COMPREHENSIVE OVERVIEW OF RISK FACTORS, DIAGNOSIS, AND TREATMENT FOR COLORECTAL CANCER

Ms. Nandhini devi M*, Dr .G. Ariharasivakumar, D. K. Shanmuganathan, M.Tamilarasan
Department of Pharmacology, KMCH College of Pharmacy.
Kalapatti Road, Coimbatore – 641048.

Corresponding Author – Ms. Nandhini devi M*
Department of Pharmacology, KMCH College of Pharmacy, Coimbatore – 641048.

Abstract: Colorectal cancer (CRC) originates in the colon or rectum, often starting as benign polyps that may become cancerous. Major risk factors include genetics, family history, obesity, inflammatory bowel diseases, and lifestyle choices such as poor diet, lack of exercise, and smoking. Early detection through screening methods like colonoscopy, stool DNA tests, and sigmoidoscopy is essential for improving outcomes. Epidemiological data show that CRC is the third most common cancer globally and a leading cause of cancer-related deaths, with rising incidence among younger populations. Management typically involves surgery, chemotherapy, and immunotherapy, tailored to the patient's tumor characteristics and genetic profile. Recent advancements, such as laparoscopic surgery and targeted therapies, have improved survival and quality of life. Prevention strategies focus on lifestyle modifications, emphasizing a healthy diet, regular physical activity, and routine screenings. Further research is needed to develop more effective treatments and improve prevention strategies for diverse populations.

Keywords: Colorectal cancer, Carcinoma, Aberrant Crypt Foci, Chemotherapy, Immunotherapy ,Genetic mutations.

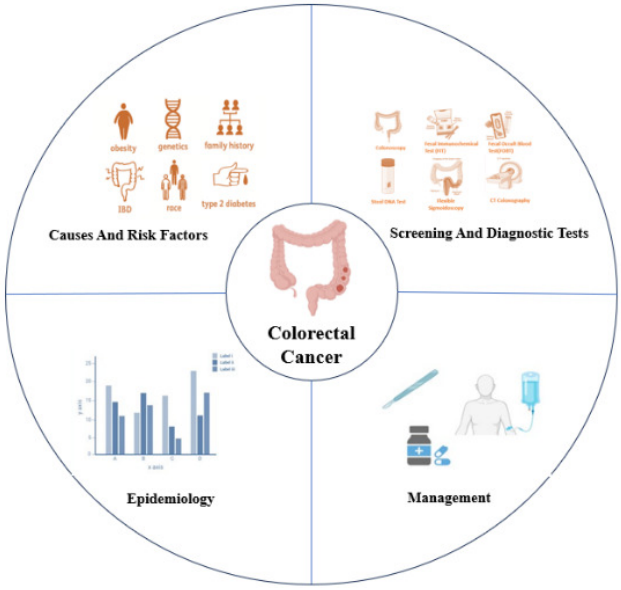

Figure 1: Classifications of colorectal cancer

INTRODUCTION

In 2020, Colorectal cancer (CRC) is the third most common cancer worldwide and the second highest cause of cancer-related deaths, with more than 1.9 million new cases and 0.9 million fatalities. Reported were differences in occurrence and death rates, temporal patterns, and projected impact of CRC between various nations and areas [1,2,3,4]. Alterations in the demographic distribution of colorectal cancer (CRC) emerged, showing a rise in cases among young individuals, particularly in developed nations [5]. These variations could be a result of differences in being exposed to risk factors for CRC such as lifestyle and environmental factors. Recognizing and steering clear of changeable risk factors, particularly lifestyle choices (like alcohol use, tobacco use, being overweight, and poor diet) while also enhancing protective elements (such as regular exercise, using certain medications like aspirin, and a nutritious diet) [6,7]. They play a crucial part in preventing CRC from developing in the first place. Screening for CRC (secondary prevention) is considered an effective method in CRC control programs for detecting and removing premalignant colorectal lesions. Even though numerous studies have been carried out on various aspects of CRC, there are still obstacles when it comes to identifying the key risk factors and best preventive measures for CRC in diverse populations [8]. In 2024, the American Cancer Society predicts 152,810 new cases of colorectal cancer in the US. 81,540 cases are expected in males, and 71,270 in females. Colon cancer will make up 106,590 cases, rectal cancer 46,220. It is the second-highest cause of cancer deaths, with 53,010 expected fatalities. Women have a 1 in 25 chance of developing CRC, and men 1 in 23. Risk factors include genetics, family history, and lifestyle choices [9]. Colorectal cancer (CRC) comprises both colon cancer (CC) and rectal cancer (RC) viewed as a single entity due to their origin in the colon, shared anatomical composition, and similar functions in the colorectal tract. CC accounts for 72% of CRC cases, with RC making up the remaining 28%. Most CRC cases involve adenocarcinoma in the colon and rectum, progressing from precancerous lesions. Metastasis to distant organs, particularly the liver, is common, driven by genetic mutations over a prolonged period [10].As cancer progresses, symptoms appear and vary based on type and location. Colorectal cancer patients report differing rates of symptoms, with blood in stool (32.8%) being most common, followed by bowel habit changes (28.9%), fatigue, decreased appetite, fever, nausea, and abdominal pain [11]. Timely identification of colorectal cancer is crucial for improving survival rates. Treatment options for CRC include chemotherapy, surgery, and radiation, tailored to factors such as tumor size and location. Advances in laparoscopic surgery, neoadjuvant chemotherapy, and radiotherapy have expanded treatment choices for patients with colorectal cancer. Research focused on developing gentler and more effective treatments is needed to enhance survival and quality of life for CRC patients in the future [10]. CEA, MSI, KRAS, and BRAF biomarkers aid in predicting outcomes in CRC post-surgery. CEA levels reflect CRC advancement but lack sensitivity in recurrence detection. Mutations in MSI, KRAS, and BRAF impact survival in metastatic CRC but don't predict chemotherapy efficacy while combining biomarkers improves prognosis precision [12].

EPIDEMIOLOGY

In 2020, colorectal cancer was the second deadliest cancer globally, causing 935,173 deaths with an ASMR of 9.0. Europe had the highest ASMR at 12.3, while Africa and the eastern Mediterranean had the lowest rates at 5.6 and 5.3. Men had an ASMR of 11.0, resulting in 515,637 deaths, compared to 419,536 deaths in women [13]. Colorectal cancer was the third most prevalent cancer globally in 2020, with 1,931,590 new cases and an ASR of 19.5 per 100,000 person-years. Europe had the highest rates [14]. Global trends in colorectal cancer (CRC) vary by country, with transitioned countries seeing stable or slightly decreasing rates while developing countries experience rising rates. GLOBOCAN 2020 projects a 63.3% increase in global new cases by 2040, with the highest increases in historically lower-risk regions. Early-onset CRC rates are highest in North America and Oceania [15]. Colorectal cancer rates are dropping in developed nations but rising in early-onset cases globally, possibly due to genetic factors [20]. Studies suggest that exposure to factors like hyperlipidemia, overweight, drinking alcohol, metabolic disorder, colon inflammation, lack of exercise, insufficient vitamin D consumption, excessive red meat consumption, and high consumption of sugary drinks may contribute to increased rates of EOCRC. Variations in gut bacteria diversity are also implicated in the trend. More research is needed to identify and address these risk factors worldwide [21].

PATHOGENESIS

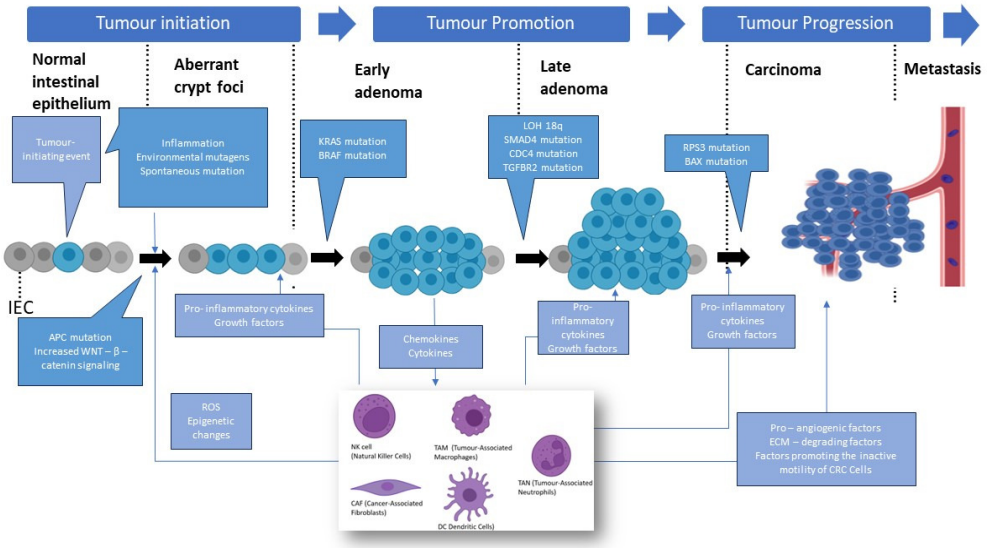

Figure 2: Pathogenesis of colorectal cancer

The development of colorectal cancer (CRC) is a multi-step process divided into three main phases: tumor initiation, promotion, and progression. It begins with normal intestinal epithelial cells (IECs), where mutations in genes such as APC led to the formation of aberrant crypt foci. Environmental factors, inflammation, and spontaneous mutations contribute to early adenoma formation. As the tumor grows, mutations in genes like KRAS and BRAF further promote tumor proliferation during the early adenoma stage. In the late adenoma phase, additional mutations such as SMAD4 and SOX9 occur, driven by pro-inflammatory cytokines, chemokines, and growth factors that fuel tumor growth. Finally, in the tumor progression phase, mutations in TP53 and RB1 allow the tumor to transition into carcinoma, with invasive characteristics. Eventually, the tumor metastasizes, spreading through the bloodstream to other organs. Throughout this process, inflammation and growth signals are critical in promoting the transition from benign lesions to malignant cancer.

ETIOLOGY

Genetic predispositions and lifestyle factors greatly influence colorectal cancer risk, especially for those with a family history. Factors to consider include age differences, relatives' age at diagnosis, and family history of tumors. Those with one affected first-degree relative, particularly before age 60, are at higher risk. Genetic syndromes like HNPCC and FAP contribute to elevated risks, with specific gene mutations causing increased risks for developing colorectal cancer [(23)]. Inflammatory bowel disease (IBD) is the third most significant risk factor for CRC after HNPCC and FAP. It includes chronic conditions affecting the digestive system's immune system, causing inflammation. Crohn's disease and ulcerative colitis are the main types. The exact cause is unknown but believed to involve genetics, immunity, and environment. Chronic inflammation from IBD increases CRC risk 2-6 times, with risk rising with IBD duration and severity [(24)]. Colon polyps are abnormal tissue growths from the mucous layer and can be non-neoplastic or neoplastic. Adenomatous polyps, a neoplastic type, pose a high cancer risk, with 95% of colorectal cancer cases stemming from them. However, only 5% of adenomatous polyps progress to cancer, usually within 5-15 years. Larger size, dysplasia level, and age increase the chance of malignancy. Removing adenomatous polyps in individuals aged 50+ is crucial for prevention [(25)]. Diabetes mellitus, a metabolic disorder resulting in high glucose levels due to insulin issues, affects 460 million people globally, with growing numbers. It significantly increases the risk of gastrointestinal cancers like colorectal cancer. Individuals with type 2 diabetes are three times more likely to develop colorectal cancer due to elevated insulin and inflammation levels. High insulin levels promote cell growth in the colon, while chronic inflammation facilitates cancer development and spread through proinflammatory cytokines [(27)]. The association between gallbladder removal and colorectal cancer

is inconclusive. Some research suggests an increased risk post-cholecystectomy, while other studies find no connection. Bile acid changes may play a role, as constant bile flow without a gallbladder can lead to harmful effects on colonic cells, potentially raising the risk of CRC through DNA damage and apoptosis [28].

DIETARY PATTERNS

Red and processed meats can increase cancer risk due to added preservatives and cooking methods. Heme in red meat causes genetic mutations, while high-fat consumption leads to obesity and cancer progression [29]. Consuming high dietary fiber lowers colorectal cancer risk by 50%. Mechanisms not fully understood. Fiber may reduce carcinogen exposure, add water to stool, promote gut bacteria. Dietary guidelines recommend 20-30 grams daily from fruits and vegetables. These foods provide vitamins, minerals, and antioxidants, reducing inflammation, protecting DNA, and lowering chronic disease risk. Maintaining a fiber-rich, plant-based diet essential for colorectal cancer prevention and overall health [30]. Consuming dairy, especially milk, is recommended by WCRF/AICR for colorectal cancer prevention due to its high calcium content. Calcium in dairy binds to bile acids/fatty acids, reducing cancer risk. Vitamin D in milk aids calcium absorption, prevents cancer, influences gene expression, boosts immunity, inhibits angiogenesis [31]. Excess weight, especially obesity, raises colorectal cancer risk. Adipose tissue produces substances promoting cancer growth and progression. Physical inactivity is linked to increased colorectal cancer rates worldwide. Those who are less active face a higher risk of developing the disease. Regular exercise can improve immune function, reduce inflammation and stress, and prevent obesity [33]. Tobacco smoke significantly increases the risk of colorectal cancer, with smokers being 2-3 times more likely to develop CRC compared to non-smokers. Smoking is believed to be responsible for 12% of colorectal cancer deaths, as it contains over 1,000 substances, including more than 60 known cancer-causing agents that can harm DNA in colorectal cells, leading to tumor growth and progression [51]. Alcohol intake, especially 2-3 drinks daily, correlates with increased colorectal cancer risk due to various mechanisms. Most colorectal cancer cases occur in people over 50, with those over 65 at highest risk. Incidence rising in younger adults, leading to updated screening guidelines. Men have a 30% higher risk of colorectal cancer and 40% higher mortality rate. Women have differing cancer locations. Individuals with low socioeconomic status are at a higher risk of developing cancer due to limited access to healthcare and poorer lifestyle habits. In North America, colon cancer rates are higher in those with low SES, while in Europe, high SES groups are more likely to develop colorectal cancer. Additional research is needed to verify these findings and understand the impact of SES on cancer incidence. CLOCK and BMAL1 control molecular clock, impact gene expression, colorectal cancer development. Disruptions accelerate tumor growth, Wnt signaling affects patient survival. [34].

Others

Table 1:

E. coli	Pcs + E. coli is linked to CRC and IBD, more common in CRC biopsies than healthy tissue, prevalent in advanced CRC stages, and produces colibactin. Consistent occurrence in CRC, adenoma, and healthy individuals.	Double-strand DNA breaks and alkylation occur, leading to decreased tumor-infiltrating T lymphocytes and increased colonic inflammation. Angiogenesis and epithelial-mesenchymal transition are promoted. Rho GTPases are altered, affecting cell adhesion and mobility.
F. nucleatum	Alkylated DNA causes double-strand breaks. Tumor-infiltrating lymphocytes decrease and colonic inflammation increases. Angiogenesis, and epithelial-mesenchymal transition are stimulated, altering Rho GTPases and impacting cell adhesion, and cytoskeleton. Phagocytosis decreases, cell growth is promoted, and host mismatch repair proteins are reduced.	It is connected to the BRAF mutation, CIMP-H, MSI-H, and MLH1 methylation. Activation of the p38 MAPK and NF-κB signaling pathways leads to the synthesis of IL-6, IL-8, and IL-18; the Fap2 protein inhibits NK cell cytotoxicity FadA-dependent activation of the E-cadherin/β-catenin pathway results in cell proliferation and increased myeloid suppressor cell count.
Streptococcus gallolyticus (Sg)	It is connected to the BRAF mutation, CIMP-H, MSI-H, and MLH1 methylation.	It is including the Wnt/β-catenin, KRAS, PI3K/AKT, and TGF-β signaling

	Stimulation of the p38 MAPK and NF-κB pathways results in the production of IL-6, IL-8, and IL-18; Fap2 protein hinders NK cell activity and boosts myeloid suppressor cell count; Activation of E-cadherin/β-catenin pathway by FadA promotes cell growth and E-cadherin expression.	pathways, along with p53 mutations and microsatellite instability (MSI). Epigenetic changes such as CpG island methylator phenotype (CIMP) and chromosomal instability (CIN)
Enterococcus faecalis (Ef)	Has superoxide: Encourages CRC induction in the experimental model using Il10-/-mice	BRAF mutation, CIMP-H, MSI-H, MLH1 methylation linked to p38 MAPK, NF-κB pathway activation producing IL-6, IL-8, IL-18; Fap2 reduces NK cell cytotoxicity, boosts myeloid suppressor cells; FadA activates E-cadherin/β-catenin pathway, promoting cell growth.

SCREENING METHODS

The USPSTF suggests various screening tests such as guaiac fecal occult blood testing, FIT, sDNA-FIT, sigmoidoscopy, CT colonography, and colonoscopy. Other screening tests that have been researched include the colon capsule (PillCam COLON 2, Medtronic) and the methylated serum septin 9 (EpiproColon, Epigenomics).

A guaiac developer is used with a stool sample in a gFOBT to check for a peroxidase reaction with heme, with sensitivities for advanced colorectal neoplasia and cancer ranging from 6-17% and 50-75% respectively. Results may be affected by diet and medications[35]. Fecal occult blood tests (FITs) are used to detect hidden blood in stools using antibodies. In the US, the performance of FITs varies among manufacturers. Two popular FITs, OC-Sensor and OC-Light by Polymedco, have high sensitivity for colorectal neoplasia (25-27%) and cancer (74-81%) with 95-96% specificity. Results on the impact of aspirin or antithrombotic drugs on test accuracy are inconclusive. The USPSTF recommends yearly FIT testing, while others suggest testing every two years. Tests can be done in person or by mail without any dietary or medication changes required. The sDNA-FIT by Exact Sciences combines DNA marker and FIT analysis to detect colorectal cancer and advanced neoplasia, with reported sensitivities of 47% and 93% and 89% specificity for advanced neoplasia. Other screening methods include sigmoidoscopy with a flexible endoscope, with high sensitivity for large adenomas and CRC. Colonoscopy is recommended every 5 years by the USPSTF, with CT colonography showing sensitivity for detecting polyps and cancer, recommended every 5 years as well. A colon capsule with a tiny camera is approved by the FDA for incomplete colonoscopy or lower GI bleeding, not for screening average-risk individuals. Studies show varying sensitivity and specificity for colon capsules compared to colonoscopy [36]. EpiproColon by Epigenomics AG uses blood to detect methylation in the septin 9 gene promoter region. The test has 25% sensitivity for advanced neoplasia, 68% for CRC, and 79% specificity. FDA approved for average-risk individuals declining standard screening. Yearly testing is recommended, with colonoscopy following abnormal results. USPSTF has not been approved for average-risk screening.

MANAGEMENT

Endoscopic Treatment

Advances in flexible endoscopes have increased demand for minimally invasive treatments for T1 CRC. Block EMR or ESD can resect the tumor, while peranal EM is used for rectal lesions with fibrosis. EFR is an option for smaller lesions. Endoscopic removal by experts is safer and cheaper than surgery. Combination endoscopic and laparoscopic surgery can reduce costs and complications. Japanese guidelines consider factors like lymph vascular invasion and histological grade. AI systems may be better at predicting lymph node metastasis in T1 CRC patients than current guidelines, as per a recent study.

Surgery

Surgery remains the most effective treatment for advanced colorectal cancer, with Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy (HIPEC) being crucial options. Lymph node dissection during colorectal cancer surgery is essential for accurate staging. Complete mesocolic excision (CME) is favored for curative colon cancer surgery, improving specimen quality and survival. Laparoscopic surgery offers short-term advantages and is recommended in

Japan and globally. Total mesorectal excision (TME) is the standard for rectal cancer surgery, with TaTME helping maintain margins. Navigation technology advancements, including Indocyanine green (ICG) and artificial intelligence (AI), have improved surgical outcomes. Three-dimensional CT angiography assists with preoperative planning. Rectal cancer patients often undergo surgery with radiation therapy. Peritoneal carcinomatosis, common in colorectal cancer, can be treated with cytoreductive surgery with HIPEC, considered the best option based on recent studies [37].

CHEMOTHERAPY

Since the 1950s has aimed to understand why some colorectal cancer patients do not respond well to 5-FU chemotherapy by identifying biomarkers predicting treatment effectiveness. Studies have shown the important role of p53 in colorectal cancer and its impact on 5-FU response, including activating cancer stem cells. P53 regulates the WNT/β-catenin pathway by promoting WNT3 expression, affecting anti-cancer effects and stem cell activation. Combining 5-FU with a WNT/β-catenin inhibitor could reduce cancer stem cells and prevent recurrence in CRC. Targeting WNT ligands along with 5-FU may further decrease recurrence risk, showing potential as a therapeutic approach against colorectal cancer recurrence. Irinotecan, derived from camptothecin, is a widely studied CRC treatment. It inhibits Topoisomerase I without DNA interaction. Irinotecan forms a ternary complex with SN-38 and DNA, blocking replication and killing cancer cells [38].

COMBINATION THERAPIES BASED ON IRINOTECAN

FOLFIRI, a CRC chemotherapy with irinotecan and leucovorin, utilizes 5-FU to hinder pyrimidine synthesis by transforming into active metabolites and inhibiting thymidylate synthase, leading to cancer cell damage and death. FOLFOXIRI is a strong chemotherapy blend of irinotecan, oxaliplatin, and 5-FU/leucovorin. Oxaliplatin creates Pt-DNA adducts, disrupting transcription and replication to kill cancer cells effectively. The treatment shows high response rates, PFS, and OS with tolerable side effects, involving drug absorption, activation, DNA binding, and intracellular processing. The XELIRI regimen, combining irinotecan and capecitabine, showed positive results in a phase II study with mCRC patients. Capecitabine, an oral prodrug of fluorouracil, has high bioavailability and offers ease of use, safety, and potent anti-tumor effects. Its rapid metabolism in the liver minimizes systemic toxicity in normal tissues [39].

IMMUNOTHERAPY

In 2004, the FDA approved bevacizumab for CRC treatment, starting a new era in anti-cancer drugs. It targets VEGF, aiding tumor growth[89]. VEGF-A in the group stimulates angiogenesis by binding to VEGFR-1 and VEGFR-2, activating VEGF signaling in endothelial cells[90]. VEGFR-2 and VEGFR-1 have distinct roles in tumors, VEGFR-2 in angiogenesis and VEGFR-1 in tumor growth. Bevacizumab blocks VEGF-A, stopping angiogenesis and tumor growth by preventing VEGF-A from binding to VEGFR-1 and VEGFR-2. It improves outcomes in colorectal cancer but can cause proteinuria and thromboembolism. Bevacizumab's cost-effectiveness prolongs overall survival, especially in second-line therapy [91]. Bevacizumab and FOLFIRI combined led to a higher response rate (44.8% vs. 34.8%, $p = 0.004$) and longer median progression-free survival (10.6 vs. 6.2 months, $p < 0.001$) and overall survival (20.3 vs. 15.6 months, $p = 0.00003$) in patients with mCRC. Bevacizumab, with FOLFIRI or XELIRI, proved an effective initial treatment for mCRC with comparable safety profiles. A 2020 meta-analysis favored bevacizumab plus irinotecan-based chemotherapy for improving PFS in mCRC patients. TRIBE-2 trials showed FOLFOXIRI + bevacizumab improved mCRC patient survival over FOLFIRI + bevacizumab, though with more adverse events. Initial treatment option supported. Palliative care, maintenance therapy, and progression treatment with FOLFOXIRI + bevacizumab possible for suitable patients. TRIBE-C study evaluates Chinese-adapted FOLFOXIRI regimen + bevacizumab in advanced CRC [40]. Panitumumab targets EGFR in mCRC by inhibiting its growth-promoting functions, commonly overexpressed in cancer cells, aiding growth and spread. Panitumumab, as the first fully human monoclonal antibody approved for treating CRC, is less likely to cause an immune response. Panitumumab, an IgG2 monoclonal antibody, has a higher affinity for EGFR compared to cetuximab, attaches more easily to EGFR, and efficiently inhibits the attachment of EGF or TGF-α ligands to EGFR. Panitumumab blocks EGF and TGF-α, leading to inhibition of EGFR signaling, preventing tumor growth, and encouraging cell death. It enhances the effects of other drugs and can be used alone or with irinotecan for mCRC. Panitumumab is more effective than

Cetuximab post-bevacizumab treatment. It does not affect irinotecan pharmacokinetics and shows no synergistic effects. Panitumumab plus irinotecan improves progression-free survival in KRAS mutant colorectal cancer patients and significantly in non-mutated individuals. It increases the response rate but not overall survival, reduces side effects like diarrhea, and is well-tolerated in RAS wild-type mCRC patients. Panitumumab and modified FOLFOXIRI regimen improved response and metastasis resection rates in RAS wild-type mCRC. Combined with potent chemotherapy, panitumumab effectively treats advanced colorectal cancer, providing prolonged progression-free survival despite skin side effects. Anti-EGFR therapy is more cost-effective than anti-VEGF in RAS wild-type mCRC. Panitumumab with irinotecan extends PFS with manageable toxicity, showing potential as salvage treatment [41]. Cetuximab, a monoclonal antibody targeting EGFR, was first approved in 2004 for mCRC treatment. It inhibits tumor growth by blocking the EGFR pathway and induces ADCC for immune responses. NK cells become activated through interaction with cetuximab attached to EGFR, leading to the secretion of IFN-γ that triggers the activation of dendritic cells, subsequently enhancing NK cell activity[101]. Cetuximab-activated dendritic cells release antigens that activate T cells. Mature dendritic cells then trigger immune responses by presenting antigens to cytotoxic T cells and activating NK cells [102]. Macrophages and immune cells interact through IFN-γ to recruit cytotoxic T cells to tumors. T cells attack tumor cells, generating antigens and boosting immune responses. Research compared cetuximab alone versus combined with irinotecan for treating CRC. The combination group had better response rates and longer progression times. Cetuximab increases irinotecan sensitivity, improving outcomes for mCRC patients. Combining cetuximab with irinotecan is more effective in treating RAS wild-type mCRC than using either drug alone. Studies like FIRE-3, AIO KRK-0306, and POCHER show improved outcomes. Trials like MACBETH and a Japanese study also show positive results with cetuximab combinations in treating mCRC. Further studies needed for survival extension [42].PHY-906 originates from the ancient recipe Huang Qin Tang (HQT), which has been utilized for millennia in Zhang Zhongjing's Treatise on Typhoid Fever of the Eastern Han Dynasty. It is mainly employed in managing gastrointestinal issues like diarrhea, stomach pain, nausea, and vomiting. PHY-906, an anti-cancer medication candidate, is a blend of Paeonia lacriform Pall, Glycyrrhiza ruralness Fisch, Scutellarin baicalensis Georgi, and Ziziphus jujuba Mill, made following quality guidelines. It enhances irinotecan's cancer-fighting abilities and reduces its side effects, including weight loss and mortality. PHY-906 lessens inflammation, promotes intestinal cell regeneration, and boosts immune responses. Baicalin, baicalein, glycyrrhizic acid, and wogonin in PHY-906 are crucial for enhancing irinotecan's effects and reducing intestinal toxicity. In a phase I trial with CRC patients, PHY-906 showed no severe adverse events compared to a placebo group. Further research is needed to fully understand PHY-906's potential in enhancing chemo effectiveness and reducing side effects [43]. Silymarin, a natural flavonoid present in the perennial herb artichoke, has silybin as its primary active component. Silymarin is safe at high levels and can interfere with cycle control, cell death, blood vessel formation, and the production of proteins linked to resistance to multiple drugs. However, silymarin's excellent antioxidant properties make it a valuable tool in both cancer prevention and treatment. Silymarin inhibits Wnt signaling in human CRC cells, reducing β-catenin and TCF4 levels. It also suppresses blood vessel growth and cell proliferation in Lovo CRC cells. In AOM-induced colon cancer models, silymarin decreases ACF numbers and colonic BGUS activity. It reduces IL-6 and inhibits IL-1β and TNF-α in colitis-associated cancer models, blocking IL-6/STAT3 signaling. Silymarin also affects intestinal enzymes, oxidative stress, and Wnt/β-catenin signaling to prevent DMH-induced colon cancer. In CT26 CRC cells, silymarin induces apoptosis and autophagy by altering Bax, Caspase-3, and Bcl-2 levels.Silymarin is not effective alone for cancer treatment but may help in combination plans. FOLFIRI chemo with irinotecan is effective for mCRC but causes severe intestinal toxicity, impacting treatment for CRC patients due to BGUS converting irinotecan to SN-38. Silymarin in diet suppresses chemically induced colon cancer in rats by inhibiting the breakdown of glucuronides. Silymarin may enhance the effectiveness of irinotecan in colon cancer treatment. The clinical trial studied silymarin with irinotecan-based therapy in mCRC patients. The study group received silymarin capsules with chemotherapy while the control group received FOLFIRI and bevacizumab [110]. Adding silymarin reduced toxic incidents in the study group, lowering nausea (27.0% vs. 40.2%, $p = 0.005$) and diarrhea (5.4% vs. 14.6%, $p = 0.002$) occurrences without affecting irinotecan clearance [44]. Silymarin proves effective and well-tolerated in irinotecan-based chemotherapy. It's a cost-effective natural product with great potential as a complementary therapy.

GENE THERAPY

IL-15 mRNA Nano formulation

Immunogene therapy is a novel treatment approach for colorectal cancer using the cytokine IL-15 to stimulate the immune system. Traditional studies have used IL-15 plasmid DNA, which has limitations. This study demonstrates the use of IL-15 mRNA for colon cancer treatment using the CLPP system for efficient delivery. The system successfully delivered IL-15 mRNA into cancer cells, stimulating immune response and inducing cytotoxicity. In preclinical models, the CLPP/mIL-15 complex showed significant inhibitory effects on cancer growth, highlighting its potential for treating colorectal cancer [45].

Bacteriotherapy in CRC

In recent years, there has been a rise in cancer mortality rates, prompting scientists to explore new treatment methods. Bacteriotherapy shows promise for CRC, a common deadly cancer, offering an innovative and affordable approach. Research shows differences in microbiomes of CRC patients and healthy individuals, with some bacteria playing healing roles with minimal side effects. Based on this background further research on bacteria's role in treating CRC in patients shows great potential based on this information. Various bacterial mechanisms have been identified and are being utilized in the treatment of CRC through bacteriotherapy, including cell membrane pore formation, metastasis inhibition, tumor necrosis, and apoptosis. Apoptosis is a crucial aspect of cancer therapy, aiming to kill tumor cells by activating programmed cell death. This process involves two main pathways: the intrinsic pathway, which releases cytochrome c from mitochondria, and the extrinsic pathway, triggered by death receptors on the cell membrane. Both pathways activate Caspases, leading to cell death. It is essential to understand the interconnectedness of these pathways, as metabolites from one pathway can impact the other [46]. Altogether, utilizing bacteria-induced apoptosis and metastasis suppression for effective therapy requires meeting framework conditions like high cytotoxicity against cancer cells, low cytotoxicity towards healthy tissue, and selective targeting of carcinomas.

Suicide Gene therapy

Gene therapy is a new method for cancer treatment using messenger RNA (mRNA). However, challenges remain in finding efficient mRNA transporters. The DMP backbone is created from lipid and polymer self-assembly. The addition of the peptide increases mRNA delivery effectiveness. DMP-039 nanoparticles successfully deliver mRNA through cell membranes. In tests, the system effectively suppressed colon cancer growth and inhibited pulmonary metastatic tumors with high safety. DMP-039 shows promise for mRNA-based suicide gene therapy. Overall, the peptide-modified hybrid nanoparticle is a potential cancer treatment [47].

PPAR inhibitors can inhibit the growth of CRC organoids

Differentially expressed genes in normal and cancer tissues showed an increase in PPAR signaling pathway-related genes in tumor epithelial cells compared to normal cells. PPARs regulate metabolism and balance glucose/lipids through three subtypes. Research on PPAR's impact on colorectal cancer using agonists or gene knockdown led to disputes, especially on cancer cell lines. Tumor organoids treated with PPAR antagonist FH535 showed reduced growth and increased apoptosis, similar to GW9662 inducing tumor cell death. Combining PPAR and WNT inhibitors enhanced tumor-killing efficacy at low doses. FH535 primarily acts through PPARγ inhibition, suggesting PPAR inhibitors may be effective in treating colorectal cancer [48].

CRISPR/Cas9 system in CRC

Gene mutations play a key role in colorectal cancer (CRC), with the exact effects still unclear. CRISPR/Cas9 technology revolutionizes genome editing by enabling precise gene manipulation with SGRNA. Successfully used in CRC cell lines, mouse models, and human organoid models, it enhances gene therapy and screening. Animal models and 3D cell cultures improve drug assessment. CRISPR/Cas9 creates precise gene knockout models, studying cancer behavior and genetic factors in CRC. Organoids combined with CRISPR/Cas9 offer insights into tumor development and drug resistance [49].

ADJUVANT CHEMOTHERAPY

Adjuvant Therapy for Stage II Colon Cancer
Adjuvant chemotherapy is generally not advised for stage II colon cancer patients unless they are considered high risk. T4 tumor patients and those with other high-risk factors like inadequate lymph node sampling or tumor invasion may benefit from ACT. Adding oxaliplatin to fluoropyrimidine-based ACT is typically not recommended, but could be considered after consultation. Patients with mismatch repair deficiency or microsatellite instability may require oxaliplatin-containing chemotherapy if considered necessary. Guidelines also mention the duration of oxaliplatin-based treatment depending on factors like disease-free survival and adverse effects.

ctDNA Guiding Adjuvant Therapy
CTDNA presence post-surgery is linked to poor outcomes in CRC patients. A study of 1,039 surgically treated CRC patients found higher recurrence risk in those testing positive 4 weeks post-surgery. CtDNA was a key predictor, more so than BRAF and RAS status. Integrating ctDNA into TNM staging may improve criteria. High detection rates were found in pre-surgery testing, with personalized testing emphasized. CtDNA positivity post-surgery indicates benefit from adjuvant chemotherapy, especially in high-risk patients. Atezolizumab may impact outcomes in ctDNA-positive patients with urothelial cancer. Adjuvant trifluridine/tipiracil is being investigated for CRC patient's post-standard treatment. CtDNA-negative patients may only need observation for positive outcomes, while ongoing trials explore this further. Multivariate analysis supports the benefits of adjuvant chemotherapy in reducing recurrence risk. Future studies will evaluate optimal ctDNA-guided treatment strategies [(50)].

Cyclooxygenase 2 inhibition as an adjuvant in stage III colon cancer with PIK3CA activation
Observational studies suggest that aspirin or COX-2 inhibitors may reduce colorectal cancer recurrence, with PIK3CA mutations potentially indicating a positive response to COX-2 inhibitors. The CALGB/SWOG 80702 trial, funded by the National Cancer Institute, aimed to investigate the addition of celecoxib to standard chemotherapy for stage III colon cancer. While overall disease-free survival did not significantly improve with celecoxib, patients with PIK3CA mutations showed better outcomes. However, the interaction test did not reach significance. Patients with PIK3CA mutations also had improved overall survival. These results highlight the potential of using PIK3CA status to guide COX-2 inhibitor treatment in stage III colon cancer [(51)].

Prevention
Preventing colorectal cancer is essential for public health. Evidence shows that quitting Preventing colorectal cancer is crucial for public health, with evidence indicating that lifestyle changes such as quitting smoking, healthy eating, and regular exercise can decrease the risk. Recommendations include daily physical activity, a diet rich in milk, whole grains, fruits, nuts, and vegetables, as well as calcium and fibre intake. Chemopreventive agents, hormone replacement therapy, and aspirin may also reduce the risk, especially for high-risk individuals with genetic predispositions. Vitamin D and calcium levels are associated with a decreased risk of colorectal neoplasma. Screening for colorectal cancer, including colonoscopy, is essential for early detection and prevention. High-risk individuals should undergo routine screenings, with various alternatives available. Post-treatment, following a healthy lifestyle and adherence to aspirin and NSAIDs can improve survival rates. Lifestyle changes post-diagnosis can significantly reduce future deaths. Further research is needed to confirm the effectiveness of these interventions. Ultimately, personalized prevention strategies based on individual risk factors are key to effectively preventing colorectal cancer and improving outcomes for patients [(51)].

CONCLUSION

Colorectal cancer (CRC) is a significant health issue globally, ranking third in common cancers and second in cancer deaths. The rates vary due to genetics, lifestyle, and environment, with a worrying rise in younger people in developed countries. Key risk factors are genetics, family history, inflammatory bowel diseases, poor diet, lack of exercise, smoking, and alcohol use. Prevention involves maintaining a nutritious diet, participating in consistent physical activity, refraining from smoking and consuming too much alcohol, and adequate calcium and vitamin D intake. Screening and early detection are crucial. Treatment advancements in chemotherapy,

surgery, and radiation have improved survival. Biomarkers like CEA, MSI, KRAS, and BRAF help in predicting outcomes and tailoring treatments. Understanding CRC's molecular pathways aids in targeted therapies. More research is needed to improve prevention, detection, and treatment, especially for diverse populations. Awareness and advancements in medical research are key to fighting colorectal cancer.

CONFLICTS OF INTEREST

There is no declaration of a conflict of interest

ACKNOWLEDGEMENT

The author thanks to college management, principal, teaching and non-teaching staff, and colleagues for their support.

REFERENCES

1. Ervik, M.; Lam, F.; Laversanne, M.; Ferlay, J.; Bray, F. Global Cancer Observatory: Cancer Over Time. Available online: https://gco.iarc.fr/overtime (accessed on 23 March 2023).
2. Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today. Available online: https://gco.iarc.fr/today (accessed on 10 October 2023).
3. Ferlay, J.; Laversanne, M.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Tomorrow. Available online: https://gco.iarc.fr/tomorrow (accessed on 10 October 2023).
4. Eng, C.; Hochster, H. Early-Onset Colorectal Cancer: The Mystery Remains. *J. Natl. Cancer Inst.* 2021, *113*, 1608–1610. [Google Scholar] [CrossRef] [PubMed]
5. Siegel, R.L.; Wagle, N.S.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics, 2023. *CA A Cancer J. Clin.* 2023, *73*, 233–254. [Google Scholar] [CrossRef] [PubMed]
6. Siegel, R.L.; Miller, K.D.; Goding Sauer, A.; Fedewa, S.A.; Butterly, L.F.; Anderson, J.C.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics, 2020. *CA A Cancer J. Clin.* 2020, *70*, 145–164.
7. Shaukat, A.; Kahi, C.J.; Burke, C.A.; Rabeneck, L.; Sauer, B.G.; Rex, D.K. ACG Clinical Guidelines: Colorectal Cancer Screening 2021. *Am. J. Gastroenterol.* 2021, *116*, 458–479. [Google Scholar] [CrossRef] [PubMed]
8. Shaukat, A.; Kahi, C.J.; Burke, C.A.; Rabeneck, L.; Sauer, B.G.; Rex, D.K. ACG Clinical Guidelines: Colorectal Cancer Screening 2021. *Am. J. Gastroenterol.* 2021, *116*, 458–479. [Google Scholar] [CrossRef] [PubMed]
9. https://colorectalcancer.org/article/acs-releases-colorectal-cancer-estimates 2024#:~:text=Colorectal%20cancer%20is%20common%20and,cases%20will%20be%20rectal %20cancer.
10. Recio-Boiles A, Kashyap S, Tsoris A, Babiker HM. Rectal Cancer. [Updated 2020 Dec. 17]. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK493202/. [Google Scholar] [Ref list]
11. Mullangi S, Lekkala MR. Adenocarcinoma. [Updated 2021 Feb. 22]. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562137/. [Google Scholar] [Ref list]
12. Formica V, Sera F, Cremolini C, Riondino S, Morelli C, Arkenau HT, Roselli M. KRAS and BRAF mutations in stage II and III Colon cancer: A systematic review and Meta-analysis. *JNCI: Journal of the National Cancer Institute*. 2022;114:517–527. doi: 10.1093/jnci/djab190.
13. Sun Q, Pastor L, Du J, Powell MJ, Zhang A, Bodmer W, Wu J, Zheng S, Sha MY. A novel xenonucleic acid-mediated molecular clamping technology for early colorectal cancer screening. *Plos One*. 2021;16:e0244332. doi: 10.1371/journal.pone.0244332
14. IARC: Colorectal cancer screening. Vol. 17. IARC Handb Cancer Prev, pp1-300, 2019. Available from: http://publications.iarc.fr/573. [Google Scholar] [Ref list]
15. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2020;76:182–188. doi: 10.1111/his.13975. WHO Classification of Tumours Editorial Board. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

16. Recio-Boiles A, Cagir B. Colon cancer. [Updated 2021 Jan. 25]. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470380/. [PubMed] [Google Scholar] [Ref list]
17. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2020;76:182–188. doi: 10.1111/his.13975. WHO Classification of Tumours Editorial Board. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]
18. Dekker E, Tanis PJ, Vleugels JL, Kasi PM, Wallace MB. Colorectal cancer. *Lancet*. 2019;394:1467–1480. doi: 10.1016/S0140-6736(19)32319-0. [PubMed] [CrossRef] [Google Scholar] [Ref list]
19. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and MortalityWorldwide for 36 Cancers in 185 Countries. CA A Cancer J. Clin. 2021, 71, 209–249. [CrossRef]
20. Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Pineros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. Global Cancer Observatory: Cancer Today. Available online: https://gco.iarc.fr/today (accessed on 10 October 2023).
21. Ervik, M.; Lam, F.; Laversanne, M.; Ferlay, J.; Bray, F. Global Cancer Observatory: Cancer Over Time. Available online: https://gco.iarc.fr/overtime (accessed on 23 March 2023).
22. Siegel, R.L.; Miller, K.D.; Goding Sauer, A.; Fedewa, S.A.; Butterly, L.F.; Anderson, J.C.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics, 2020. CA A Cancer J. Clin. 2020, 70, 145–164
23. Arnold, M.; Abnet, C.C.; Neale, R.E.; Vignat, J.; Giovannucci, E.L.; McGlynn, K.A.; Bray, F. Global Burden of 5 Major Types of Gastrointestinal Cancer. Gastroenterology 2020, 159, 335–349.e315
24. Ghasemi-Kebria, F.; Jafari-Delouie, N.; Semnani, S.; Fazel, A.; Etemadi, A.; Norouzi, A.; Khandoozi, R.; Besharat, S.; Shokouhifar, N.; Mirkarimi, H.; et al. Colorectal cancer incidence trends in Golestan, Iran: An age-period-cohort analysis 2004–2018. Cancer Epidemiol. 2023, 86, 102415
25. Clinton, S.K.; Giovannucci, E.L.; Hursting, S.D. The world cancer research fund/American institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: Impact and future directions. J. Nutr. 2020, 150, 663–671.
26. Montminy, E.M.; Zhou, M.; Maniscalco, L.; Abualkhair, W.; Kim, M.K.; Siegel, R.L.; Wu, X.C.; Itzkowitz, S.H.; Karlitz, J.J. Contributions of Adenocarcinoma and Carcinoid Tumors to Early-Onset Colorectal Cancer Incidence Rates in the United States. Ann. Intern. Med. 2021, 174, 157–166. [CrossRef] [PubMed]
27. Howren, A.; Sayre, E.C.; Loree, J.M.; Gill, S.; Brown, C.J.; Raval, M.J.; Farooq, A.; De Vera, M.A. Trends in the Incidence of Young-Onset Colorectal Cancer with a Focus on Years Approaching Screening Age: A Population-Based Longitudinal Study. J. Natl. Cancer Inst. 2021, 113, 863–868. [CrossRef]
28. Emile, S.H.; Horesh, N.; Freund, M.R.; Garoufalia, Z.; Gefen, R.; Silva-Alvarenga, E.; Maron, D.J.; DaSilva, G.; Wexner, S.D. Trends in the Characteristics, Treatment, and Outcomes of Rectal Adenocarcinoma in the US From 2004 to 2019: A National Cancer Database Analysis. JAMA Oncol. 2023, 9, 355–364. [CrossRef]
29. Eng, C.; Hochster, H. Early-Onset Colorectal Cancer: The Mystery Remains. J. Natl. Cancer Inst. 2021, 113, 1608–1610. [CrossRef] [PubMed]
30. Murphy, N.; Campbell, P.T.; Gunter, M.J. Unraveling the Etiology of Early-Onset Colorectal Cancer. J. Natl. Cancer Inst. 2021, 113, 505–506. [CrossRef]
31. Cercek, A.; Chatila, W.K.; Yaeger, R.; Walch, H.; Fernandes, G.D.S.; Krishnan, A.; Palmaira, L.; Maio, A.; Kemel, Y.; Srinivasan, P.; et al. A Comprehensive Comparison of Early-Onset and Average-Onset Colorectal Cancers. J. Natl. Cancer Inst. 2021, 113,1683–1692. [CrossRef]
32. Zheng, X.; Hur, J.; Nguyen, L.H.; Liu, J.; Song, M.; Wu, K.; Smith-Warner, S.A.; Ogino, S.; Willett, W.C.; Chan, A.T.; et al. Comprehensive Assessment of Diet Quality and Risk of Precursors of Early-Onset Colorectal Cancer. J. Natl. Cancer Inst. 2021, 113, 543–552. [CrossRef]
33. Hur, J.; Otegbeye, E.; Joh, H.K.; Nimptsch, K.; Ng, K.; Ogino, S.; Meyerhardt, J.A.; Chan, A.T.; Willett, W.C.; Wu, K.; et al. Sugar-sweetened beverage intake in adulthood and adolescence and risk of early-onset colorectal cancer among women. Gut 2021, 70, 2330–2336. [CrossRef] [PubMed]
34. Sinicrope, F.A. Increasing Incidence of Early-Onset Colorectal Cancer. N. Engl. J. Med. 2022, 386, 1547–1558. [CrossRef] [PubMed]

35. Lin JS, Perdue LA, Henrikson NB, Bean SI, Blasi PR. Screening for colorectal cancer: updated evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2021;325(19):1978–1998. [PubMed: 34003220]
36. Cash BD, Fleisher MR, Fern S, et al. Multicentre, prospective, randomised study comparing the diagnostic yield of colon capsule endoscopy versus CT colonography in a screening population (the TOPAZ study). Gut. 2020.
37. Bartoş A, Bartoş D, Raluca S, Mitre C, Hadade A, Iancu I, Cioltean C, Iancu C, Militaru C, Părău A, Breazu C. Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for the Treatment of Peritoneal Carcinomatosis: Our Initial Experience. Chirurgia (Bucur). 2019 Mar-Apr;114(2):222-233. doi: 10.21614/chirurgia.114.2.222. PMID: 31060655.
38. Kciuk, M., Marciniak, B., and Kontek, R. (2020). Irinotecan-still an important player in cancer chemotherapy: a comprehensive overview. *Int. J. Mol. Sci.* 21 (14), E4919. doi:10.3390/ijms21144919
39. García-Alfonso, P., Muñoz Martín, A. J., Ortega Morán, L., Soto Alsar, J., Torres Pérez-Solero, G., Blanco Codesido, M., et al. (2021). Oral drugs in the treatment of metastatic colorectal cancer. *Ther. Adv. Med. Oncol.* 13, 17588359211009001. doi:10.1177/17588359211009001
40. Cremolini, C., Antoniotti, C., Rossini, D., Lonardi, S., Loupakis, F., Pietrantonio, F., et al. (2020). Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, randomized, controlled trial. *Lancet Oncol.* 21 (4), 497–507. doi:10.1016/S1470-2045(19)30862-9
41. Koilakou, S., and Petrou, P. (2021). Economic evaluation of monoclonal antibodies in metastatic colorectal cancer: a systematic review. *Mol. Diagn. Ther.* 25 (6), 715–734. doi:10.1007/s40291-021-00560-4
42. Kadowaki, S., Masuishi, T., Ura, T., Sugiyama, K., Mitani, S., Narita, Y., et al. (2021). A triplet combination of FOLFOXIRI plus cetuximab as first-line treatment in RAS wild-type, metastatic colorectal cancer: a dose-escalation phase Ib study. *Int. J. Clin. Oncol.* 26 (4), 701–707. doi:10.1007/s10147-020-01842-3
43. Qin, H.-R., Sun, W.-J., Zhang, H., Yan, H., Zhang, S.-W., Wang, H., et al. (2022). Overview of the mechanism and clinical application of Huang Qin Tang on the potentiation and toxicity reduction of chemotherapeutic drugs. *China J. Chin. Med.* 37 (1), 293–296.
44. Huang, C.-Y., and Zhu, J. (2021). Irinotecan in neoadjuvant radiotherapy for rectal cancer. *J. Chin. Oncol.* 27 (10), 791–797. doi:10.11735/j.issn.1671-170X.2021.10.B001
45. Mol. Pharmaceutics 2020, 17, 9, 3378-3391https://doi.org/10.1021/acs.molpharmaceut.0c00451
46. Soleimanpour, S.; Hasanian, S.M.; Avan, A.; Yaghoubi, A.; Khazaei, M. Bacteriotherapy in gastrointestinal cancer. *Life Sci.* 2020, *254*, 117754. [Google Scholar] [CrossRef]
47. Carneiro, B.A.; El-Deiry, W.S. Targeting apoptosis in cancer therapy. *Nat. Rev. Clin. Oncol.* 2020, *17*, 395–417. [Google Scholar] [CrossRef] [PubMed]
48. Yan Gao, Ke Men, Congbin Pan, Jingmei Li, Jieping Wu, Xiaohua Chen, Sibei Lei, Xiang Gao & Xingmei Duan (2021) Functionalized DMP-039 Hybrid Nanoparticle as a Novel mRNA Vector for Efficient Cancer Suicide Gene Therapy, International Journal of Nanomedicine, 5211-5232, DOI: 10.2147/IJN.S319092
49. Wang, R., Li, J., Zhou, X. *et al.* Single-cell genomic and transcriptomic landscapes of primary and metastatic colorectal cancer tumors. *Genome Med* 14, 93 (2022). https://doi.org/10.1186/s13073-022-01093-z
50. Hu, Y., Liu, L., Jiang, Q. *et al.* CRISPR/Cas9: a powerful tool in colorectal cancer research. *J Exp Clin Cancer Res* 42, 308 (2023). https://doi.org/10.1186/s13046-023-02901-z.
51. Nancy N. Baxter et al., Adjuvant Therapy for Stage II Colon Cancer: ASCO Guideline Update. *JCO* 40, 892-910(2022). DOI:10.1200/JCO.21.02538.