis, we now have a clearer view of the entire picture, upon triggering of death receptors by TRAIL, the receptors TRAIL-R1/R2 go through homo trimerization. This leads to the recruitment of their intracellular part or Death Domains (DD) to activate pro caspase 8 via the death effector domain as a direct result of activation of FADD (FAS-associated death domain protein). The entire process is to create DISC (death-inducing signaling complex) to further employ pro-caspase-8. The activated form of Pro-caspase-8 is a dimeric entity that is then liberated to break down and activate the effector caspase 3, thus initiating apoptosis in a certain type of cell (type I).[80] For the other type of cells (Type II) activation of the mitochondrial pathway is another necessity to initiate the movement of apoptosis reactions. In this cascade of events, the Pro-caspase-8 breaks down the BH3 Interacting domain death agonist (Bid), which is followed by oligomerization of BAK and BAX (BCL2 antagonist/Killer and BCL2 associated X respectively) takes place in the outer mitochondrial membrane which leads to the formation of pores. Facilitating the release of cytochrome C takes place and in conjunction with apoptotic peptidase activating factor 1 (Apaf-1) and pro-caspase-9 forms an assembly structure known as the apoptosome. This structure further goes on to activate the various other effector caspases and even increases the cleavage of caspase-3. This entire mechanism results in a superfluity of the destruction of cellular proteins that induces apoptosis.[81-83] The mechanism of TRAIL is depicted in Figure 4 in a simplified way.

## TRAIL in the Anticancer Action Plan and Clinical Trials

Two major forms of TRAIL are present in our system, soluble TRAIL (sTRAIL) and membrane-bound TRAIL. When it comes to strategies for delivering the TRAIL for anti-cancer action there have been 2 approaches that are pursued in clinical trials, they are recombinant s-TRAIL like Apo2L.0 or AMG-951 also called dulanermin and TRAIL-R targeting agonistic antibodies. Among these, the latter treatment showed promising results in pre-clinical studies but when it comes to human trials antibody treatments have failed to induce apoptosis. This is because most of the tumors get resistance against TRAIL during ongoing therapy.[84,85] The second hurdle faced in TRAIL was the discovery of non-apoptotic pathways. Trauzold *et al.*[86] showed that TRAIL-R agonist treatment helped in inducing metastases the in liver for an animal xenograft model of pancreatic adenocarcinoma. In another study, the possibility of TRAIL being exploited by malignant cells to increase proliferation and invasion.[87] To overcome this problem TRAIL deliveries are designed in combination with sensitizing agents. With proper caution sensitization of vital normal cells against cells must be avoided. Drug delivery systems can be designed for TRAIL keeping a few shortcomings in mind as explored in the clinical trials for Apo2L.0 or dulanermin such as rapid clearance, reduced plasma $T_{1/,2,}$ and the problem of low accumulation of TRAIL-Death receptors. When it comes to TRAIL-R antibodies, despite having a higher plasma half-life, practical problems were observed when the antibodies failed to accumulate near the DRs cause of a lack of external crosslinking.[68,88] Various TRAIL-R antibodies, recombinant, TRAIL, and multivalent molecules have entered clinical trials for various malignancies and are listed in Table 1.

TRAIL-induced apoptosis is also sensitive to synergistic action by other drugs, Caldiran *et al.*[89] showed that the combination treatment of bortezomib and epirubicin can enhance the TRAIL-sensitized apoptosis via upregulating the death receptors in CRC. 5-Fluorouracil and genistein can enhance DR4, DR5 regulated TRAIL-induced apoptosis via XIAP, DcR1, and MMP reduction, enhanced ROS.[90]

## TRAIL Formulations for the Improved Mechanism

Formulators have designed the TRAIL drug delivery system to lead over the main 2 hurdles, (I) reduced stability and (II) less accumulation in DRs, to overcome these problems modification in valency with stability and conjugations to

**Table 1.** Details of clinical trials conducted for R-TRAIL and TRAIL-R antibodies for various types of malignancies, data obtained from clinicaltrial.gov.[91]

| Drug Name | Disease for trial | Mechanism of action | Trial design | Trial ID |
|---|---|---|---|---|
| | | **Trail-R agonistic antibodies** | | |
| Mapatumumab | Advanced hepatocellular carcinoma | Monoclonal antibody targeting TRAIL-R1 | A randomized, multicenter, blinded, placebo-controlled study | NCT01258608 |
| Mapatumumab | Multiple myeloma | Monoclonal antibody targeting TRAIL-R1 | Multi-centre, open-label, randomized study | NCT00315757 |
| Mapatumumab | Relapsed or refractory non-Hodgkin's lymphoma | Monoclonal antibody targeting TRAIL-R1 | A multi-center, open-label, dose-escalation study | NCT00094848 |
| Mapatumumab | Advanced non-small cell lung cancer | Monoclonal antibody targeting TRAIL-R1 | Randomized, Multi-Centre, Open-Label Study | NCT00583830 |
| Tigatuzumab | Metastatic or unresectable non-small cell lung cancer | Monoclonal antibody targeting TRAIL-R2 | Randomized, double-blinded, placebo-controlled | NCT00991796 |
| Tigatuzumab | Pancreatic Cancer | Monoclonal antibody targeting TRAIL-R2 | Phase 2 multi-center, open-label study | NCT00521404 |
| Tigatuzumab | Metastatic triple-negative breast cancer | Monoclonal antibody targeting TRAIL-R2 | An open-label, randomized study | NCT01307891 |
| Conatumumab | Pancreatic cancer | Monoclonal antibody targeting TRAIL-R2 | A randomized, double-blind study | NCT00630552 |
| | | **Multivalent antibodies** | | |
| Gen1029 | Colorectal cancer non-small cell lung cancer triple negative breast cancer renal cell carcinoma gastric cancer pancreatic cancer | 1:1 mixture of two humanized noncompeting DR5- specific mAbs, each carrying an E430G epimerization enhancing mutation | Randomized, open-label, multicenter study | NCT03576131 |
| | | **Recombinant TRAIL** | | |
| Dulanermin | B-Cell non-Hodgkins lymphomas that have progressed following previous rituximab therapy. | Recombinant TRAIL triggering apoptosis via activation of DR4 and DR5 | A randomized, open-label, multicenter study | NCT01258608 |
| Dulanermin | Previously untreated stage IIIb/IV non-small cell lung cancer (NSCLC) | Recombinant TRAIL triggering apoptosis via activation of DR4 and DR5 | A multi-center, open-label, randomized study | NCT00508625 |
| Dulanermin | Advanced non-small cell lung cancer | Recombinant TRAIL triggering apoptosis via activation of DR4 and DR5 | A randomized, double-blind, placebo-controlled study | NCT03083743 |
| | | **Trail derivates and modifications** | | |
| MSCTRAIL | Non-small cell lung cancer (NSCLC) | Targeted stem cells expressing TRAIL | Multicentre, randomized double-blind placebo-controlled | NCT03298763 |

increase specificity in carcinoma targeting. Engineering of different such formulations.[71]

### *Formulations enhancing stability*

The first problem that hindered the bioavailability in TRAIL monotherapy was the unstable nature of the molecule. This issue was addressed by engineering various moieties along with TRAIL or recombinant TRAIL or TRAIL-R antibodies over the years. The first TRAIL recombinant was however not to enhance stability but to facilitate the purification process, it was the THD or TNF homology domain bound with poly-histidine at the N-terminus of the amino acid chain, termed as His-TRAIL[68] and the second recombination was a short octapeptide, having a sequence of DYKDDDDK, also called as FLAG tag bound at the same site,[92] singularly it showed poor activity but when tagged with M2 antibodies. It indicated high efficacy in in-vitro

and in non-human models but it has high hepatocellular cytotoxic activity when tested against isolated human liver cells that limited the use of macromolecule tagged TRAIL recombinant forms. To date, the only approved recombinant TRAIL molecule is dulanermin of Apo2L.0 which is an untagged soluble residue of TRAIL containing amino acids from 114 to 281.[12] Dulanermin even though it was taken to clinal trials, showed a significant amount of issues with stability and bioavailability, including distribution $T_{1/2}$ of about 3-5 minutes and elimination of half $T_{1/2}$ of an estimated 20 minutes.[12]

Addressing these issues, several strategies have been followed over time for formulations, such as the addition of a trimerization motif to enhance stability at the N terminus of the chain. Creation of single chain TRAIL (scTRAIL), the strategy behind this is to form the TRAIL by translation of a single sequence of side-by-side extracellular TRAIL domains which are then ligated in a head-to-tail coupling manner with linkers in between the domains. This not only created a highly stable trimer but also reduced hepatotoxicity was observed, surprisingly some of the resistant tumors against dulanermin also was inhibited by this approach.[12,93,94] The third strategy for improving stability is the linkage of known ligands such as human serum albumin or PEG.[95,96] Details of formulations tackling stability issues are given in Table 2.

### *Formulations enhancing targeting*

Most of the primary malignancies are TRAIL resistance and chemotherapeutic drugs are not tumor-specific, these two problems together gave rise to targeted chemotherapy with TRAIL. Chemotherapeutic drugs enhanced TRAIL sensitization in malignant cells where the TRAIL provided the agents a specified target, therefor conjugation of TRAIL or TRAIL-R antibodies along with chemotherapeutic drugs in a nanomedicine format opened a new era of target-specific cancer therapy for different malignancies. 2 major targeting modes have been pursued in this case, they are actively targeting, where fragments of biological macromolecules are used to target TRAIL towards the specific tumor by using the surface proteins as antigens, and passive targeting based on enhanced permeability and retention (EPR) effect.

#### *Active targeting of TRAIL*

Active targeting approach is defined as, the utilization of TRAIL as the apoptosis-inducing agent by combining it with biological macromolecules or motifs that can target specifically the tumor of interest. In this regard the first candidate that comes into mind are antibodies, but the large molecular weight of whole immunoglobulins (150KDa) increases steric hindrance, therefore, making them less than useful when it comes to conjugation with TRAIL, this was the point that single-chain variable fragment (scFv) of immunoglobulins that have the same targeting profile in 1/6$^{th}$ of the size of the full immunoglobulin (25KDa) that facilitates in easy fusion with biological molecules like TRAIL. Molecules such as "melanoma-associated chondroitin sulfate proteoglycan" or MCSP, CD19, etc. are examples of such fusion drug deliveries.[104,105]

Fn14 is the receptor for TNF-related weak inducer of apoptosis (TWEAK) or Apo3L, a fusion of peptides that correspond to the extracellular part of the receptor Fn14 with TRAIL is another approach of engineering of the delivery system of TRAIL with active targeting.[106] The formulations used for active targeting of TRAIL in CRC are summarized in Table 3.

MicroRNAs (miRNA) are often found dysregulated in cancers, especially in TRAIL resistance cell lines. Another approach to achieving synergy and increasing the effectiveness of TRAIL in those cancers includes combination therapy of miRNA with TRAIL. miRNA-128 (miR-128) is a specific miRNA that has been seen to be under-expressed in the case of colorectal cancers. Under-expression of miR-128 causes over-expression of SIRT1, SIRT1 is a cellular histone deacetylate. SIRT1 overexpression increases the expression of superoxide dismutase, thereby reducing the ROS (reactive oxygen species) stress and hence promoting growth in cancer cells by suppression of apoptosis. The combination treatment of miR-128 with TRAIL provided a balance in depleted miR-128, which resulted in downregulation of SIRT1,

**Table 2.** Formulations of TRAIL to enhance stability.

| TRAIL type | Combined molecule | Formulation type | Ref. |
|---|---|---|---|
| TRAIL homotrimer | leucin zipper | Chimera, recombinant TRAIL | 97 |
| TRAIL homotrimer | Iso-Leucine zipper motif | Chimera, recombinant TRAIL | 93 |
| Iso-leucine TRAIL recombinant | PEG conjugated with TRAIL | Poly lactic-co-glycolic acid sustained-release microspheres | 98 |
| single-chain TRAIL-receptor-binding domain (scTRAIL-RBD) | Fusion with ImmunoglobulinG1 | Fusion Protein | 99 |
| Soluble TRAIL | FN-14 peptide | Fusion soluble protein chimera | 100 |
| Bioactive TRAIL | Lipid | TRAIL-coated Lipid NPs | 101 |
| Apo2L.0 / Dulenermin | PEG | Synthetic lipid bilayer nanospheres/liposomes | 102 |
| sTRAIL | PEG | Coated stealth liposome | 103 |

**Table 3.** Formulations aimed to enhance TRAIL action by active targeting of CRC.

| TRAIL type | Combined molecule | Formulation type | Reference |
|---|---|---|---|
| PEG-TRAIL | Chondroitin sulfate | PLGA nanoparticles | [107] |
| PEG-TRAIL | Doxorubicin | PLGA microspheres | [108] |
| TRAIL | PEGylated heparin (PEG-HE) and poly-l-lysin | Sustained release PEG nanoparticles | [109] |
| TRAIL | The single-chain anti-EGFR antibody fragment | Immunoliposomes | [110] |
| Recombinant soluble TRAIL | Antibodies | Glass-supported lipid bilayer/ lipid bilayer liposomes | [102] |
| Single-chain recombinant TRAIL | Anti-EGFR antibody fragments | Dimer fusion protein | [111] |

further increasing ROS stress and at last, leading to DR5 overexpression, thus even the TRAIL resistance cancer cell lines could be targeted, and apoptosis could be induced by TRAIL, miR-128 combination therapy[112] another active targeting approach was TRAIL combined with a muc2 inhibitor, *Akkermansia muciniphilia* is a natural intestinal microbiota that helps in degrading mucin by inhibiting Muc2, a main component of mucin by its proteolytic enzymatic action. It has been seen that colorectal cancer cells overexpress mucin, thereby creating a sheath of protective coat around them which helps them thrive.[113-115] The only known protease enzyme capable of degrading the core Muc2 identified till now known as Amuc_1434* is a recombinant version of the original enzyme.[116] As the immunity of the cell lines is also negatively linked with p53 genes, which have been found to have a proportional link to Muc2,[76,117] and also a failure of apoptosis is one of the leading causes of conversion of adenoma to carcinoma in CRC,[118,119] therefore, treatment with Amuc_1434* has shown to increase apoptosis through TRAIL-mediated caspases pathway in even the resistance cases of CRC.[120]

### *Passive targeting of TRAIL*

Passive targeting of TRAIL is when TRAIL itself is used as the targeting agent coupled with chemotherapeutic agents in a delivery form of nanoparticles like liposomes. Other delivery systems like micelles and microspheres are also explored in this process, passive targeting with nanoformulations has the distinct advantage of using an EPR mechanism to easily penetrate the tumor and having a high retention time as well, particles that possess a size of around 10-150 nm have this added advantage. PEG and HSA are added in these formulations for extra stability and ease of surface modification. Passive targeting has been the most popular approach for researchers in CRC.

TRAIL nanoformulations conjugated with other anticancer medications or small molecules, even microRNAs, and in a few cases coupled with radiotherapy are gaining an increasing amount of popularity for specific targeting ability using TRAIL and improved action against both nonresistant and resistant malignancies. Iron oxide cluster-based nanoparticles have improved significantly the antitumor activity of TRAIL/Apo2L, which was confirmed in both TRAIL-resistant HT-29, intermediately resistant SW-480, and sensitive HCT-116 cells.[121]

Jo *et al.*[122] showed reactive oxygen species modulator-1 (Romo1) to be an effective sensitizer for TRAIL, increasing its half-life in patients with Colorectal cancer, it was seen that Romo1 inhibition induces TRAIL-mediated apoptosis. Utilizing passive targeting drug delivery for treatment of CRC using TRAIL.[122] A drug delivery combination of TRAIL and RUNX3 (RUNT-related transcription factor 3) was developed by Kim *et al.*[123] and RUNX3 overexpression markedly reduced the transcription of superoxide dismutase, thereby increasing the production of reactive oxygen species, which led to an increase in DR5 receptors. It confirmed a reduction in tumor growth in colorectal cancer xenografted mice.

Another TRAIL combination therapy was evaluated of cannabidiol, which is a non-psychotomimetic compound obtained from *Cannabis sativa* can enhance the effect of TRAIL in inducing apoptosis in colon cancer, in xenografted mice. However, this synergy did not show any effect on normal colon cells. It was suggested that this synergy is due to the enhancement of ER stress by the cannabidiol, upregulating the DR5 receptor and sensitizing the cells for TRAIL.[124] The combination of TRAIL with Diallyl Disulfide (DADS) boosts the apoptosis-inducing activity of TRAIL even if the resistance species of colorectal cancer cell lines. DADS is a major component of the oil obtained by the distillation of garlic. When treated with only DADS cell lines of colorectal cancer showed a slower growth rate, however, in combination with the TRAIL, it was seen to be enhancing the cytotoxic activity even if resistant cell lines. This action is due to the downregulation of the BCL2 gene and the initiation of caspases by DADS. Therefore, it caused the degradation of PARP (Poly – ADP Ribose Polymerase), leading to the inability of the cells to repair any damage. Which may increase stress and thus enhance the sensitivity of TRAIL for the colorectal cancer cell lines.[125]

Trametinib, an anticancer drug that acts by MEK1 and MEK2 (mitogen induced protein kinase) enzymes and is used for the treatment of melanoma, thyroid, and lung cancers was co-administered along with TRAIL for colorectal cancer and the results were rather promising. This study suggested that Trametinib and TRAIL showed very high synergistic activity in cell viability study against HCT116 cell lines. It was found that this synergistic activity

is due to the suppression the of MCL1 protein, which is coded by the MCL1 (myeloid cell leukemia 1) gene, a pro-survival member of the BCL2 family of genes. When MCL1 is overexpressed that affects TRAIL-induced apoptosis but the treatment comprising TRAIL/Trametinib showed enhanced apoptosis and reduced growth.[126]

Farnesoid X Receptor, when activated causes the suppression of autophagy in tumor cells. Autophagy is the process by which a cell removes or digests its damaged organelles. Reduction or inhibition in autophagy leads to increased expression of death receptors. It was seen that GW4064, a ligand with a particular aim to show agonistic activity on Farnesoid X Receptors (FXR) can greatly potentiate the activity of TRAIL molecules on colorectal cell lines. This synergistic activity is the result of the upregulation of the DR5 receptors and thereby can be useful in the treatment of colorectal cancers with TRAIL resistance.[127] In a more recent study, Joshua et.al produced "super natural killer cells" where NK cell-targeted liposomes are fused with TRAIL using thiolation and CD 335 antibodies that showed higher activity against oxaliplatin resistant CRC,[128] TRAIL gene have also been used as a therapeutic agent via infection mode, Jung et.al, showed that the Newcastle disease virus (NDV) containing TRAIL can effectively enhance apoptosis in TRAIL-resistant CRC as NDV is known to upregulate death receptors in CRC, rNDV-TRAIL showed higher efficacy than that of only rNDV[89] Few more formulation strategies, that are used in passive targeting are mentioned in Table 4.

As both active and passive targeting play a pivotal role in the TRAIL formulation, conjugated systems, and their corresponding mechanisms are illustrated in Figure 5.

## Conclusion

As seen in this study, despite of emergence of various TRAIL resistance mechanisms, the way by which TRAIL shows selectivity and induces apoptosis is still a major field of interest in anti-cancer therapy. TRAIL from being used as a therapeutic agent is now proven to be an even more efficient biomarker or an adjuvant to other cancer drug therapy. It is clear that molecules that can enhance cytoplasmic free radical concentration, could initiate the expression of TRAIL receptors (DR4 and DR5) thereby overcoming the resistance to TRAIL, and showing a synergistic cytotoxic action. TRAIL can add an edge to conventional chemotherapy and radiotherapy by being an active synergistic agent. However, the administration of TRAIL alone is not an advantageous way of handling cancer, but it can be a successful biomarker. Future of the cell-specific cancer treatment can go to the path of using TRAIL molecule as a conjugate to the active moiety, just to reduce dosage and increase specificity.

The studies covered in this article showed TRAIL actions can be enhanced by natural anticancer drugs such as cannabidiol, *Codium fragile* extract, DADS from garlic oil and etc. It was also used in combination with miRNAs, and rAD-TRAIL adenovirus. TRAIL apoptotic action can even be enhanced by conjugating it with novel synthetic molecules such as GW4064, Romo 1 inhibitors, CBUD and etc. This clearly shows the huge diversity of compatibility of TRAIL for both co-administration as well as conjugation, which in turn can be marked by how wide of an approach this particular path of treatment might have in store for the future of specified cancer chemotherapy.

There have been significant numbers of clinical trials that involved TRAIL in different types of carcinogenesis, both directly and indirectly, singularly as well as in combination. Such as the ones that are mentioned above in Table 1, apart from that, TRAIL sensitization using drugs like Bortezomib and then treatment with NK cells have also been tested for chronic myeloid leukemia (CML)

This being discussed, we can conclude that TRAIL might be like a process that we only understand on its surface, there is a lot of depth yet to explore. Not only for colorectal cancer but TRAIL can be successfully incorporated for all the different types of carcinomas. The future may lie modification of TRAIL, semisynthetic TRAIL, or maybe even chemically synthesized TRAIL analogs. The future aim for this approach should be creating a molecule capable of targeted delivery and activation of death receptors with minimum binding with healthy cells and possible resistance, then that could be incorporated into various types of dosage forms containing conventional chemotherapeutic agents, SiRNA, or even phytomedicines

**Table 4.** Formulations of TRAIL enhancing efficacy by passive targeting of CRC.

| TRAIL type | Combined molecule | Formulation type | Ref. |
|---|---|---|---|
| sTRAIL | Quercetin | Lipid Raft | 129 |
| Recombinant sTRAIL | TRVP1 antagonist capsazepine | Conjugation | 130 |
| Plasmid encoding TRAIL | Doxorubicin | Polyamidoamine dendrimer modified with cholesteryl chloroformate | 131 |
| Gene coding TRAIL | Oncolytic adenovirus encoding gene PZD55 joined with PCA13 gene containing TRAIL code | Targeted oncolytic adenovirus with TRAIL | 132 |
| sTRAIL | Receptor binding domain of SARS-CoV-2 spike protein | Lipid nanoparticle | 133 |
| sTRAIL | Imatinib | Liposomes (150nm) | 134 |
| TRAIL mRNA | - | Ionizable lipid nanoparticles | 135 |

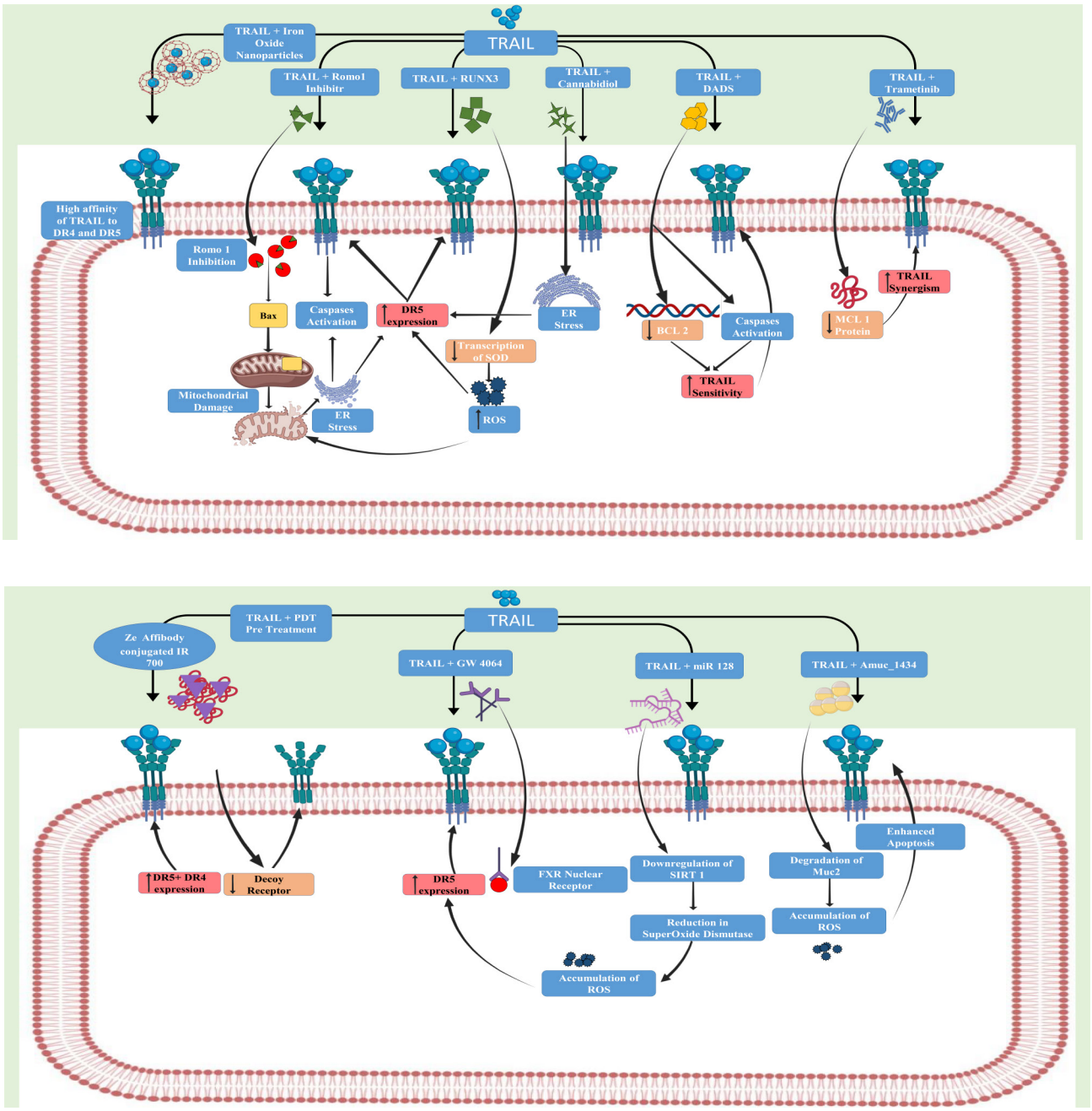


**Figure 5.** Mechanisms of notable active and passive targeting systems of TRAIL.

to open a low cost, effective, targeted, rapid and site-specific treatment of carcinomas.

## Acknowledgements

The authors would like to express their heartfelt gratitude towards the Department of Biotechnology for their kind fund support through the DBT-NER Twinning Project bearing the ID BT/PR23634/NER/95/786/2017. The authors are also grateful for the support given by the central instrumental facility and their team at Himalayan Pharmacy Institute, Sikkim, for providing valuable inputs and corrections in the paper. The authors would like to thank the Department of Biotechnology - Boost to University Interdisciplinary Life Science Departments for Education and Research program (DBT-BUILDER) for the facilities provided for conducting the research. The authors would like to thank the Department of Science and Technology - Fund for Improvement of Science and Technology Infrastructure (DST-FIST) and Promotion of University Research and Scientific Excellence (DST-PURSE) for the facilities provided for conducting the research.

## Author Contributions

Parikshit Roychowdhury: Conceptualization, Methodology, Validation, Writing - Original Draft, Visualization, Project administration. Indhumathi Thirugnanasambandham: Conceptualization, Supervision, correcting draft. Anindita

De: Conceptualization, Methodology, Validation, Writing - Original Draft, Visualization, Project administration. Mirunalini Gobinath: Conceptualization, Methodology, Validation, Writing - Original Draft, Visualization. Samanwita Khanra: Writing - Review & Editing. Veera Venkata Satyanarayana Reddy Karri: Writing - Review & Editing. Nihar Ranjan Bhuyan: Writing - Review & Editing. Gowthamarajan Kuppusamy: Conceptualization, Supervision, Project administration, Funding acquisition.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References


1. Melero I, Berman DM, Aznar MA, Korman AJ, Perez Garcia JL, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2015;15(8):457-72. doi:10.1038/nrc3973
2. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ. 2014;21(1):15-25. doi:10.1038/cdd.2013.67
3. Karpisheh V, Nikkhoo A, Hojjat-Farsangi M, Namdar A, Azizi G. Prostaglandin E2 as a potent therapeutic target for the treatment of colon cancer. Prostaglandins Other Lipid Mediat. 2019;144:106338. doi:10.1016/j.prostaglandins.2019.106338
4. Mehta A, Patel BM. Therapeutic opportunities in colon cancer: Focus on phosphodiesterase inhibitors. Life Sci 2019;230:150-61. doi:10.1016/j.lfs.2019.05.043
5. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174. doi:10.1016/j.tranon.2021.101174
6. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012;3(3):153-73. doi:10.3978/j.issn.2078-6891.2012.030
7. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. Gastroenterology. 2010;138(6):2044-58. doi: 10.1053/j.gastro.2010.01.054
8. Graff RE, Möller S, Passarelli MN, Witte JS, Skytthe A. Familial risk and heritability of colorectal cancer in the nordic twin study of cancer. Clin Gastroenterol Hepatol. 2017;15(8):1256-64. doi:10.1016/j.cgh.2016.12.041
9. Nauts HC, Swift WE, Coley BL. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, M.D., was reviewed in the light of modern research. Cancer Res. 1946;6:205-16.
10. Walczak H. Death receptor-ligand systems in cancer, cell death, and inflammation. Cold Spring Harb Perspect Biol. 2013;5(5):a008698. doi:10.1101/cshperspect.a008698.
11. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975;72(9):3666-70. doi:10.1073/pnas.72.9.3666
12. Walczak H, Miller RE, Ariail K, Gliniak B, Griffith TS. The tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo. Nat Med. 1999;5(2):157-63. doi:10.1038/5517
13. Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest. 1999;104(2):155-62. doi:10.1172/JCI6926
14. Cancer Today. Available from: http://gco.iarc.fr/today/home [Last accessed: 12/5/2022].
15. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet 2019;394(10207):1467-80. doi:10.1016/S0140-6736(19)32319-0
16. Henrikson NB, Webber EM, Goddard KA, Scrol A, Piper M. Family history and the natural history of colorectal cancer: a systematic review. Genet Med 2015;17(9):702-12. doi:10.1038/gim.2014.188
17. Schoen RE, Razzak A, Yu KJ, Berndt SI, Firl K, Riley TL et al. Incidence and mortality of colorectal cancer in individuals with a family history of colorectal cancer. Gastroenterology 2015;149(6):1438-45.e1. doi:10.1053/j.gastro.2015.07.055
18. Czene K, Lichtenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer. 2002;99(2):260-6. doi:10.1002/ijc.10332
19. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000;343(2):78-85. doi:10.1056/NEJM200007133430201
20. Jiao S, Peters U, Berndt S, Brenner H, Butterbach K. Estimating the heritability of colorectal cancer. Hum Mol Genet. 2014;23(14):3898-905. doi:10.1093/hmg/ddu087
21. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. Am J Gastroenterol. 2015;110(2):223-62. doi:10.1038/ajg.2014.435
22. Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. Clin Gastroenterol Hepatol. 2012;10(6):639-45. doi:10.1016/j.cgh.2012.01.010
23. Brenner H, Chang-Claude J, Seiler CM, Rickert A, Hoffmeister M. Protection from colorectal cancer after colonoscopy: a population-based, case-control study. Ann Intern Med. 2011;154(1):22-30. doi:10.7326/0003-4819-154-1-201101040-00004
24. Vasen HFA, Blanco I, Aktan-Collan K, Gopie JP, Alonso A. Revised guidelines for the clinical management of

Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut. 2013;62(6):812-23. doi:10.1136/gutjnl-2012-304356
25. Kyrgiou M, Kalliala I, Markozannes G, Gunter MJ, Paraskedaidis E. Adiposity and cancer at major anatomical sites: umbrella review of the literature. BMJ. 2017;356:j477. doi:10.1136/bmj.j477
26. Cai S, Li Y, Ding Y, Chen K, Jin M. Alcohol drinking and the risk of colorectal cancer death: a meta-analysis. Eur J Cancer Prev. 2014;23(6):532-39. doi:10.1097/CEJ.0000000000000076
27. Botteri E, Iodice S, Bagnardi V, Raimondi S, Lowenfels AB, Maisonneuve P. Smoking and colorectal cancer: a meta-analysis. JAMA. 2008;300(23):2765-78. doi:10.1001/jama.2008.839
28. Chan DSM, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, et al. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. PloS One. 2011;6(6):e20456. doi:10.1371/journal.pone.0020456
29. Krämer HU, Schöttker B, Raum E, Brenner H. Type 2 diabetes mellitus and colorectal cancer: meta-analysis on sex-specific differences. Eur J Cancer. 1990 2012;48(9):1269-82. doi:10.1016/j.ejca.2011.07.010
30. Kostic AD, Chun E, Robertson L, Glickman JN, Galini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe. 2013;14(2):207-15. doi:10.1016/j.chom.2013.07.007
31. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WKK, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. Nat Commun. 2015;6:8727. doi:10.1038/ncomms9727
32. Nassar D, Blanpain C. Cancer Stem Cells: Basic Concepts and Therapeutic Implications. Annu Rev Pathol. 2016;11:47-76. doi:10.1146/annurev-pathol-012615-044438
33. Medema JP. Cancer stem cells: the challenges ahead. Nat Cell Biol. 2013;15(4):338-44. doi:10.1038/ncb2717
34. Muzny DM, Bainbridge MN, Chang K, Dinh HH, Drummond JA, Fowler G, et al. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012;487(7407):330-7. doi:10.1038/nature11252
35. Loree JM, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A, et al. Classifying Colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation Profiles and consensus molecular subtypes. Clin Cancer Res. 2018;24(5):1062-72. doi:10.1158/1078-0432.CCR-17-2484
36. Venook AP, Niedzwiecki D, Innocenti F, Fruth B, Greene C, O'Neil BH, et al. Impact of primary (1o) tumor location on overall survival (OS) and progression-free survival (PFS) in patients (pts) with metastatic colorectal cancer (mCRC): Analysis of CALGB/SWOG 80405 (Alliance). J Clin Oncol. 2016;34(15_suppl):3504. doi:10.1200/JCO.2016.34.15_suppl.3504
37. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21(11):1350-6. doi:10.1038/nm.3967
38. Fijten GH, Starmans R, Muris JW, Schouten JH, Blijham GH, Knottnerus JA. Predictive value of signs and symptoms for colorectal cancer in patients with rectal bleeding in general practice. Fam Pract. 1995;12(3):279-86. doi:10.1093/fampra/12.3.279
39. Kaminski MF, Regula J, Kraszewska E, Polkowski M, Wojciechowska A, Didkowska J, et al. Quality indicators for colonoscopy and the risk of interval cancer. N Engl J Med. 2010;362(19):1795-803. doi:10.1056/NEJMoa0907667
40. Benson AB, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, et al. NCCN Guidelines Insights: Colon Cancer, Version 2.2018. J Natl Compr Cancer Netw. 2018;16(4):359-69. doi:10.6004/jnccn.2018.0021.
41. Beets-Tan RGH, Lambregts DMJ, Maas M, Bipat S, Barbaro B, Curvo-Semedo L, et al. Magnetic resonance imaging for clinical management of rectal cancer: Updated recommendations from the 2016 European Society of Gastrointestinal and Abdominal Radiology (ESGAR) consensus meeting. Eur Radiol. 2018;28(4):1465-75. doi:10.1007/s00330-017-5026-2
42. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandalà M, Cervantes A, et al. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24 Suppl 6:vi64-72. doi:10.1093/annonc/mdt354
43. Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol Off J Am Soc Clin Oncol 2010;28(20):3219-26. doi:10.1200/JCO.2009.27.1825
44. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391(10135):2128-39. doi:10.1016/S0140-6736(18)30789-X
45. Moss A, Nalankilli K. Completing the circle of informed consent for EMR versus surgery for nonmalignant large or complex colorectal polyps. Gastrointest Endosc. 2016;84(2):304-6. doi:10.1016/j.gie.2016.02.039
46. Jayanna M, Burgess NG, Singh R, Hourigan LF, Brown GJ, Zanati SA, et al. Cost Analysis of Endoscopic Mucosal Resection vs Surgery for Large Laterally Spreading Colorectal Lesions. Clin Gastroenterol Hepatol. 2016;14(2):271-8.e1-2. doi:10.1016/j.cgh.2015.08.037
47. Emmanuel A, Haji A. Complete mesocolic excision and extended (D3) lymphadenectomy for colonic

cancer: is it worth that extra effort? A review of the literature. Int J Colorectal Dis. 2016;31(4):797-804. doi:10.1007/s00384-016-2502-0
48. Cleary RK, Morris AM, Chang GJ, Halverson AL. Controversies in surgical oncology: does the minimally invasive approach for rectal cancer provide equivalent oncologic outcomes compared with the open approach? Ann Surg Oncol. 2018;25(12):3587-95. doi:10.1245/s10434-018-6740-y
49. Rullier E, Rouanet P, Tuech J-J, Valverde A, Lelong B, Rivoire M, et al. Organ preservation for rectal cancer (GRECCAR 2): a prospective, randomized, open-label, multicentre, phase 3 trial. Lancet. 2017;390(10093):469-79. doi:10.1016/S0140-6736(17)31056-5
50. Taylor FGM, Quirke P, Heald RJ, Moran B, Blomqvist L, Swift I, et al. Preoperative high-resolution magnetic resonance imaging can identify good prognosis stage I, II, and III rectal cancer best managed by surgery alone: a prospective, multicenter, European study. Ann Surg. 2011;253(4):711-9. doi:10.1097/SLA.0b013e31820b8d52
51. Abdel-Rahman O, Cheung WY. Integrating Systemic Therapies into the Multimodality Treatment of Resectable Colorectal Liver Metastases. Gastroenterol Res Pract. 2018;2018:4326082. doi:10.1155/2018/4326082.
52. Baratti D, Kusamura S, Pietrantonio F, Guaglio M, Niger M, Deraco M. Progress in treatments for colorectal cancer peritoneal metastases during the years 2010-2015. A systematic review. Crit Rev Oncol Hematol. 2016;100:209-22. doi:10.1016/j.critrevonc.2016.01.017
53. André T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009;27(19):3109-16. doi:10.1200/JCO.2008.20.6771
54. André T, Iveson T, Labianca R, Meyerhardt JA, Souglakos I, Yoshino T, et al. The IDEA (International Duration Evaluation of Adjuvant Chemotherapy) Collaboration: Prospective Combined Analysis of Phase III Trials Investigating Duration of Adjuvant Therapy with the FOLFOX (FOLFOX4 or Modified FOLFOX6) or XELOX (3 versus 6 months) Regimen for Patients with Stage III Colon Cancer: Trial Design and Current Status. Curr Colorectal Cancer Rep. 2013;9(3):261-9. doi:10.1007/s11888-013-0181-6.
55. Meyers BM, Cosby R, Quereshy F, Jonker D. Adjuvant Chemotherapy for Stage II and III Colon Cancer Following Complete Resection: A Cancer Care Ontario Systematic Review. Clin Oncol (R Coll Radiol). 2017;29(7):459-65. doi:10.1016/j.clon.2017.03.001
56. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, Taib J, et al. Duration of Adjuvant Chemotherapy for Stage III Colon Cancer. N Engl J Med. 2018;378(13):1177-88. doi:10.1056/NEJMoa1713709.
57. Mehrgou A, Ebadollahi S, Seidi K, Ayoubi-Joshaghani M, Yazdi AA, Zare P, et al. Roles of miRNAs in Colorectal Cancer: Therapeutic Implications and Clinical Opportunities. Adv Pharm Bull. 2021;11:233-47. doi:10.34172/apb.2021.029
58. Hurwitz H, Fehrenbacher L, Novotny W, Carwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350(23):2335-42. doi:10.1056/NEJMoa032691
59. Arnold D, Lueza B, Douillard J-Y, Peeters M, Lenz H-J, Venook A, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol. 2017;28(8):1713-29. doi:10.1093/annonc/mdx175
60. Pietrantonio F, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer. 2015;51(5):587-94. doi:10.1016/j.ejca.2015.01.054
61. Mayer RJ, Van Cutsem E, Falcone A, Yoshino T, Garcia-Carbonero R, Mizunuma N, et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. N Engl J Med. 2015;372(20):1909-19. doi:10.1056/NEJMoa1414325
62. Housini M, Dariya B, Ahmed N, Stevens A, Fiadjoe H, Nagaraju GP, et al. Colorectal cancer: Genetic alterations, novel biomarkers, current therapeutic strategies and clinical trials. Gene. 2024;892:147857. doi:10.1016/j.gene.2023.147857
63. Alshehri S, Imam SS, Rizwanullah Md, Akhter S, Mahdi W, Kazi M, et al. Progress of cancer nanotechnology as diagnostics, therapeutics, and theranostics nanomedicine: preclinical promise and translational challenges. Pharmaceutics. 2020;13(1):24. doi:10.3390/pharmaceutics13010024
64. Yao Y, Zhou Y, Liu L, Xu L, Chen Q, Wang Y, et al. Nanoparticle-based drug delivery in cancer therapy and its role in overcoming drug resistance. Front Mol Biosci. 2020;7:193. doi:10.3389/fmolb.2020.00193
65. Din FU, Aman W, Ullah I, Qureshi OS, Mustapha O, Shafique S, et al. Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomedicine. 2017;12:7291-309. doi:10.2147/IJN.S146315
66. Younis NK, Roumieh R, Bassil EP, Ghoubaira JA, Kobeissy F, Eid AH. Nanoparticles: Attractive tools to treat colorectal cancer. Semin Cancer Biol 2022;86(Pt 2):1-13. doi:10.1016/j.semcancer.2022.08.006
67. Sharifi-Azad M, Fathi M, Cho WC, Barzegari A, Dadashi H, Dadashpour M, et al. Recent advances in targeted drug delivery systems for resistant colorectal cancer. Cancer Cell Int. 2022;22(1):196. doi:10.1186/

s12935-022-02605-y
68. Pitti RM, Marsters SA, Ruppert S, Donahue CJ, Moore A, Ashkenazi A. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem. 1996;271(22):12687-90. doi:10.1074/jbc.271.22.12687
69. Chaudhary PM, Eby M, Jasmin A, Bookwalter A, Murray J, Hood L. Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. Immunity. 1997;7(6):821-30. doi:10.1016/s1074-7613(00)80400-8
70. Kayagaki N, Yamaguchi N, Nakayama M, Takeda K, Akiba H, Tsutsui H, et al. Expression and function of TNF-related apoptosis-inducing ligand on murine activated NK cells. J Immunol. 1999;163(4):1906-13.
71. de Miguel D, Lemke J, Anel A, Walczak H, Martinez-Lostao L. Onto better TRAILs for cancer treatment. Cell Death Differ. 2016;23(5):733-47. doi:10.1038/cdd.2015.174
72. Naval J, de Miguel D, Gallego-Lleyda A, Anel A, Martinez-Lostao L. Importance of TRAIL molecular anatomy in receptor oligomerization and signaling. Implications for cancer therapy. Cancers. 2019;11(4):444. doi:10.3390/cancers11040444
73. Cha S-S, Kim M-S, Choi YH, Sung BJ, Shin NK, Shin HC, et al. 2.8 Å Resolution crystal structure of human TRAIL, a cytokine with selective antitumor activity. Immunity. 1999;11(2):253-61. doi:10.1016/S1074-7613(00)80100-4
74. Pan G, O'Rourke K, Chinnaiyan AM, Gentz R, Ebner R, Ni J, et al. The Receptor for the Cytotoxic Ligand TRAIL. Science. 1997;276(5309):111-3. doi:10.1126/science.276.5309.111
75. Pan G, Ni J, Wei Y-F, Yu G-L, Gentz R, Dixit VM. An antagonist decoy receptor and a death domain-containing receptor for TRAIL. Science. 1997;277(5327):815-8. doi:10.1126/science.277.5327.815
76. Marsters SA, Sheridan JP, Pitti RM, Huang A, Skubatch M, Baldwin D, et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol. 1997;7(12):1003-6. doi:10.1016/S0960-9822(06)00422-2
77. Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, et al. Osteoprotegerin Is a Receptor for the Cytotoxic Ligand TRAIL J Biol Chem. 1998;273(23):14363-7. doi:10.1074/jbc.273.23.14363
78. Morizot A, Mérino D, Lalaoui N, Jacquemin G, Granci V, Iessi E, et al. Chemotherapy overcomes TRAIL-R4-mediated TRAIL resistance at the DISC level. Cell Death Differ. 2011;18(4):700-11. doi:10.1038/cdd.2010.144
79. Wang S, El-Deiry WS. TRAIL and apoptosis induction by TNF-family death receptors. Oncogene 2003;22(53):8628-33. doi:10.1038/sj.onc.1207232
80. Maji A, Paul A, Sarkar A, Nahar S, Bhowmik R, Samanta A, et al. Significance of TRAIL/Apo-2 ligand and its death receptors in apoptosis and necroptosis signalling: Implications for cancer-targeted therapeutics. Biochem Pharmacol. 2024;221:116041. doi:10.1016/j.bcp.2024.116041
81. Dianat-Moghadam H, Heidarifard M, Mahari A, Shahgolzari M, Keshavarz M, Nouri M, et al. TRAIL in oncology: From recombinant TRAIL to nano- and self-targeted TRAIL-based therapies. Pharmacol Res. 2020;155:104716. doi:10.1016/j.phrs.2020.104716
82. von Karstedt S, Montinaro A, Walczak H. Exploring the TRAILs less travelled: TRAIL in cancer biology and therapy. Nat Rev Cancer. 2017;17(6):352-66. doi:10.1038/nrc.2017.28
83. Yuan X, Gajan A, Chu Q, Xiong H, Wu K, Wu G. Developing TRAIL/TRAIL death receptor-based cancer therapies. Cancer Metastasis Rev. 2018;37(4):733-48. doi:10.1007/s10555-018-9728-y
84. Dimberg LY, Anderson CK, Camidge R, Behbakht K, Thorburn A, Ford HL. On the TRAIL to successful cancer therapy? Predicting and counteracting resistance against TRAIL-based therapeutics. Oncogene. 2013;32(11):1341-50. doi:10.1038/onc.2012.164
85. Lemke J, von Karstedt S, Zinngrebe J, Walczak H. Getting TRAIL back on track for cancer therapy. Cell Death Differ. 2014;21(9):1350-64. doi:101038/cdd.2014.81
86. Trauzold A, Siegmund D, Schniewind B, Sipos B, Egberts J, Zorenkov D, et al. TRAIL promotes metastasis of human pancreatic ductal adenocarcinoma. Oncogene. 2006;25(56):7434-9. doi:10.1038/sj.onc.1209719
87. Pimentel JM, Zhou J-Y, Wu GS. The Role of TRAIL in apoptosis and immunosurveillance in cancer. Cancers. 2023;15(10):2752. doi:10.3390/cancers15102752
88. Jain N, Parikshit P, Ranjan Bhuyan N, Kuppusamy G. 'TRAIL' of targeted colorectal cancer therapy. Indian J Biochem Biophys. 2023;60(2):95-8. doi:10.56042/ijbb.v60i2.70737
89. Caldiran F, Berkel C, Yilmaz E, Kucuk B, Cacan AH, Citli S, et al. Combination treatment of bortezomib and epirubicin increases the expression of TNFRSF10 A/B, and induces TRAIL-mediated cell death in colorectal cancer cells. Biochem Biophys Res Commun. 2023;675:33-40. doi:10.1016/j.bbrc.2023.06.015
90. Çal Doğan T, Aydın Dilsiz S, Canpınar H, Ündeğer Bucurgat Ü. Genistein enhances TRAIL-mediated apoptosis through the inhibition of XIAP and DcR1 in colon carcinoma cells treated with 5-fluorouracil. Turk J Pharm Sci 2024;21(1):7-24. doi:10.4274/tjps.galenos.2023.60543
91. ClinicalTrials. https://clinicaltrials.gov. [Last accessed: 12/13/2022].
92. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3(6):673-82. doi:10.1016/1074-

7613(95)90057-8
93. Ganten TM, Koschny R, Sykora J, Schulze-Bergkamen H, Büchler P, Haas TL, et al. Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs. Clin Cancer Res. 2006;12(8):2640-6. doi: 10.1158/1078-0432.CCR-05-2635
94. Berg D, Lehne M, Müller N, Siegmund D, Münkel S, Sebald W, et al. Enforced covalent trimerization increases the activity of the TNF ligand family members TRAIL and CD95L. Cell Death Differ. 2007;14(12):2021-34. doi:10.1038/sj.cdd.4402213
95. Crawford J. Clinical benefits of pegylated proteins in oncology. Cancer Treat Rev. 2002;28(Suppl A):1-2. doi:10.1016/s0305-7372(02)80001-9
96. Müller N, Schneider B, Pfizenmaier K, Wajant H. Superior serum half life of albumin tagged TNF ligands. Biochem Biophys Res Commun. 2010;396(4):793-9. doi:10.1016/j.bbrc.2010.04.134
97. Rozanov DV, Savinov AY, Golubkov VS, Rozanova OL, Postnova TI, Sergienko EA, et al. Engineering a leucine zipper-TRAIL homotrimer with improved cytotoxicity in tumor cells. Mol Cancer Ther. 2009;8(6):1515-25. doi:10.1158/1535-7163.MCT-09-0202
98. Kim TH, Jiang HH, Park CW, Youn YS, Lee S, Chen X, et al. PEGylated TNF-related apoptosis-inducing ligand (TRAIL)-loaded sustained release PLGA microspheres for enhanced stability and antitumor activity. J Control Release. 2011;150(1):63-9. doi:10.1016/j.jconrel.2010.10.037
99. Gieffers C, Kluge M, Merz C, Sykora J, Thiemann M, Schaal R, et al. APG350 induces superior clustering of TRAIL receptors and shows therapeutic antitumor efficacy independent of cross-linking via Fcγ receptors. Mol Cancer Ther. 2013;12(12):2735-47. doi:10.1158/1535-7163.MCT-13-0323
100. Razmara M, Hilliard B, Ziarani AK, Murali R, Yellayi S, Ghazanfar M, et al. Fn14-TRAIL, a chimeric intercellular signal exchanger, attenuates experimental autoimmune encephalomyelitis. Am J Pathol. 2009;174(2):460-74. doi:10.2353/ajpath.2009.080462
101. De Miguel D, Gallego-Lleyda A, Anel A, Martinez-Lostao L. Liposome-bound TRAIL induces superior DR5 clustering and enhanced DISC recruitment in histiocytic lymphoma U937 cells. Leuk Res. 2015;39(6):657-66. doi:10.1016/j.leukres.2015.03.019
102. Nair PM, Flores H, Gogineni A, Marsters S, Lawrence DA, Kelley RF, et al. Enhancing the antitumor efficacy of a cell-surface death ligand by covalent membrane display. Proc Natl Acad Sci U S A. 2015;112(18):5679-84. doi:10.1073/pnas.1418962112
103. Loi M, Becherini P, Emionite L, Giacomini A, Cossu I, Destefanis E, et al. sTRAIL coupled to liposomes improves its pharmacokinetic profile and overcomes neuroblastoma tumour resistance in combination with Bortezomib. J Control Release. 2014;192:157-66. doi:10.1016/j.jconrel.2014.07.009
104. de Bruyn M, Rybczynska AA, Wei Y, Schwenkert M, Fey GH, Dierckx RAJO, et al. Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP)-targeted delivery of soluble TRAIL potently inhibits melanoma outgrowth in vitro and in vivo. Mol Cancer. 2010;9:301. doi:10.1186/1476-4598-9-301
105. Stieglmaier J, Bremer E, Kellner C, Liebig TM, Ten CB, Peipp M, et al. Selective induction of apoptosis in leukemic B-lymphoid cells by a CD19-specific TRAIL fusion protein. Cancer Immunol Immunother. 2008;57(2):233-46. doi:10.1007/s00262-007-0370-8
106. Aronin A, Amsili S, Prigozhina TB, Tzdaka K, Rachmilewitz J, Shani N, et al. Fn14•Trail Effectively Inhibits Hepatocellular Carcinoma Growth. PLoS One. 2013;8(10):e77050. doi:10.1371/journal.pone.0077050
107. Kim H, Jeong D, Kang HE, Lee KC, Na K. A sulfate polysaccharide/TNF-related apoptosis-inducing ligand (TRAIL) complex for the long-term delivery of TRAIL in poly(lactic-co-glycolic acid) (PLGA) microspheres. J Pharm Pharmacol. 2013;65(1):11-21. doi:10.1111/j.2042-7158.2012.01564.x
108. Jiang HH, Kim TH, Lee S, Chen X, Youn YS, Lee KC. PEGylated TNF-related apoptosis-inducing ligand (TRAIL) for effective tumor combination therapy. Biomaterials. 2011;32(33):8529-37. doi:10.1016/j.biomaterials.2011.07.051
109. Lim SM, Kim TH, Jiang HH, Park CW, Lee S, Chen X, et al. Improved biological half-life and anti-tumor activity of TNF-related apoptosis-inducing ligand (TRAIL) using PEG-exposed nanoparticles. Biomaterials. 2011;32(13):3538-46. doi:10.1016/j.biomaterials.2011.01.054
110. Seifert O, Pollak N, Nusser A, Steiniger F, Ruger R, Pfizenmaier K, et al. Immuno-LipoTRAIL: Targeted delivery of TRAIL-functionalized liposomal nanoparticles. Bioconjug Chem. 2014;25(5):879-87. doi:10.1021/bc400517j
111. Siegemund M, Pollak N, Seifert O, Wahl K, Hanak K, Vogel A, et al. Superior antitumoral activity of dimerized targeted single-chain TRAIL fusion proteins under retention of tumor selectivity. Cell Death Dis. 2012;3(4):e295. doi:10.1038/cddis.2012.29
112. Lian B, Yang D, Liu Y, Shi G, Li J, Yan X, et al. miR-128 Targets the SIRT1/ROS/DR5 Pathway to Sensitize Colorectal Cancer to TRAIL-Induced Apoptosis. Cell Physiol Biochem. 2018;49(6):2151-62. doi:10.1159/000493818
113. Huang K, Wang MM, Kulinich A, Yao HL, Ma HY, Martinez JER, et al. Biochemical characterisation of the neuraminidase pool of the human gut symbiont Akkermansia muciniphila. Carbohydr Res. 2015;415:60-5. doi:10.1016/j.carres.2015.08.001
114. Passel MWJ van, Kant R, Zoetendal EG, Plugge CM, Derrien M, Malfatti SA, et al. The genome of Akkermansia muciniphila, a dedicated intestinal

mucin degrader, and its use in exploring intestinal metagenomes. PLoS One. 2011;6(3):e16876. doi:10.1371/journal.pone.0016876
115. Shin J, Noh J-R, Chang D-H, Kim Y, Kim MH, Lee ES, et al. Elucidation of Akkermansia muciniphila probiotic traits driven by mucin depletion. Front Microbiol. 2019;10:1137. doi:10.3389/fmicb.2019.01137
116. Meng X, Wang W, Lan T, Yang W, Yu D, Fang X, et al. A Purified Aspartic Protease from Akkermansia muciniphila plays an important role in degrading Muc2. Int J Mol Sci. 2019;21(1):72. doi:10.3390/ijms21010072
117. Ookawa K, Kudo T, Aizawa S, Saito H, Tsuchida S. Transcriptional activation of the MUC2 gene by p53. J Biol Chem. 2002;277(50):48270-5. doi:10.1074/jbc.M207986200
118. Manne U, Weiss HL, Grizzle WE. Racial differences in the prognostic usefulness of MUC1 and MUC2 in colorectal adenocarcinomas. Clin Cancer Res. 2000;6(10):4017-25
119. Robinson JR, Newcomb PA, Hardikar S, Cohen SA, Phipps AI. Stage IV colorectal cancer primary site and patterns of distant metastasis. Cancer Epidemiol. 2017;48:92-5. doi:10.1016/j.canep.2017.04.003
120. Manne U, Shanmugam C, Katkoori VR, Bumpers HL, Grizzle WE. Development and progression of colorectal neoplasia. Cancer Biomark. 2011;9(1-6):235-65. doi:10.3233/CBM-2011-0160
121. Shi Y, Wang J, Liu J, Lin G, Xie F, Pang X, et al. Oxidative stress-driven DR5 upregulation restores TRAIL/Apo2L sensitivity induced by iron oxide nanoparticles in colorectal cancer. Biomaterials. 2020;233:119753. doi:10.1016/j.biomaterials.2019.119753
122. Jo MJ, Kim BG, Park SH, Kim HJ, Jeong S, Kim BR, et al. Romo1 inhibition induces TRAIL-mediated apoptosis in colorectal cancer. Cancers. 2020;12(9):2358. doi:10.3390/cancers12092358
123. Kim BR, Park SH, Jeong YA, Na YJ, Kim JL, Jo MJ, et al. RUNX3 enhances TRAIL-induced apoptosis by upregulating DR5 in colorectal cancer. Oncogene. 2019;38(20):3903-18. doi:10.1038/s41388-019-0693-x
124. Kim JL, Kim BR, Kim DY, Jeong Y, Jeong S, Na YJ, et al. Cannabidiol enhances the therapeutic effects of TRAIL by upregulating DR5 in colorectal cancer. Cancers. 2019;11(5):642. doi:10.3390/cancers11050642
125. Kim HJ, Kang S, Kim DY, You S, Park D, Oh SC, et al. Diallyl disulfide (DADS) boosts TRAIL-Mediated apoptosis in colorectal cancer cells by inhibiting Bcl-2. Food Chem Toxicol. 2019;125:354-60. doi:10.1016/j.fct.2019.01.023
126. Lin L, Ding D, Xiao X, Li B, Cao P, Li S. Trametinib potentiates TRAIL-induced apoptosis via FBW7-dependent Mcl-1 degradation in colorectal cancer cells. J Cell Mol Med. 2020;24(12):6822-32. doi:10.1111/jcmm.15336
127. Hotta M, Sakatani T, Ishino K, Wada R, Kudo M, Yokoyama Y, et al. Farnesoid X receptor induces cell death and sensitizes to TRAIL-induced inhibition of growth in colorectal cancer cells through the up-regulation of death receptor 5. Biochem Biophys Res Commun. 2019;519(4):824-31. doi:10.1016/j.bbrc.2019.09.033
128. Greenlee JD, Zhang Z, Subramanian T, Liu K, King MR. TRAIL-conjugated liposomes that bind natural killer cells to induce colorectal cancer cell apoptosis. J Biomed Mater Res A. 2024;112(1):110-20. doi:10.1002/jbm.a.37621
129. Psahoulia FH, Drosopoulos KG, Doubravska L, Andera L, Pintzas A. et al. Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. Mol Cancer Ther. 2007;6(9):2591-9. doi:10.1158/1535-7163.MCT-07-0001
130. Sung B, Prasad S, Ravindran J, Yadav VR, Aggarwal BB. Capsazepine, a TRPV1 antagonist, sensitizes colorectal cancer cells to apoptosis by TRAIL through ROS–JNK–CHOP-mediated upregulation of death receptors. Free Radic Biol Med. 2012;53(10):1977-87. doi:10.1016/j.freeradbiomed.2012.08.012
131. Pishavar E, Ramezani M, Hashemi M. Co-delivery of doxorubicin and TRAIL plasmid by modified PAMAM dendrimer in colon cancer cells, in vitro and in vivo evaluation. Drug Dev Ind Pharm. 2019;45(12):1931-9. doi:10.1080/03639045.2019.1680995
132. Gao H, Zhang X, Ding Y, Qiu R, Hong Y, Chen W. Synergistic suppression effect on tumor growth of colorectal cancer by combining radiotherapy with a TRAIL-armed oncolytic adenovirus. Technol Cancer Res Treat. 2019;18:1533033819853290. doi:10.1177/1533033819853290
133. Gu T, Wang M, Fu X, Tian X, Bi J, Lu N, et al. Intratumoural delivery of TRAIL mRNA induces colon cancer cell apoptosis. Biomed Pharmacother. 2024;174:116603. doi:10.1016/j.biopha.2024.116603
134. Fu R, Chang R, Peng A, Feng C, Zhu W, Chen Y, et al. Efficient treatment of colon cancer with codelivery of TRAIL and imatinib by liposomes. Pharm Dev Technol. 2024;29(1):52-61. doi:10.1080/10837450.2024.2301763
135. da Silva WN, Carvalho Costa PA, Scalzo Júnior SRA, Ferreira HAS, Prazeres PHDM, Campos CLV, et al. Ionizable lipid nanoparticle-mediated TRAIL mRNA delivery in the tumor microenvironment to inhibit colon cancer progression. Int J Nanomedicine. 2024;19:2655–73. doi:10.2147/IJN.S452896