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Theranostic A New Approaches For Colorectal Cancer Treatment: Molecular Biomarkers, Challenges And Future Directions

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Abstract

Colorectal cancer (CRC) presents a significant global health challenge, being one of the most prevalent malignancies and a leading cause of cancer-related mortality worldwide. Despite advances in screening initiatives and therapeutic interventions, CRC incidence persists, particularly among younger demographics and in transitioning regions. The multifaceted etiology of CRC involves a complex interplay of genetic predisposition, environmental influences, and lifestyle factors, contributing to its heterogeneous nature. Understanding the traditional adenoma-carcinoma sequence provides a framework for comprehending the stepwise progression of CRC, characterized by the accumulation of genetic and epigenetic alterations driving tumorigenesis. This review delves into the emerging paradigm of theranostics in CRC, which integrates targeted therapies with diagnostic tools to tailor treatment strategies based on individual molecular profiles. Theranostics revolutionizes CRC care by enhancing precision through early detection, guided therapy selection, and real-time monitoring of treatment responses. By ident[1]ifying specific biomarkers and employing personalized therapeutic agents, theranostics minimizes unnecessary exposure to ineffective treatments and their associated toxicities, while optimizing therapeutic outcomes. Various theranostic strategies are explored, including the utilization of nanomaterials such as iron oxide nanoparticles, quantum dots, dendrimers, carbon nanotubes, and liposomes, along with the assessment of epigenetic markers such as CpG island methylator phenotype and microRNAs. Additionally, novel potential markers for CRC, such as phosphatidylinositide-3-kinases (PI3K), PTEN, TP53, and IGFR-1R, are investigated, alongside the expression of protein markers like carcinoembryonic antigen (CEA) and heat shock proteins (HSP). Furthermore, the review elucidates the role of molecular imaging biomarkers in CRC management, encompassing techniques such as computed tomographic colonography, dual-energy spectral CT, diffusion-weighted imaging, and positron emission tomography. The integration of machine learning algorithms enhances the predictive accuracy of imaging modalities, facilitating early detection and precise treatment planning. Personalized treatment strategies in CRC are discussed, focusing on biomarker-driven targeted therapies such as

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RAS mutations, BRAF V600E mutation, microsatellite instability (MSI), and deficiency in mismatch repair (dMMR). Moreover, the emergence of immunotherapy and immune checkpoint inhibitors, including PD-1/PD-L1 pathway inhibitors and CTLA-4 blockade, represents a promising frontier in CRC treatment, offering improved outcomes and prolonged survival for patients.

Key words: *Theranostic Approaches, Colorectal Cancer, Heat Shock Proteins, CTLA-4 blockade and NTSD4.*

1.Introduction

CRC is a major public health problem worldwide and one of the most common malignancies and the leading cause of cancer death. Despite advances in screening programs and treatment strategies, the incidence of CRC continues to increase, particularly in younger populations and developing regions undergoing epidemiological change. The etiology of CRC is complex, involving genetic susceptibility, environmental factors, and lifestyle behaviors, and the interaction of these factors contributes to its heterogeneity. The traditional adenoma-carcinoma sequence provides a framework for understanding the stepwise progression of CRC, which is characterized by the accumulation of genetic and epigenetic changes that drive tumorigenesis. In particular, CRC exhibits considerable molecular diversity, with distinct molecular subtypes with unique genetic signatures and clinical behavior. This molecular heterogeneity underlines the need for precision medicine approaches adapted to individual tumor biology [1].

CRC, the leading cause of cancer-related death worldwide, arises in the colon or rectum. It usually starts as non-cancerous (benign) polyps and can develop into malignant cancer over time. This progression is often caused by a combination of genetic mutations, lifestyle factors and environmental influene [2]. The adenoma-carcinoma sequence is a well-recognized pathway in the development of CRC, in which benign adenomatous polyps transform into invasive adenocarcinomas. This transformation involves the accumulation of genetic changes in key oncogenes and tumor suppressor genes, such as APC, KRAS, and TP53, which regulate cell growth and apoptosis [3]. The variability of CRC is highlighted by the complexity of its molecular landscape, which may be seen in differences in tumour behaviour, prognosis, and response to therapy. The hunt for more targeted treatment approaches has been fueled by this heterogeneity, and as a result, theranostics a combination of medicine and diagnostics—has emerged. The goal of theranostics in CRC is to customise treatment according to unique tumour features, perhaps enabling personalised medicine. It makes use of developments in targeted treatment and molecular imaging to precisely detect, track, and treat CRC, underscoring the urgent need for continued study and innovation in this area [4].

1.1. Significance of theranostics in CRC

Theranostics appears as a crucial advancement in personalised medicine when it comes to CRC, combining targeted medicines with diagnostics to customise treatment regimens based on each patient's unique molecular profile. The accuracy of CRC care is greatly increased by this strategy, starting with early identification and continuing through therapy and beyond. Theranostics reduces needless exposure to unsuccessful medicines and their related toxicities by helping doctors choose treatments with a better chance of success by finding distinct biomarkers and using tailored therapeutic agents. Moreover, it offers a structure for continuous observation and real-time therapy modification, guaranteeing that therapeutic approaches stay in sync with the ever-changing dynamics of tumour biology [5]. The incorporation of theranostics in CRC therapy and care represents a paradigm change towards precision oncology. This novel method combines therapeutic and diagnostic modalities to allow for a highly customised treatment plan based on the distinct molecular features of each patient's tumour. Theranostics improves the accuracy of CRC care by using certain biomarkers to direct the use of tailored treatments. This allows for early identification, educated therapy selection, and real-time monitoring of therapeutic

responses [6]. The adoption of theranostics in CRC is a major step forward in addressing the drawbacks of conventional therapy paradigms, which frequently take a broad approach with notable adverse effects and unpredictable success. Theranostics seeks to enhance treatment results, lower toxicity, and enhance patients' overall quality of life by identifying molecular targets and implementing appropriate tailored treatments. Adaptive and responsive patient care is embodied by this method, which also enables the dynamic modification of treatment regimens in response to tumour behaviour changes or the onset of resistance [7]. Therefore, theranostics in the context of CRC represents a new era in cancer treatment that places a strong emphasis on understanding and treating the illness at a molecular level, rather than just improving therapeutic efficacy. Theranostics integration into CRC management is set to play a major role in the progress of personalised medicine, providing promise for more effective, efficient, and patient-centered treatment regimens as research and clinical practice continue to evolve.

1.2 Purpose and scope of the review

This review aims to provide a comprehensive overview of biomarkers, theranostic approaches, and personalized treatment strategies in CRC. The review aims to synthesise clinical ideas and current research to:

Examine Biomarkers in CRC

This section covers the basics of biomarkers, the ones that are often employed in CRC, and the difficulties in identifying and validating them. We'll talk about the roles that genetic mutations, epigenetic markers, protein biomarkers, mismatch repair (MMR) status, microsatellite instability (MSI) in CRC diagnosis, prognosis, and therapy choices.

Analyse Theranostic Approaches:

This review will explain and describe theranostics while emphasising its benefits for the management of CRCs. In order to highlight the potential of theranostic tactics in CRC to improve patient outcomes and treatment efficacy, examples will be discussed, including molecular imaging approaches. Identify Limitations and Future Directions: The review will point out the difficulties and obstacles in the areas of biomarker development, validation, and theranostics' clinical integration. In order to shed light on overcoming challenges and expanding precision medicine techniques, prospective future advances and emerging trends in CRC research and clinical practice will also be covered.

In order to enlighten clinicians, researchers, and policymakers as well as drive future research directions in the area, this review aims to give a thorough and informative assessment of the state-of-the-art in biomarkers, theranostics, and personalised therapy methods in CRC.

2. Molecular Biomarkers in CRC

2.1 Overview of biomarkers

A biomarker is a biological molecule present in blood, body fluids, or tissues that indicates the presence of a normal or abnormal process, condition, or disease like cancer. These biomarkers help distinguish between individuals with the disease and those who do not have it. They can vary due to factors such as germline or somatic mutations, transcriptional changes, and post-translational modifications. Biomarkers play a crucial role in various clinical scenarios, such as evaluating the likelihood of disease, conducting screenings for hidden primary cancers, differentiating between benign and malignant findings or different types of malignancies, predicting prognosis for diagnosed cancer patients, and monitoring the disease's status, whether to identify potential recurrence or assess the response and progression of treatment[8]. Biomarkers come in various forms, serving distinct purposes in disease detection and patient prognosis. Some function as diagnostic markers, aiding in identifying diseases like prostate cancer using Prostate-specific antigen (PSA) or detecting inflammation and infection through C-reactive protein (CRP)[9]. Others, such as HER2/neu, offer valuable prognostic insights into breast cancer outcomes[10], while

predictive biomarkers like EGFR mutation status guide targeted therapy decisions for lung cancer and HER2 assists in tailored breast cancer treatment [11].

2.2 Commonly used biomarkers in CRC

CRC is considered as one of frequently diagnosed cancer and remains the third leading cause of cancer-related mortality globally, affecting a significant number of adults worldwide. Hence it is very important for early diagnosis which helps in detection and treatment, improve prognosis, prevention of metastasis and increase chances of survival[12]. The current preferred approach for CRC screening involves colonoscopy. However, due to low patient compliance, its invasive nature, and potential risks like bleeding, colon perforation, and cardiorespiratory complications, its usage is limited[13]. As an alternative, the most commonly used non-invasive method is the guaiac fecal occult blood test (gFOBT), which identifies hemoglobin peroxidase activity in stool. While gFOBT is a cost-effective and straightforward screening option, its sensitivity and specificity are not ideal, leading to significant rates of false-positive and false-negative results[14]. Consequently, there is an urgent need for alternative, accurate, easily applicable, and cost-effective screening methods for CRC. Biomarkers hold promise not only for early detection of the disease but also for prognostic stratification, monitoring, and guiding treatment decisions, making their clinical application in CRC essential[15].This biomarkers can be differentiated into diagnostics, predictive, prognostics markers etc as discussed in the previous sections. The list of biomarkers that have been employed for CRC includes genetic mutations such as KRAS, KRAS G12C, KRAS wild type, NRAS, NRAS wild type, BRAF, BRAF V600E, PIK3CA, MSI-high, CEA, HER2, TRK fusions[16-17].

2.3 Challenges in biomarker discovery and validation

Biomarkers have emerged as vital tools in precision medicine across various medical fields. In oncology, biomarkers have particularly influenced disease management for cancer patients through genetic profiling and targeted cell therapies. Nonetheless, discovering potential biomarkers involves complex pre-clinical research on a pool of candidates, facing numerous challenges that can hinder the reliability of newly identified markers[18]. Biomarker discovery encounters a significant obstacle in the form of numerous false discoveries, which arise when initial research findings related to a potential biomarker cannot be replicated by other laboratories or independent sample sets. Various factors, such as implementation errors, inadequate study design, and limitations in bioinformatics, can contribute to the occurrence of false discoveries[19].Bias stands as a prominent reason for the shortcomings in biomarker discovery and validation endeavors. A major contributor to this bias is the study design, particularly in the analysis of biospecimens[20].

The complexity and heterogeneity of diseases, especially in cases like cancer with multifactorial attributes, present a significant challenge in identifying precise biomarkers that can effectively represent the diverse disease states[21]. Additionally, there exist various obstacles that require attention, encompassing the reproducibility and standardization of biomarkers, the analysis of high-throughput data, resource allocation and costs, as well as the specificity and sensitivity of biomarkers[22].

3. Theranostic approaches for CRC

3.1 Definition and concept of theranostics

Theranostics is an innovative medical strategy that seamlessly integrates diagnostic and therapeutic functions within a unified system. This interdisciplinary approach, aptly named "theranostics," originates from the fusion of "therapy" and "diagnostics." The primary objective behind this cutting-edge methodology is to employ advanced diagnostic tools to discern specific biomarkers or molecular targets present in a patient's body. Subsequently, this valuable information is harnessed to administer personalized and tailored therapies, ensuring enhanced treatment efficacy while minimizing potential side effects associated with conventional generalized treatments[23]. By synergizing diagnosis and therapy,

theranostics holds immense promise in revolutionizing personalized medicine and substantially improving patient outcomes across various medical domains, including oncology, neurology, cardiology, and infectious diseases. This harmonious integration of diagnostic precision and therapeutic effectiveness signifies a remarkable advancement in healthcare, ultimately enabling clinicians to deliver more precise and optimal treatments catered to individual patient needs and characteristics[24].

3.2 Advantages of theranostics approaches in CRC

Theranostic approaches in CRC offer a host of advantages for patients and healthcare management by integrating diagnostic and therapeutic strategies. Personalized medicine is a key benefit as theranostics enable tailored treatment plans, leveraging individual cancer characteristics and genetic profiles. Through the identification of specific biomarkers and mutations, clinicians can opt for targeted therapies with higher efficacy and reduced side effects [5-7]. Furthermore, these techniques facilitate early CRC detection and diagnosis via advanced imaging and biomarker-based tests, enabling timely interventions and better survival rates. Continuous treatment monitoring provides real-time feedback, allowing for timely adjustments to treatment plans, potentially enhancing treatment effectiveness and disease control. Although theranostics may not be universally applicable to all CRC patients due to factors such as biomarker availability and costs, ongoing research aims to expand their scope, advancing precision medicine and empowering patients to make informed treatment decisions.

3.3 Theranostics strategies in CRC

In the field of treating cancer, scientists have developed tiny materials called nanomaterials and specialized formulations known as nanoformulations. These are becoming important tools because they can precisely deliver drugs and help in diagnosing CRC. This array of innovative technologies encompasses carbon nanotubes, dendrimers, liposomes, silica nanoparticles, gold nanoparticles, metal-organic frameworks, core-shell polymeric nano-formulations, and nano-emulsion systems, among others. Integrating these cutting-edge approaches into the field of nanomedicine, theranostic strategies have been proposed as a groundbreaking avenue to revolutionize CRC detection and treatment, marking a remarkable advancement in the fight against this debilitating disease[7].

Iron oxide nanoparticles, both IONS and SPIONS, have garnered considerable attention from researchers in the biomedical domain owing to their wide array of applications. Notably, these nanoparticles demonstrate biocompatibility and pose no toxicity concerns[25]. In the field of cancer research, the utilization of IONS loaded with 5-fluorouracil (5-FU) in conjunction with magnetic hyperthermia has proven highly effective in reducing the growth of heterotopic human colon tumors in mouse models[26]. Another noteworthy innovation involves the tertiary complex formed by combining the Epirubicin-5TR1 aptamer with SPIONs, enabling based tumor detection and facilitating the efficient delivery of Epirubicin to murine C26 colon carcinoma cells[25]. Moreover, chitosan-coated IONS exposure induced an increase in reactive oxygen species (ROS) levels in human colorectal cell lines (HCT 116), thereby triggering apoptotic cell death through the activation of Caspase 9/3[27].

Quantum dots, a class of zero-dimensional nanomaterials with particle sizes ranging from 2–10 nm, possess exceptional optical and chemical properties that make them highly valuable in cancer research, particularly for CRC[28]. In the context of CRC, QDot655 specifically targeted to the vascular endothelial growth factor receptor 2 (VEGFR2) has demonstrated its efficacy in detecting VEGFR2 expression within CRC tumors in vivo[29]. In other study which investigated the interaction between IgG-functionalized Boron carbide (B4C) nanoparticles (NPs) synthesized from B4C powder produced through the direct reaction between boron and soot under argon flow, two cell lines were utilized: MC38 murine colon cancer and RAW 264.7 mouse macrophages cell line. Flow cytometry analysis revealed that macrophages absorbed a higher quantity of fluorescently tagged NPs

compared to cancer cells. These findings hold tremendous potential for advancing targeted cancer therapies and diagnostics in the ongoing fight against CRC[30].

Dendrimers are complex 3D molecules with highly branched structures, offering biodegradable backbones. They find significant application in nanopharmaceuticals, particularly for targeted anticancer drug delivery and theranostic applications in cancer therapy[31]. Dendrimers have also been employed in in vitro experimental anticancer therapy. Specifically, G4-PAMAM dendrimers, when combined with capecitabine, demonstrate the ability to reduce tumors and minimize capecitabine by-products[32]. In both colorectal C26 and HT29 cells, the combination of curcumin with Au NPs of PAMAM dendrimers exhibited notable cellular uptake, internalization, and demonstrated substantial cytotoxic effects[33].

Carbon nanotubes (CNTs) exhibit diverse functionalities as carriers in drug administration, gene therapy, immunotherapy, and diagnostics, owing to their exceptional attributes such as remarkable optical qualities, thermal conductivity, chemical stability, and functional versatility[34]. The composites of single-walled carbon nanotubes (SWCNTs) with II-NCC, functionalized with fluorescein, exhibited natural effectiveness against cancer cell lines, particularly colon cancer cell lines (Caco-2). When these SWCNT nano biocomposites were paired with the C225 antibody, which targets epidermal growth factor receptors on CRC cells, they demonstrated an increased ability to kill colon cancer cells during photodynamic treatment[35].

Liposomes are artificial, lipid-based, non-toxic vesicle carriers. The FDA authorised the use of liposomes as medication delivery nanocarriers in 1961. They are composed of phospholipid bilayers and have a tiny, spherical aqueous centre[36]. The specificity of the TF-receptor mediated binding in colon cancer cells was confirmed in vitro using PEGylated liposomes functionalized with transferrin (TF-PEG liposomes) and sodium borocaptate (BSH) at concentrations of 107-123 nm/26-30 g/mol of lipid 6-8%. Male BALB/c mice having ex vivo colon-26-containing cells and in vivo colon-26-bearing cells showed slower tumour progression[30].

Table 1: Advancements of Nanomaterial and Nanoformulation under the theranostics approaches in CRC.

Nanomaterial/ Nanoformulation	**Key Findings/Advancements**
Iron Oxide Nanoparticles (IONS/SPIONS)	IONS loaded with 5-fluorouracil (5-FU) in magnetic hyperthermia effectively reduced colon tumor growth in mouse models. Epirubicin-5TR1 aptamer complex with SPIONs facilitated tumor detection and efficient drug delivery to colon carcinoma cells. Chitosan-coated IONS induced apoptotic cell death in colorectal cell lines by increasing reactive oxygen species (ROS) levels.
Quantum Dots (QDot655)	QDot655 targeted to VEGFR2 successfully detected VEGFR2 expression within CRC tumors in vivo.
Boron Carbide Nanoparticles (B4C NPs)	IgG-functionalized B4C NPs showed higher uptake by macrophages compared to cancer cells, holding potential for targeted therapies and diagnostics.
Dendrimers (G4-PAMAM)	G4-PAMAM dendrimers combined with capecitabine reduced tumors and minimized capecitabine by-products.

	Curcumin combined with Au NPs of PAMAM dendrimers exhibited significant cytotoxic effects in colorectal cells.
Carbon Nanotubes (SWCNTs)	SWCNT nano biocomposites, when paired with the C225 antibody, demonstrated increased ability to kill colon cancer cells during photodynamic treatment.
Liposomes (TF-PEG Liposomes)	TF-PEG liposomes showed specificity in binding to colon cancer cells in vitro, leading to slower tumor progression in mouse models.

4. Molecular Biomarkers in CRC

4.1 Epigenetic Markers

Epigenetic markers encompass chemical alterations occurring on DNA or associated proteins, orchestrating the intricate regulation of gene expression independent of DNA sequence changes. These modifications wield significant influence over diverse cellular functions, spanning developmental pathways, cellular differentiation processes, and disease progression. The different epigenetic markers in CRC includes CpG island methylator phenotype, Adenomatous polyposis coli (APC), Micro RNAs.

CpG island methylator phenotype:

Epigenetics, as a concept, encompasses alterations in phenotype or gene expression that occur independently of changes in DNA sequence. Among these epigenetic mechanisms, DNA methylation stands out as a highly investigated biomarker in CRC, exerting a significant influence on the changes observed in gene expression during cancer development [37]. Hypermethylation of CpG islands situated within the promoter regions of tumor suppressor genes stands as a widely acknowledged mechanism for inducing gene inactivation. This process triggers alterations in the chromatin structure of gene promoters, impeding accessibility to crucial transcription factors [38]. As a consequence, this epigenetic alteration has the potential to disrupt numerous vital cellular pathways, encompassing DNA repair mechanisms (like hMLH1 and MGMT), apoptosis regulation (involving DAPK), inhibition of angiogenesis (related to THBS1), suppression of metastasis (including TIMP3), regulation of cell cycle progression (through p14 ARF, p15 INK4b, and p16 INK4a), as well as modulation of cell adhesion (involving CDH1 and CDH13) [39].

This epigenetic dysregulation underscores the intricate interplay between genetic and epigenetic factors in the development and progression of cancer. There exists compelling evidence indicating a robust association between CIMP-high status and right colon cancer, microsatellite instability, and an elevated incidence of BRAF mutations. Certain studies have suggested that aberrant DNA methylation patterns in DNA repair genes, such as MGMT and MLH1, may contribute to the progression from adenoma to carcinoma [40]. Lee et al. [41] proposed the potential utility of CIMP as a predictive marker for anti-EGFR therapy, although further prospective investigations are warranted to validate this hypothesis. Additionally, methylated genes like MLH1, VIM, and SEPT9 hold promise as biomarkers for CRC and DNA-based screening tests for colon cancer. Notably, methylated Vimentin (mVim) has been established as a validated stool-based biomarker for the early detection of CRC, now accessible in the US. The presence of methylated VIM gene is observed in a significant majority of CRC cases, ranging from 53% to 84% [42]. In a systematic analysis, Perez-Carbonell and colleagues investigated a panel of methylated genes specific to CRC, including SEPT9, TWIST1, IGFBP3, GAS7, ALX4, and miR137. They found significantly elevated methylation levels of all these genes in CRC patients compared to normal individuals ($P < 0.0001$), particularly notable for miR137 and IGFBP3 (86.7% and 83%, respectively). Notably, the combined assessment of miR137 and IGFBP3 exhibited a sensitivity of 95.5% and a specificity of 90.5% for CRC detection, suggesting a promising diagnostic biomarker. Furthermore, their findings highlighted that

hypomethylation of IGFBP3 could serve as an independent prognostic factor for poor outcomes in stage II and III CRC patients.

Interestingly, among stage II and III CRC patients exhibiting hypermethylation of IGFBP3, the study revealed that adjuvant chemotherapy with 5FU did not confer improvements in overall survival or disease-free progression, underscoring the complexities in treatment response and the potential implications of specific epigenetic alterations in CRC prognosis [43]. Tang et al.'s research emphasized the significance of methylated secreted frizzled-related protein 2 (SFRP2) as a potential indicator for detecting and determining the stage of CRC [44]. Other promising blood markers include methylated thrombomodulin (THBD), which detected 71% of CRC cases with an 80% [45] specificity, and methylated syndecan 2 (SDC2), demonstrating a sensitivity of 92% for CRC detection, particularly at early stages [46]. Watson et al.'s study revealed that CRC patients with TYMS amplification who received 5-FU-based chemotherapy after surgery exhibited shorter median survival times [47]. Additionally, various genes involved in pyrimidine metabolism, such as thymidine phosphorylase (TYMP), uridine monophosphate/cytidine monophosphate kinase (UMPK), and dehydrogenase (DYPD) genes, may influence resistance to 5-FU, thus aiding in the selection of appropriate chemotherapy regimens for CRC treatment [44].

Adenomatous polyposis coli (APC):

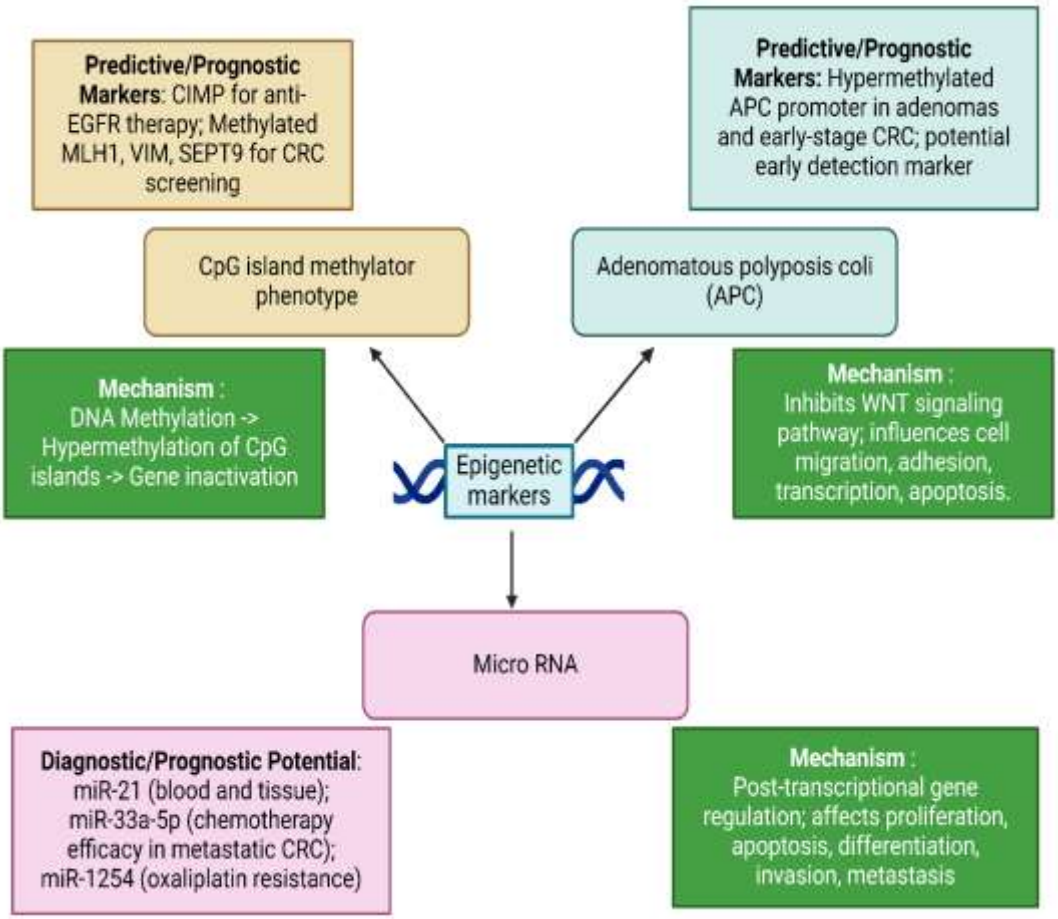
The tumor suppressor gene known as Adenomatous polyposis coli (APC) was identified through genetic linkage analysis within familial adenomatous polyposis (FAP). Mutations in APC are implicated in the majority of sporadic cases of CRC [48]. APC plays a crucial role as an inhibitor of the WNT signaling pathway, governing various cellular processes including cell migration, adhesion, transcriptional activation, and programmed cell death (apoptosis) [49]. A significant portion, approximately 70% to 80%, of CRC patients exhibit APC loss. Liang et al.'s meta-analysis examined the correlation between three APC polymorphisms (D1822V, E1317Q, and I1307K) and CRC susceptibility. Their findings revealed a weak link between the E1317Q variant and CRC risk, particularly concerning adenomas. Notably, carriers of the I1307K variant among Ashkenazi Jews displayed a notably elevated CRC risk compared to those without the variant. The meta-analysis detected no notable variation across studies; however, it's important to note that all studies included were case-control studies, possibly prone to recall and selection biases [50]. A recent meta-analysis revealed a higher occurrence of hypermethylated APC promoter in adenomas compared to normal tissue samples. Additionally, CRC patients at stage I exhibited elevated levels of APC hypermethylation compared to normal controls. This suggests APC hypermethylation could serve as a valuable biomarker for early CRC detection and a potential target for personalized therapies. Interestingly, no significant link was found between APC promoter methylation and overall survival in CRC patients. While the meta-analysis detected a heterogeneity of 43% and no publication bias [51].

Micro RNA:

MicroRNAs (miRNAs) are diminutive non-coding RNA molecules implicated in the post-transcriptional regulation of gene expression, wielding significant influence over diverse cellular processes pivotal in cancer biology such as proliferation, apoptosis, differentiation, invasion, and metastasis [52]. Mounting evidence underscores their pertinence in carcinogenesis and tumor progression, thereby accentuating their potential utility as discerning biomarkers for early cancer detection, prognostic stratification, and prediction of therapeutic response [53]. A recent investigation has delineated a panel of 19 disparately expressed miRNAs, notable among which are the upregulation of hsa-miR-183-5p and hsa-miR-21-5p, alongside the downregulation of hsa-miR-195-5p and hsa-miR-497-5p, which orchestrate their effects in CRC through intricate interactions with pivotal signaling pathways encompassing the MMR pathway, and transforming growth factor β, WNT, RAS, MAPK, and PI3K pathways [54]. Of particular scrutiny, miR-21, being among the most extensively scrutinized [55]. miRNAs, has garnered attention in a recent meta-analysis by Peng et al., unveiling its diagnostic potential in CRC. Notably, the meta-analysis unveils

the diagnostic accuracy metrics for miR-21, divulging a sensitivity of 0.64, specificity of 0.85, and an area under the curve (AUC) of 0.85, with analogous values observed in blood-derived samples demonstrating enhanced diagnostic prowess.

Furthermore, combinatorial biomarker analyses incorporating miR-21 underscore its augmented predictive capability, particularly in circulating milieu, thus advocating for its clinical utility as a promising diagnostic biomarker, particularly in serum, while also extolling its prognostic value in CRC tissue [56]. Another study led by Sasaki et al [57]. has shed light on the significance of plasma miR-33a-5p as a discerning biomarker for predicting the efficacy of chemotherapy in metastatic CRC, specifically focusing on the conventional first-line treatment regimen comprising fluoropyrimidine + oxaliplatin + bevacizumab. The findings elucidate that individuals exhibiting non-responsive outcomes to CRC chemotherapeutic regimens tend to display elevated levels of miR-33a-5p in plasma. Conversely, patients manifesting diminished plasma levels of this particular miRNA are poised to exhibit a more favorable response to CRC chemotherapy, thereby underscoring its potential as a predictive biomarker for therapeutic success in metastatic CRC [57]. Moreover, an investigation undertaken by Mou et al. showcased that miR-1254 exerts regulatory control over the development of oxaliplatin resistance in human CRC cell



lines [58]. The Figure 1 shows the summary of epigenetic markers and their role in CRC.

Figure 1: The figure depicts the different epigenetic markers and their mechanism in CRC along with predictive markers that can target CRC for therapy.

Newer Potential Markers for CRC

Phosphatidylinositide-3-kinases (PI3K)

Phosphatidylinositol-3-kinases (PI3K) play a crucial role in regulating cell functions such as growth, adhesion, and survival. This signalling pathway is a key player in the

proliferation and progression of tumors driven by RAS mutations [59]. In various cancers, including CRC, abnormalities in PI3K signalling are common, often linked to mutations in the PIK3CA gene encoding the p110alpha subunit [60] [61]. These mutations, particularly prevalent in CRCs located on the right side, associated with mucinous histology, KRAS mutations, loss of MGMT expression, and high methylation levels, notably the CpG island methylator phenotype (CIMP) [60]. Mutation in PIK3CA is linked to a notable decrease in survival among CRC patients lacking mutations in BRAF [62]. Studies indicate that PIK3CA mutations could serve as an independent prognostic factor, suggesting better survival rates and potential benefits from adjuvant therapy. Furthermore, emerging research suggests that mutated PIK3CA could potentially identify CRC patients who are responsive to aspirin therapy [63]. In a study involving 964 CRC patients, Liao et al. found that those with mutated PIK3CA who initiated aspirin therapy post-diagnosis exhibited higher CRC-specific survival and overall survival compared to patients with wild-type PIK3CA [64].

PTEN

PTEN serves as a vital tumor suppressor gene, regulating the cell-survival pathway initiated by PI3K. Mutations in PTEN are often linked to advanced and metastatic tumors, while hypermethylation of its promoter is frequently observed in MSI-high sporadic CRC's [64]. Patients expressing PTEN typically experience longer overall survival compared to those with PTEN loss tumors. However, some studies suggest that PTEN loss may predict poor prognosis, particularly in stage II patients or those with liver metastasis [65]. Notably, PTEN could potentially serve as a predictive marker for KRAS wild-type patients undergoing anti-EGFR therapy [66].

TP53

The TP53 gene is responsible for encoding a crucial tumor suppressor protein that orchestrates various cellular processes, including cell cycle regulation, apoptosis, senescence, and DNA repair mechanisms. When mutations occur within the TP53 gene, they often lead to functional alterations in the TP53 protein, which in turn significantly contribute to the initiation and progression of tumors. Notably, TP53 mutations are prevalent in approximately 60% of colorectal tumors, manifesting in both precancerous adenomas and malignant cells. This underscores the pivotal role played by TP53 in colorectal tumorigenesis, highlighting its significance as a potential therapeutic target and diagnostic marker in combating this disease [67]. Research suggests that the levels of p53 mRNA expression could serve as a valuable prognostic indicator for the survival outcomes of individuals diagnosed with stage III or rectal cancer [68].

NTSD4

NDST4, situated on chromosome 4q26, emerges as a significant player in CRC as a tumor suppressor gene. Comparative analyses reveal a conspicuous reduction in NDST4 expression within CRCs in contrast to normal colonic mucosa. Moreover, studies underscore a correlation between NDST4 loss and advanced pathological stages, indicative of a potential link to poorer survival outcomes [68]. Belonging to the N-deacetylase/N-sulfotransferase (heparan glucosaminyl) family, NDST4 governs heparan sulfate (HS) biosynthesis, pivotal for the formation of heparan sulphate proteoglycans (HSPGs) [69]. The functional deficit of NDST4 may instigate heightened invasiveness among cancer cells by altering the dynamics of cell adhesion receptor-ligand interactions. Consequently, the genetic absence of NDST4 emerges as a promising biomarker signaling an adverse prognosis for CRC patients, urging further exploration into its clinical implications [70].

Chromosome 18q loss of heterozygosity

Frequent in CRC, loss of heterozygosity of chromosome 18q (18qLOH) denotes a common genetic alteration [71]. Notably, several critical genes implicated in CRC development, such as DCC, SMAD2, and SMAD4, reside on chromosome 18q [72]. Sarli et al.'s study on 118 CRC patients revealed diminished overall survival among stage III CRC patients with 18qLOH compared to those without. This finding underscores the potential of 18qLOH as a predictive genetic marker, offering insights into recurrence risk and survival rates post-resection [73]. Similarly, Boulay et al.'s analysis of 202 colorectal tumor biopsies from a prior study on adjuvant chemotherapy revealed that patients

exhibiting 18q loss, alongside SMAD4 deletion, may experience reduced benefits from adjuvant 5-FU treatment [74].

IGFR-1R

The transmembrane glycoprotein known as type 1 insulin-like growth factor receptor (IGF-1R) comprises two extracellular and two cytoplasmic subunits with tyrosine kinase activity. Elevated levels of IGF-1R have been documented in various tumor types, including primary renal cancer cells and preinvasive breast lesions, where its activation influences crucial cellular processes like proliferation, differentiation, angiogenesis, and apoptosis [75]. Emerging as a focal point in novel treatments, particularly monoclonal antibodies or tyrosine kinase inhibitors, IGF-1R has garnered attention. In vitro investigations have revealed a correlation between chemotherapy resistance in cell lines and heightened nuclear IGF-1R expression. Recent research by Codony-Servat et al. involving four cohorts of metastatic CRC patients indicates that nuclear localization of IGF-1R may contribute to resistance against chemotherapy and targeted agents, exacerbating overall survival rates. Metastatic CRCs displayed elevated IGF-1R levels compared to untreated primary cancers, signaling a grim prognosis. Intriguingly, ganitumab, an IGF-1R blocking monoclonal antibody, and dasatinib, an SRC inhibitor, were found to enhance the nuclear accumulation of IGF-1R. These findings suggest the potential of IGF-1R as a novel biomarker for predicting poor prognosis in metastatic CRC patients [76].

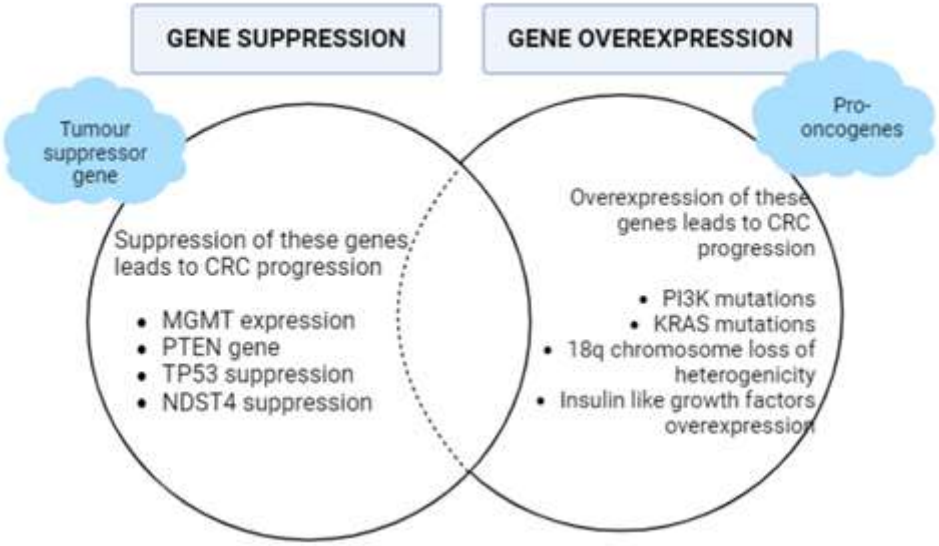


Figure 2: Genes play a role in development of CRC via its suppression and over expression.

4.3 Microsatellite instability (MSI) and Mismatch repair (MMR) status

Microsatellites, which are brief, repetitive DNA sequences dispersed across the genome, are susceptible to length variations caused by inaccuracies during DNA replication. When these variations occur at an elevated frequency,3 typically due to deficiencies in DNA mismatch repair mechanisms, it leads to MSI, characterizing a condition where the length of these sequences becomes altered [77]. In sporadics CRC, MSI manifests in roughly 15% to 20% of cases. Furthermore, MSI is prevalent in hereditary non-polyposis colorectal cancer (HNPCC), or Lynch syndrome, where its occurrence is even more pronounced [78]. Lynch syndrome, comprising 3%–5% of CRCs, results from autosomal dominant mutations in various MMR genes, leading to heightened susceptibility to cancer. This syndrome presents an elevated risk primarily for colon cancer but also encompasses malignancies affecting the endometrium, ovary, stomach, small intestine, hepatobiliary tract, urinary tract, brain, and skin. Individuals with Lynch syndrome face lifetime and endometrial cancer risks ranging from 60%–80% and 40%–60%, respectively [79]. In CRC, tumors displaying MSI are notably prevalent in the right colon. These MSI-positive tumors often exhibit mucinous characteristics coupled with signet ring cell morphology, alongside features such as poor differentiation and robust lymphocyte infiltration.

Furthermore, CRC patients harboring MSI tend to experience a more favorable prognosis compared to those lacking MSI, and they demonstrate distinct responses to chemotherapy regimens, emphasizing the significance of tailored treatment approaches based on MSI status [80]. Recent studies have indicated that individuals diagnosed with stage II or stage III CRC who have microsatellite stable or low MSI status may derive significant benefits from adjuvant chemotherapy involving 5-fluorouracil. However, for patients with stage II CRC and high MSI status, there appears to be a concerning threefold rise in mortality rates. This elevated risk is believed to stem from potential immunosuppressive effects associated with the therapy, highlighting the need for personalized treatment strategies based on MSI status to optimize patient outcomes [81].

MMR status refers to the functionality of the DNA MMR system within tumor cells. This status is assessed using techniques like immunohistochemical staining or molecular testing to evaluate the levels of MMR proteins like MLH1, MSH2, MSH6, and PMS2 [82]. These proteins are vital for correcting errors that occur during DNA replication, ensuring the accuracy of the genetic code and maintaining genomic stability. When the MMR system fails to function properly, errors in DNA replication can accumulate, leading to a condition known as MSI [83].This instability manifests as variations in the length of repetitive DNA sequences, termed microsatellites, throughout the genome. The identification of mutations in key MMR genes—MLH1, MSH2, MSH6, and PMS2—via molecular testing aids in diagnosing Lynch syndrome, an inherited predisposition to certain cancers. Lynch syndrome is characterized by an increased risk of and other malignancies [84]. Individuals with inherited loss of DNA mismatch repair function often develop CRC by the age of 40 in approximately 80% of instances [85]. In sporadic CRC cases, the majority of DNA MMR defects result from the suppression of MLH1 gene expression through promoter hypermethylation—a process termed epigenetic silencing [86]. It's noteworthy that both alleles of MMR genes must be affected for MMR function to be lost, as having one wild-type allele is adequate to maintain a microsatellite stable (MSS) genotype, both in HNPCC and sporadic CRC cases.

4.4 Expression of Protein Markers in CRC

Protein markers, referred to as biomarkers, are distinct proteins identifiable in bodily fluids or tissues. They act as signposts for various biological functions, disease conditions, or treatment responses. In CRC, these biomarkers play pivotal roles in diagnosis, prognosis, and treatment evaluation [87]. For example, they can pinpoint specific molecular pathways driving cancer growth or highlight potential therapeutic targets. Additionally, protein markers enable clinicians to tailor treatment strategies based on individual patient profiles, enhancing precision medicine approaches. Moreover, advancements in proteomics technologies continue to uncover novel protein markers with diagnostic and prognostic significance, offering promising avenues for improved CRC management.

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA)is a glycoprotein generated by a range of cells, including cells and fetal tissue during development. It serves as a widely employed tumor marker, especially for monitoring CRC. Elevated CEA levels in blood samples can suggest the potential existence of CRC. Moreover, beyond its diagnostic role, CEA is also explored for its utility in predicting treatment response and disease progression, contributing to more personalized treatment approaches for patients with [88]. CEA, a recognized serum protein marker, is a member of the immunoglobulin (Ig) superfamily, known to facilitate cell adhesion in cancer cells. Recent research indicates that overexpression of CEA is prevalent in over 90% of s and approximately 60% of other cancer types, such as gastric, lung, and pancreatic cancers [89]. Interestingly, studies suggest that elevated levels of CEA play a role in promoting metastasis, particularly in metastatic CRC, by facilitating colonization in the liver and fostering the development of spontaneous metastases in the pancreas and lungs. This underscores the multifaceted role of CEA in cancer progression and metastatic dissemination [90].

Heat Shock Proteins (HSP)

Heat shock proteins (HSP) comprise a diverse group found in all multicellular organisms and are categorized based on their molecular weight, including hsp10, hsp40, hsp60, hsp70, hsp90, and hsp110 [91] [92]. While the exact role of HSP overexpression in cancer remains elusive, it is hypothesized that factors such as stress and temperature within the tumor microenvironment may trigger the induction of HSP. Moreover, emerging research suggests that HSPs not only respond to cellular stress but also play intricate roles in various cellular processes, including protein folding, degradation, and immune responses, thereby potentially influencing cancer progression and therapeutic responses. HSPs are evolutionarily conserved proteins implicated in diverse cellular signaling pathways that contribute to cell survival, particularly in cancer. They exist in various molecular weight forms and are present across different cancer types. For instance, inhibiting the expression of HSP70 has been shown to elevate intracellular Ca2+ levels in colon cancer cell lines, leading to intracellular Ca2+ release into the cell culture environment. This surge in Ca2+ triggers a caspase-dependent cell death mechanism specific to colon cancer cells. Additionally, HSP70 has been found to suppress programmed cell death in colon cancer cells, reduce cytosolic calcium levels in tissues, and stabilize lysosomes. Furthermore, HSP70 exhibits a similar role in promoting cell survival in other cancer types such as pancreatic and prostate cancers. This underscores the versatile and complex functions of HSPs in cancer biology, highlighting their potential as therapeutic targets across various malignancies [93]. In a study by Morita et al, it was discovered that the HSP40 family member DNAJB8 exhibits heightened expression levels in CRC. Their findings suggest that the overexpression of DNAJB8 correlates with increased tumorigenicity, thereby implicating DNAJB8 as a significant factor in the prognosis of CRC. This highlights the potential utility of DNAJB8 as a prognostic marker and underscores its importance in understanding the progression and outcome of [94].

Tissue inhibitor of Matrix metalloproteinase 1 (MMP)

Cancer cells interact with neighboring cells and the extracellular matrix (ECM) through cell receptors, a process regulated by matrix metalloproteinases (MMPs). MMPs degrade the ECM, and their activity is controlled by tissue inhibitors of metalloproteinases (TIMPs) [95]. In human colon cancer models, TIMP-1 synthesis is increased, promoting cancer growth and progression while facilitating the accumulation of Cancer Associated Fibroblasts (CAFs). Additionally, TIMP-1 exhibits protumor effects in various cancers, including prostate and colon cancer [96]. Tissue inhibitor of metalloproteinases-1 (TIMP-1) is a glycoprotein found in both cancerous and noncancerous tissues and bodily fluids. Alongside TIMP-2, it is one of four essential classes of TIMPs. TIMP-1 and TIMP-2 play crucial roles primarily in inhibiting enzymes within cancer models, exerting regulatory control over processes essential for cancer progression and metastasis [97]. TIMP-1 exhibits growth-promoting characteristics and fosters tumor growth by suppressing apoptosis specifically in colon cancer. Additionally, TIMP-1 serves as a novel and distinct protein marker for the early detection of colon cancer. Elevated levels of TIMP-1 are notably present in the blood plasma samples of patients with colon cancer compared to those of healthy individuals, offering potential diagnostic value in identifying early-stage disease [98]. depicts Protein biomarkers in CRC. **Figure 3** depicts protein biomarkers in CRC.

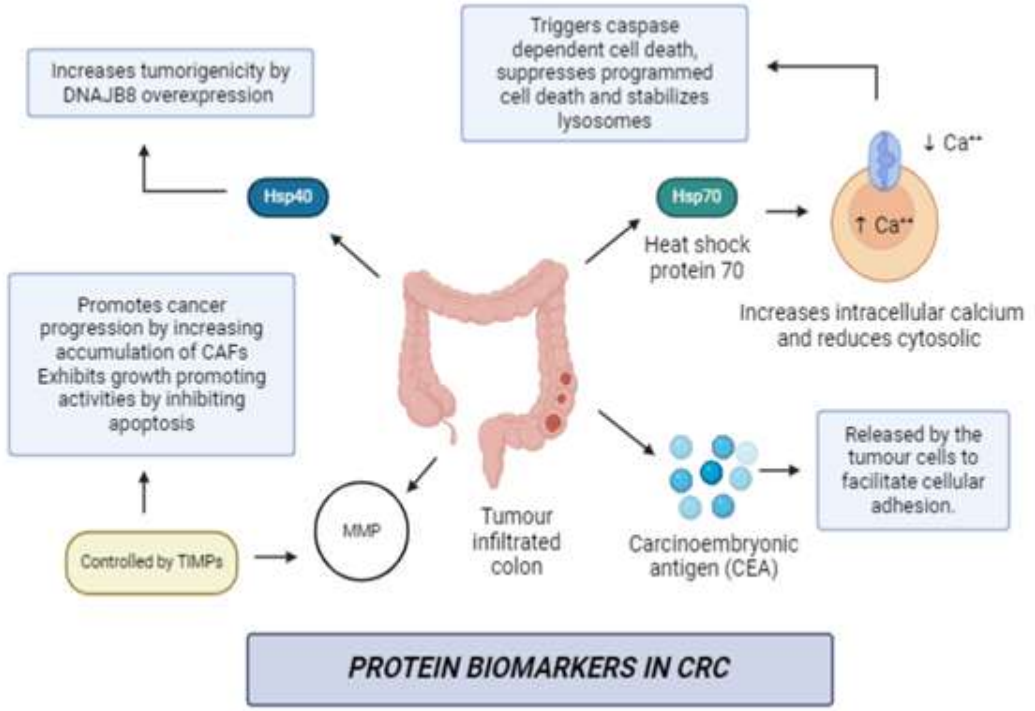


Figure 3: Protein biomarkers in CRC: Role of proteins such as CEA, HSP, and MMP released by the cancerous colonic cells in development and prognosis determination of CRC.

5. Imaging biomarkers in CRC

5.1 Overview of imaging markers

The idea of using imaging biomarkers is a recent development, but it's becoming more and more crucial in many phase II/III clinical trials as a substitute endpoint. These biomarkers help in objectively evaluating how tumors respond to treatment and/or spotting early signs of disease without invasive procedures. Currently, the imaging methods used to gauge treatment effectiveness in CRC can be broadly categorized into those that assess tumor dimensions and those that gauge tumor activity [7] [99].

Measuring Tumor Size

Diminishing tumor size has proven to be a valuable biomarker, with the potential for measurement in one, two, or three dimensions using various standard imaging methods like CT and MRI [100]. However, two commonly utilized criteria, WHO and RECIST [101], present differing characteristics, particularly regarding the method for measuring tumor size, with RECIST focusing solely on one dimension. Despite the utility of size measurements, limitations arise in determining the extent of tumor bulk reduction that signifies a clinically meaningful response. For instance, Morgan et al. [102] illustrated this challenge when investigating the impact of a VEGF receptor inhibitor on colorectal metastases, where substantial size reduction did not correspond to a similarly significant overall response (less than 10%). Conversely, the innovative MRI-based tumor regression grade (mrTRG), which evaluates response based on the degree of fibrosis observed in the tumor post-chemoradiotherapy, has emerged as a valuable clinical assessment tool [103].

Determining tumor activity

These methods entail examining images to measure the functional activity of tumors. A prevalent example is positron emission tomography (PET) with Fluorodeoxyglucose (18-FDG), which operates based on the principle of the varying glycolytic rate observed in tumor cells. By utilizing the glucose analogue 18-FDG, it provides an evaluation of tumor metabolism by quantifying standard uptake values (SUV) [104] [105].

5.2 Molecular Imaging biomarkers in CRC

Molecular imaging biomarkers are specialized instruments enabling the visualization and understanding of molecular-level biological processes in living organisms. They offer crucial insights into disease mechanisms, evolution, and reaction to therapy by focusing on particular molecules or pathways linked to the specific condition. In cancer, for example, these biomarkers aid in tumor detection, evaluation of aggressiveness, and tracking treatment response without invasive procedures. Their ability to refine and tailor disease management offers great potential for enhancing diagnostic accuracy, treatment strategy development, and patient results across diverse medical fields [106] [107].

Computed tomographic colonography

Computed tomographic colonography (CTC) employs CT scanning to generate 2D and 3D images of the entire colon and rectum, achieved through air insufflation. It stands out as a top radiological diagnostic tool for screening CRC and polyps. Its diagnostic accuracy in detecting CRC matches that of traditional colonoscopy and surpasses that of barium enemas [108]. Moreover, CTC offers less invasiveness compared to conventional colonoscopies and is user-friendly. Diverse indications, backed by robust evidence and endorsed by medical societies, have emerged, including diagnosing synchronous cancers in cases of incomplete or failed colonoscopies, assessing elderly or frail patients, investigating symptoms suggestive of CRC, localizing tumors (particularly beneficial for laparoscopic surgery), and examining diverticular disease and patients with colonic stomas [109]. Additional applications, albeit subject to ongoing debate, involve CRC screening and surveillance post-surgery or polypectomy [109] [110]. As per the meta-analysis conducted by Pickhardt et al., the combined sensitivities of CTC and OC for were approximately 96% and 95%, correspondingly. In summary, this systematic review and meta-analysis have provided point estimates of sensitivity for invasive cancer in both CTC and OC [111]. Sato et al. demonstrated that CTC accurately identified 86 out of 87 central colon tumors. Utilizing CTC and removing a single minor lesion enabled the detection of all 87 colon tumors. Consequently, they propose further exploration of clipping as a method to diagnose small tumors (less than 10 mm in diameter) [112].

Dual-energy spectral CT

Dual-energy spectral CT utilizes varying energy levels to enable material decomposition, leveraging energy-dependent attenuation profiles for precise tissue characterization. This technology facilitates the quantification of iodine contrast material attenuation and the computation of iodine concentration. The resulting color map offers enhanced visualization, providing more accurate tissue enhancement measurements. In CRC patients, dual-energy spectral CT enables the identification and characterization of CRC tumors and extracolonic findings through analysis of iodine uptake patterns [113]. Research findings indicate that dual-energy spectral CT has the potential to enhance tumor grading and staging [114] [115]. Virtual monochromatic images generated from dual-energy spectral CT reveal finer details of contrast enhancement compared to standard polychromatic images from single-energy CT scans. This improved visualization enhances the detection of liver metastatic deposits by increasing lesion conspicuity and contrast between lesions and surrounding tissue. Initial findings also suggest that iodine concentration measurements derived from dual-energy spectral CT could serve as a valuable parameter for distinguishing lymph node metastases [116].

Volumetry

Tumor burden plays a significant role in predicting outcomes for CRC patients, but changes in tumor volume are particularly crucial for assessing response to therapy. In rectal cancer (RC), the extent of reduction in post-treatment volume correlates with disease-free survival and tumor regression grade. Compared to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, utilizing the tumor volume reduction rate proves more effective in predicting the pathological response of RC to neoadjuvant radiation therapy and chemotherapy [117]. MRI volumetry based on T2-weighted MR images outperforms tumor size in indicating a pathologic complete response following radiation therapy and chemotherapy. However, relying solely on T2-weighted MR images poses challenges in

distinguishing between therapy-induced fibrosis and remaining viable tumor. Notably, functional and molecular imaging techniques such as diffusion-weighted imaging (DWI) or fluorine 18 (18F) fluorodeoxyglucose (FDG) PET offer easier tumor delineation due to their superior intrinsic contrast resolution [118] [119] [120].

Hepatic resection

Hepatic resection has significantly improved the survival rates of patients with metastatic CRC. About a quarter of CRC patients are diagnosed with liver metastases initially, and another quarter develop them later [121]. The size of the remaining liver after surgery impacts the risks of postoperative complications and mortality. Therefore, it's crucial to use CT or MR imaging to measure liver volume and simulate virtual liver resection before surgery. This preoperative planning is essential for ensuring the success of hepatic resection in treating metastatic CRC [122].

CRC and Machine Learning

Machine learning is gaining traction in CRC patient care. Researchers are investigating its application in various aspects, including computer-aided detection and diagnosis during CT colonography. Studies have demonstrated improved reader sensitivity in CRC screening with this approach. Moreover, with the growing volume of imaging data in clinical settings, there's a need for automated and intelligent systems for tasks like image segmentation, registration, fusion, and guiding therapy. These advancements hold promise for enhancing the efficiency and accuracy of CRC diagnosis and treatment by leveraging computational algorithms and artificial intelligence technologies [123].

Positron Emission Tomography (PET)

PET has marked a significant advancement in cancer treatment. Beyond the conventional radiotracer, 18F FDG, emerging interest surrounds various novel tracers for assessing diverse tumor metabolic pathways, albeit with limited clinical utilization reported thus far. PET/CT stands as a well-established tool guiding decision-making across different stages of CRC, offering potential clinical impact. Particularly, FDG PET/CT surpasses CT alone in sensitivity for detecting seemingly confined metastatic disease pre-intervention or extrahepatic involvement, potentially refining staging to prevent unnecessary surgeries [124]. While FDG PET is a primary molecular imaging modality for CRC evaluation, routine use in preoperative staging lacks robust scientific support [125]. Recent meta-analysis findings endorse FDG PET's efficacy in restaging locally advanced RC post-radiotherapy and chemotherapy [126]. Notably, PET proves valuable in preoperative staging of recurrent CRC, aiding in surgical decision-making by discerning unresectable disease, clarifying ambiguous imaging features, and guiding therapeutic approaches. Furthermore, PET's quantitative parameters demonstrate prognostic value in primary and recurrent CRC, with high accuracy in early therapy response prediction [127].

Texture Analysis

Malignant tumors exhibit significant diversity in their biological characteristics and behavior, both spatially and temporally. However, a considerable portion of the variation seen in imaging may simply be noise. Texture analysis offers a method to mitigate the impact of noise in images while highlighting biological heterogeneity. This approach involves examining the distribution and relationships of grey-level values within images. By extracting fundamental components from conventional images and generating derived sub-images, texture analysis enables the quantification of various parameters such as entropy, kurtosis, and standard deviation of pixel distribution histograms [128]. In CRC, texture features have been found to correlate with KRAS expression, patient survival, tumor staging, and response to treatment [129] [130]. For instance, studies have shown that CT texture evaluation of lymph nodes in CRC patients can reveal greater heterogeneity in malignant nodes within specific size ranges, as well as fractal dimension. However, less heterogeneous tumors have been associated with poorer outcomes[129]. Some research has indicated that certain texture parameters measured in primary CRC on contrast-enhanced CT scans, such as entropy, kurtosis, and standard deviation of pixels, are linked to overall survival rates [130]. Moreover, CRC tumors with hepatic metastases that exhibit more

homogeneity on coarse filters may possess more aggressive biology, characterized by higher tumor grade and worse overall survival [131].

Difusion-weighted Imaging (DWI)

Diffusion refers to the random movement of water molecules within tissue, driven by thermal energy. DWI is an MRI technique that enables the noninvasive mapping of water molecule diffusion in biological tissue. By capturing the Brownian motion of water molecules on a microscopic scale, DWI provides insights into various in vivo phenomena. This imaging modality offers valuable information about tissue microstructure, reflecting factors like cell membrane integrity and cell density [132]. In oncology, DWI plays a crucial role, serving purposes such as tumor detection, characterization, staging, prognosis, monitoring treatment response, and detecting recurrence in patients [133].

In CRC, DWI is highly effective in detecting tumor lesions [134]. However, while DWI offers superior contrast resolution, its spatial resolution is typically lower compared to T2-weighted imaging. Therefore, combining both datasets simultaneously yields more accurate results for tumor delineation. Additionally, quantitative parameters derived from diffusion, such as the apparent diffusion coefficient (ADC), hold promise as noninvasive biomarkers for assessing tumor aggressiveness in CRC. The ADC, representing the rate of signal intensity decrease on diffusion images, shows potential in distinguishing between poorly differentiated and well-differentiated tumors. It can also aid in predicting the extent of extramural depth invasion, assessing mesorectal fascia and lymph node involvement, and evaluating response to therapy in CRC tumors [135]. Furthermore, whole-body DWI presents an appealing alternative for staging or detecting recurrent tumors in CRC patients [136]. Numerous researchers have investigated the use of DWI to predict tumor response in CRC patients, yielding conflicting findings [118]. While some studies have indicated a significant correlation between pretreatment mean ADC values and treatment response, others have not replicated this association. Despite this inconsistency, several published studies have suggested that ADC values can effectively differentiate between good and poor responders. However, a recent systematic analysis revealed the limited accuracy of imaging modalities in confirming a pathologic complete response in rectal cancer patients. While DWI and FDG PET/CT exhibited the most potential for assessing pathologic complete response, both methods had their limitations [137]. Notably, one of the primary strengths of imaging techniques like DWI may lie in identifying nonresponders. Additionally, DWI might facilitate the differentiation of locally recurrent rectal cancer from scar tissue. For instance, Grosu et al. demonstrated that using a cutoff ADC value of 1.34 $\times$ 10−3 mm²/sec resulted in high sensitivity, specificity, and accuracy for this purpose [138].

DCE MR imaging

Dynamic contrast-enhanced (DCE) MRI is commonly employed for CRC assessment, utilizing a contrast-enhanced three-dimensional gradient-echo T1-weighted sequence. Initial findings have indicated a link between various DCE MRI parameters and angiogenic as well as genetic markers in RC patients. For instance, Yeo et al. observed correlations between mean return rate constant values and microvessel density, as well as tumor stage, while RC cases positive for epidermal growth factor receptor exhibited elevated mean volume transfer constants [139]. Similarly, Lollert et al. noted a significant association between time to peak and epidermal growth factor receptor expression [140]. Additionally, quantitative and semiquantitative parameters derived from DCE MRI have shown correlations with tumor and nodal staging in CRC [141].

Perfusion CT

Perfusion CT offers a means to assess tumor vasculature by modeling tracer kinetics in vivo. Although limited in scope, existing studies on perfusion CT in CRCpatients indicate potential roles for perfusion parameters in tumor phenotyping, evaluating tumor vascular heterogeneity, prognostication, and assessing treatment response [142]. However, the correlation between perfusion CT-derived parameters, such as blood flow and permeability, and microvessel density in CRC remains contentious [143]. Research findings regarding the utility of perfusion CT in tumor characterization indicate that perfusion CT parameters

may be linked to tumor grade and differentiation level. For example, Xu et al [144]. noted associations between blood flow and time to peak on perfusion CT images with tumor grade. Similarly, Sun et al. found significant differences in mean blood flow among well-, moderately, and poorly differentiated tumors [145]. Moreover, perfusion CT holds prognostic value, as tumors with poorer perfusion often exhibit worse outcomes. Perfusion CT's ability to accurately assess complete pathological response is limited. Additionally, the vascular response to therapies varies, influenced by factors like the therapy's mechanism of action and timing. For example, treated rectal cancer may exhibit early increases in perfusion due to radiation-induced inflammatory changes [146]. The table 2 provides summary of molecular imaging biomarkers.

Table 2: Summary of Molecular Imaging Modalities and Their Clinical Applications.

Imaging Modality	**Key features**	**Clinical Applications**
Computed Tomographic Colonography (CTC)	Non-invasive 2D and 3D imaging of colon and rectum using CT scanning High diagnostic accuracy in detecting CRC Less invasive than conventional colonoscopy	Screening and detection of CRC and polyps Identifying synchronous cancers Assessing elderly or frail patients Localizing tumors for surgery
Dual-energy Spectral CT	Enables material decomposition for precise tissue characterization. Enhances visualization with color maps Improves liver metastasis detection	Tumor grading and staging Liver metastasis detection Distinguishing lymph node metastases
Volumetry	Predicts outcomes based on tumor burden and volume changes Superior to RECIST criteria in assessing response to therapy MRI volumetry outperforms tumor size in predicting response	Predicting disease-free survival Assessing response to neoadjuvant therapy Delineating viable tumor from fibrosis
Machine Learning	Improves sensitivity in CRC screening Automates image analysis tasks Enhances efficiency and accuracy in diagnosis and treatment	Computer-aided detection and diagnosis Image segmentation and fusion Therapy guidance and monitoring
Positron Emission Tomography (PET)	Evaluates tumor metabolic pathways with various tracers PET/CT surpasses CT alone in detecting metastatic disease Quantitative parameters aid in staging and prognosis	Preoperative staging Restaging post-radiotherapy Predicting therapy response Identifying recurrent disease

Texture Analysis	Mitigates noise in images while highlighting biological heterogeneity Correlates with tumor characteristics and patient outcomes	Correlating with KRAS expression Predicting survival and staging Distinguishing aggressive tumors
Diffusion-weighted Imaging (DWI)	Noninvasive mapping of water molecule diffusion Effective in detecting CRC lesions Provides insights into tumor microstructure	Tumor detection and characterization Staging and prognosis Monitoring treatment response and recurrence
Dynamic Contrast-enhanced (DCE) MRI	Links DCE MRI parameters with angiogenic and genetic markers Correlates with tumor and nodal staging	Assessing tumor characteristics Evaluating treatment response Predicting outcomes
Perfusion CT	Models' tracer kinetics for assessing tumor vasculature - Potential roles in tumor phenotyping and treatment evaluation	Tumor characterization and grading Assessing vascular response to therapy Prognostication

6. Personalized Treatment Strategies

Personalised regimens of therapy for CRC represent a dramatic change in the way disease management is approached, emphasising the importance of genetic and molecular profiling in maximising treatment results. These approaches use the discovery of certain biomarkers to forecast responses to immunotherapeutic and targeted medicines, making it easier to choose the therapy modalities that will work best for each patient [5]. This strategy improves patient quality of life and overall survival rates by reducing the likelihood of side effects and increasing the effectiveness of treatment therapies. A new age of tailored, efficient, and patient-centred care is being ushered in by the ongoing development in genomic medicine, which is gradually revealing new biomarkers and therapeutic targets [147].

6.1 Current standard treatment approaches for colorectal cancer

Treatment for CRC often consists of a multimodal strategy that is adapted to the disease's stage and location and includes surgery, chemotherapy, and radiation therapy. The primary treatment for localised illness is surgery, which aims to remove the tumour and any surrounding lymph nodes. Adjuvant chemotherapy is recommended for higher-stage tumours to lower the chance of recurrence. It is frequently based on fluoropyrimidine-based regimens (e.g., 5-fluorouracil or capecitabine) with or without oxaliplatin [148]. Treatment regimens for advanced or metastatic CRC may combine chemotherapy drugs (FOLFOX, FOLFIRI) with targeted treatments, based on the molecular makeup of the tumour. When treating rectal cancer, radiation treatment is usually administered either surgically to reduce the chance of local recurrence or preoperatively to reduce tumour size and increase respectability [149].

6.2 Biomarker-driven targeted therapies

The emergence of biomarker-driven targeted medicines is a significant advancement in the management of CRC, providing a personalised medicine paradigm that greatly improves therapeutic effectiveness and specificity. This strategy makes use of the knowledge of the molecular changes unique to a patient's tumour to direct the choice of targeted therapy medicines that specifically target these changes, potentially improving patient outcomes and lowering the risk of needless side effects [150].

Essential Biomarkers and Allied Treatments

RAS Mutations

Lack of responsiveness to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (cetuximab and panitumumab) is predicted by mutations in the RAS genes (KRAS or NRAS), which is a crucial component in the therapy of metastatic colorectal cancer. Consequently, it is advised that patients with RAS wild-type mCRC only get these medicines, demonstrating a practical implementation of biomarker-driven treatment selection [151].

BRAF V600E Mutation

In mCRC, this mutation is linked to a worse prognosis. For tumours with the BRAF V600E mutation, targeted treatments have been developed, such as BRAF kinase inhibitors. BRAF inhibitors have been shown to be effective when combined with other medications, such as MEK inhibitors and anti-EGFR antibodies, giving individuals with this particular genetic composition a new lease on life [152].

Microsatellite instability (MSI) and deficiency in mismatch repair (dMMR)

Tumours with high MSI (MSI-H) or dMMR are more amenable to checkpoint inhibitor-based immunotherapy. This is because of their high mutation load, which raises the possibility of producing neoantigens that the immune system will recognise. Thus, determining a patient's MSI-H or dMMR status in colorectal cancer has become essential to the immunotherapy patient selection process [153].

HER2 Amplification

While less frequent, HER2 amplification in CRC is a developing target. Research indicates that individuals with HER2-amplified tumours may benefit from HER2-targeted medicines, which are widely used in the treatment of breast cancer [154].

Immunotherapy and immune checkpoint inhibitors

In the treatment of cancer, immunotherapy has shown great promise, especially in the case of CRC, where some patient groups may not respond well to conventional medicines. Immunotherapy is an innovative and possibly revolutionary approach to treating CRC by using the body's immune system to target and eliminate cancer cells. Immune checkpoint inhibitors, which have drawn a lot of interest for their capacity to alter the immune response and activate the body's natural anti-tumor defences, are essential components of this strategy [155].

Immune checkpoints are immune system regulatory circuits that support self-tolerance and guard against unchecked immunological activation. Cancer cells, however, can take advantage of these barriers to avoid immune system recognition and elimination. Two important checkpoints that act as brakes on the immune response are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death-1 (PD-1) [156].

PD-1/PD-L1 Pathway

Tumour cells and other immune cells express PD-L1 and PD-L2, the ligands of PD-1, which is expressed on the surface of T-cells. Through the inhibition of T-cell activation and the promotion of immunological tolerance, PD-1 binds to PD-L1 or PD-L2, enabling cancer cells to elude immune surveillance [157].

CTLA-4 Pathway

Activated T cells express CTLA-4, another immunological checkpoint receptor. It efficiently inhibits T-cell activation and proliferation by competing with the co-stimulatory protein CD28 for binding to CD80/86 on APCs [158].

Immune Checkpoint Inhibitors' Mode of Action

In order to suppress the immune system and trigger anti-tumor immunological responses, immune checkpoint inhibitors obstruct the interactions between checkpoint molecules and their ligands. Checkpoint inhibitors increase T-cell activation, proliferation, and effector function by interfering with these inhibitory signals, which enables the detection and destruction of cancer cells.

PD-1/PD-L1 Inhibitors
PD-1 or its ligand, PD-L1, are the targets of medications like pembrolizumab and nivolumab, which prevent T-cell activity suppression and encourage tumour cell death [157].
CTLA-4 Inhibitors
By inhibiting CTLA-4, medications such as ipilimumab improve anti-tumor immunity and maintain T-cell activation [159]

7. Challenges and Future Directions

7.1 Limitations and challenges in theranostics

Theranostics, a newly developed field of therapeutic and diagnostic approaches, emerges as a beacon of innovation in healthcare, offering tailored treatment avenues by seamlessly intertwining diagnostic capabilities with therapeutic interventions. Nonetheless, amidst its vast promise, theranostics grapples with an array of obstacles and complexities that necessitate resolution for its widespread adoption and efficacy [160]. Foremost among these challenges is the intricate nature of molecular targets, wherein diseases manifest a spectrum of molecular variations, rendering the identification of precise biomarkers for diagnosis and treatment a daunting task [161]. Furthermore, the validation of these biomarkers presents a formidable hurdle, demanding stringent validation processes to ascertain their accuracy, sensitivity, and reproducibility, thereby mitigating the risk of erroneous diagnostic outcomes [162]. Technological constraints add another layer of complexity, prompting relentless endeavors to enhance the sensitivity, precision, and specificity of imaging modalities, molecular diagnostic tools, and targeted therapeutic approaches. Additionally, navigating the regulatory landscape governing the integration of diagnostic and therapeutic elements into combined products poses significant hurdles, prolonging the journey from inception to market readiness and escalating developmental costs [163]. Theranostics places a strong emphasis on interdisciplinary collaboration, although this is filled with difficulties due to differences in terminology, approaches, and research priorities among specialists in different fields. The requirement for patient selection and stratification highlights the importance of taking clinical, molecular, and genetic aspects into account in a thorough manner.
This calls for the development of reliable algorithms that are specific to clinical settings [164]. Persistent challenges pertaining to resistance and relapse underscore the formidable nature of theranostics, with tumor cells often evolving mechanisms to thwart targeted therapies, while disease recurrence and metastasis pose enduring challenges to long-term management strategies. Addressing concerns regarding cost-effectiveness and reimbursement is paramount to ensuring the accessibility and affordability of theranostic interventions, alongside navigating ethical and legal complexities encompassing patient privacy, informed consent, and equitable resource allocation [165]. Furthermore, effective patient education and acceptance emerge as pivotal factors, demanding transparent communication of the benefits and risks associated with theranostics to empower informed decision-making. Despite these multifaceted challenges, sustained research endeavors, interdisciplinary collaborations, and technological innovations hold the promise of surmounting obstacles and unlocking the transformative potential of theranostics in enhancing patient outcomes and reshaping the landscape of disease diagnosis and treatment.

7.2 Potential future developments and emerging trends:

Theranostics is the integration of diagnostics and therapeutics, that holds significant promise in the field of CRC management. Here are some potential future developments and emerging trends: In the future, treatments for CRC are expected to become more precise, targeting specific types of the disease based on their unique genetic makeup. This could involve using advanced delivery systems like nanoparticles or antibody-drug combinations. These systems would be guided by specific genetic markers found through advanced

imaging or genetic testing [166]. For example, nanoparticles could be designed to deliver drugs directly to cancer cells with certain genetic mutations, reducing side effects and improving treatment effectiveness. Similarly, antibody-drug combinations could be used to deliver powerful drugs directly to CRC cells while leaving healthy cells unharmed [167].
Immunotherapy, a promising approach in cancer treatment, has demonstrated effectiveness in CRC and other malignancies. Moving forward, advancements could center on integrating immunotherapeutic drugs with diagnostic tools to pinpoint patients who would benefit most from these treatments. This might entail leveraging biomarkers or imaging technologies to evaluate the tumor environment accurately and forecast how well a patient would respond to immunotherapy [168]. For example, researchers could explore novel biomarkers or refine existing ones to better predict which CRC patients are likely to respond favorably to immunotherapy. Additionally, advancements in imaging techniques may enable more precise assessments of the tumor microenvironment, helping clinicians tailor immunotherapy regimens to individual patients' needs. By combining immunotherapy with sophisticated diagnostic approaches, future developments aim to enhance treatment outcomes and optimize patient care in CRC management [155].
Theranostic nanomedicine, an innovative field, integrates both diagnostic and therapeutic functions within a single nanoscale platform. In CRC, these nanoparticles offer potential for precise drug delivery and imaging. Future advancements may focus on creating multifunctional nanoparticles that not only deliver treatment but also provide real-time imaging of how tumors respond to therapy. This approach could revolutionize cancer treatment by allowing clinicians to monitor treatment effectiveness more accurately while simultaneously delivering targeted therapy to CRC tumors [7].
Incorporating Artificial Intelligence (AI) into healthcare presents a significant opportunity. AI-powered algorithms can sift through vast datasets derived from diagnostic imaging, genomic analyses, and clinical records to uncover patterns and forecast how CRC patients might respond to treatment. Looking ahead, further advancements might entail embedding AI-driven decision support systems directly into theranostic workflows. These tools could aid clinicians by providing personalized treatment recommendations, leveraging insights gleaned from AI analysis to optimize patient care in management [169].

8. Conclusion

Incorporating theranostics into colorectal cancer management marks a transformative step towards precision oncology, underscoring the pivotal role of molecular insights in guiding therapeutic strategies. By harnessing targeted therapies and diagnostic tools tailored to individual molecular profiles, theranostics offers the potential to revolutionize CRC care. This approach not only enhances treatment precision through early detection and therapy selection but also enables real-time monitoring of treatment responses, minimizing unnecessary exposure to ineffective treatments and optimizing therapeutic outcomes. From the utilization of diverse nanomaterials to the exploration of novel biomarkers and molecular imaging techniques, theranostics showcases a multifaceted approach poised to improve the effectiveness, efficiency, and patient-centeredness of CRC treatment regimens. As research and clinical practice continue to advance, the integration of theranostics holds promise for driving significant improvements in patient outcomes and enhancing quality of life in the management of CRC.

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References:

1. Qiu H, Ding S, Liu J, Wang L, Wang X. Applications of Artificial Intelligence in Screening, Diagnosis, Treatment, and Prognosis of Colorectal Cancer. Curr Oncol. 2022; **29**(3):1773-1795. doi:10.3390/curroncol29030146.
2. Hossain, M.S., et al., Colorectal Cancer: A Review of Carcinogenesis, Global Epidemiology, Current Challenges, Risk Factors, Preventive and Treatment Strategies. Cancers (Basel), 2022. **14**(7).
3. Nguyen, L.H., A. Goel, and D.C. Chung, Pathways of Colorectal Carcinogenesis. Gastroenterology, 2020. **158**(2): p. 291-302.
4. Yang W, Zou J, Li Y, et al. Longitudinal Circulating Tumor DNA Profiling in Metastatic Colorectal Cancer During Anti-EGFR Therapy. Front Oncol. 2022;12:830816. doi:10.3389/fonc.2022.830816.
5. Vaseghi Maghvan, P., et al., Personalized medicine in colorectal cancer. Gastroenterol Hepatol Bed Bench, 2020. **13**(Suppl1): p. S18-s28.
6. Parisi, A., et al., What Is Known about Theragnostic Strategies in Colorectal Cancer. Biomedicines, 2021. **9**(2).
7. Sachin M, Sachin D, Dinesh S. Voyage of theranostic liposomes for imaging and therapy. J Cosmet Laser Ther. 2017; **19**(4):245-249. doi:10.1080/14764172.2017.1279331
8. Sarhadi, V.K. and G. Armengol, Molecular Biomarkers in Cancer. Biomolecules, 2022. **12**(8).
9. Farha, M.W. and S.S. Salami, Biomarkers for prostate cancer detection and risk stratification. Ther Adv Urol, 2022. **14**: p. 17562872221103988.
10. Cooke, T., et al., HER2 as a prognostic and predictive marker for breast cancer. Ann Oncol, 2001. **12 Suppl 1**: p. S23-8.
11. Mehta, S., et al., Predictive and prognostic molecular markers for cancer medicine. Ther Adv Med Oncol, 2010. **2**(2): p. 125-48.
12. Rawla, P., T. Sunkara, and A. Barsouk, Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Prz Gastroenterol, 2019. **14**(2): p. 89-103.
13. Shaukat, A. and T.R. Levin, Current and future colorectal cancer screening strategies. Nat Rev Gastroenterol Hepatol, 2022. **19**(8): p. 521-531.
14. Ang, C.S., M.S. Baker, and E.C. Nice, Mass Spectrometry-Based Analysis for the Discovery and Validation of Potential Colorectal Cancer Stool Biomarkers. Methods Enzymol, 2017. **586**: p. 247-274.
15. Coghlin, C. and G.I. Murray, Biomarkers of colorectal cancer: recent advances and future challenges. Proteomics Clin Appl, 2015. **9**(1-2): p. 64-71.
16. Crutcher, M. and S. Waldman, Biomarkers in the development of individualized treatment regimens for colorectal cancer. Front Med (Lausanne), 2022. **9**: p. 1062423.
17. Ratovomanana T, Cohen R, Svrcek M, et al. Performance of Next-Generation Sequencing for the Detection of Microsatellite Instability in Colorectal Cancer With Deficient DNA Mismatch Repair. Gastroenterology. 2021;161(3):814-826. doi:10.1053/j.gastro.2021.05.007.
18. Silberring, J. and P. Ciborowski, Biomarker discovery and clinical proteomics. Trends Analyt Chem, 2010. **29**(2): p. 128.
19. McDermott, J.E., et al., Challenges in Biomarker Discovery: Combining Expert Insights with Statistical Analysis of Complex Omics Data. Expert Opin Med Diagn, 2013. **7**(1): p. 37-51.
20. Goossens, N., et al., Cancer biomarker discovery and validation. Transl Cancer Res, 2015. **4**(3): p. 256-269.
21. Yan, W., et al., Biological Networks for Cancer Candidate Biomarkers Discovery. Cancer Inform, 2016. **15**(Suppl 3): p. 1-7.

22. Taube, S.E., et al., A perspective on challenges and issues in biomarker development and drug and biomarker codevelopment. J Natl Cancer Inst, 2009. **101**(21): p. 1453-63.
23. Shrivastava, S., et al., A Review on Theranostics: An Approach to Targeted Diagnosis and Therapy. Asian Journal of Pharmaceutical Research and Development, 2019. **7**: p. 63-69.
24. Turner, J.H., An introduction to the clinical practice of theranostics in oncology. Br J Radiol, 2018. **91**(1091): p. 20180440.
25. Suciu, M., et al., Applications of superparamagnetic iron oxide nanoparticles in drug and therapeutic delivery, and biotechnological advancements. Beilstein J Nanotechnol, 2020. **11**: p. 1092-1109.
26. Dabaghi, M., et al., Iron Oxide Nanoparticles Carrying 5-Fluorouracil in Combination with Magnetic Hyperthermia Induce Thrombogenic Collagen Fibers, Cellular Stress, and Immune Responses in Heterotopic Human Colon Cancer in Mice. Pharmaceutics, 2021. **13**(10).
27. Alkahtane, A.A., et al., A possible theranostic approach of chitosan-coated iron oxide nanoparticles against human colorectal carcinoma (HCT-116) cell line. Saudi J Biol Sci, 2022. **29**(1): p. 154-160.
28. Gil, H.M., et al., NIR-quantum dots in biomedical imaging and their future. iScience, 2021. **24**(3): p. 102189.
29. Carbary-Ganz, J.L., et al., In vivo molecular imaging of colorectal cancer using quantum dots targeted to vascular endothelial growth factor receptor 2 and optical coherence tomography/laser-induced fluorescence dual-modality imaging. J Biomed Opt, 2015. **20**(9): p. 096015.
30. Vorobyeva MA, Dymova MA, Novopashina DS, et al. Tumor Cell-Specific 2'-Fluoro RNA Aptamer Conjugated with Closo-Dodecaborate as A Potential Agent for Boron Neutron Capture Therapy. Int J Mol Sci. 2021;22(14):7326. doi:10.3390/ijms22147326
31. Bober, Z., D. Bartusik-Aebisher, and D. Aebisher, Application of Dendrimers in Anticancer Diagnostics and Therapy. Molecules, 2022. **27**(10).
32. Nabavizadeh, F., et al., Evaluation of Nanocarrier Targeted Drug Delivery of Capecitabine-PAMAM Dendrimer Complex in a Mice Colorectal Cancer Model. Acta Med Iran, 2016. **54**(8): p. 485-493.
33. Alibolandi, M., et al., Curcumin-entrapped MUC-1 aptamer targeted dendrimer-gold hybrid nanostructure as a theranostic system for colon adenocarcinoma. Int J Pharm, 2018. **549**(1-2): p. 67-75.
34. Liu, X., Y. Ying, and J. Ping, Structure, synthesis, and sensing applications of single-walled carbon nanohorns. Biosens Bioelectron, 2020. **167**: p. 112495.
35. Lee, P.C., et al., Targeting colorectal cancer cells with single-walled carbon nanotubes conjugated to anticancer agent SN-38 and EGFR antibody. Biomaterials, 2013. **34**(34): p. 8756-65.
36. Silva, R., H. Ferreira, and A. Cavaco-Paulo, Sonoproduction of liposomes and protein particles as templates for delivery purposes. Biomacromolecules, 2011. **12**(10): p. 3353-68.
37. Kim, J.H., et al., Prognostic implications of CpG island hypermethylator phenotype in colorectal cancers. Virchows Arch, 2009. **455**(6): p. 485-94.
38. Lee, S., et al., Clinicopathological features of CpG island methylator phenotype-positive colorectal cancer and its adverse prognosis in relation to KRAS/BRAF mutation. Pathol Int, 2008. **58**(2): p. 104-13.
39. Karakaidos, P., D. Karagiannis, and T. Rampias, Resolving DNA Damage: Epigenetic Regulation of DNA Repair. Molecules, 2020. **25**(11).
40. Wu, C. and T. Bekaii-Saab, CpG Island Methylation, Microsatellite Instability, and BRAF Mutations and Their Clinical Application in the Treatment of Colon Cancer. Chemother Res Pract, 2012. **2012**: p. 359041.
41. Lee, M.S., et al., Association of CpG island methylator phenotype and EREG/AREG methylation and expression in colorectal cancer. British Journal of Cancer, 2016. **114**(12): p. 1352-1361.
42. Itzkowitz, S.H., et al., Improved fecal DNA test for colorectal cancer screening. Clin Gastroenterol Hepatol, 2007. **5**(1): p. 111-7.
43. Perez-Carbonell, L., et al., IGFBP3 methylation is a novel diagnostic and predictive biomarker in colorectal cancer. PLoS One, 2014. **9**(8): p. e104285.
44. Tang, D., et al., Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. Clin Invest Med, 2011. **34**(2): p. E88-95.

45. Lange, C.P., et al., Genome-scale discovery of DNA-methylation biomarkers for blood-based detection of colorectal cancer. PLoS One, 2012. **7**(11): p. e50266.
46. Oh, T., et al., Genome-wide identification and validation of a novel methylation biomarker, SDC2, for blood-based detection of colorectal cancer. J Mol Diagn, 2013. **15**(4): p. 498-507.
47. Watson, R.G., et al., Amplification of thymidylate synthetase in metastatic colorectal cancer patients pretreated with 5-fluorouracil-based chemotherapy. Eur J Cancer, 2010. **46**(18): p. 3358-64.
48. Zhang, L. and J.W. Shay, Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer. J Natl Cancer Inst, 2017. **109**(8).
49. Panarelli, N.C., et al., Sporadic microsatellite instability-high colon cancers rarely display immunohistochemical evidence of Wnt signaling activation. Am J Surg Pathol, 2015. **39**(3): p. 313-7.
50. Liang, J., et al., APC Polymorphisms and the Risk of Colorectal Neoplasia: A HuGE Review and Meta-Analysis. American Journal of Epidemiology, 2013. **177**(11): p. 1169-1179.
51. Liang, T.J., et al., APC hypermethylation for early diagnosis of colorectal cancer: a meta-analysis and literature review. Oncotarget, 2017. **8**(28): p. 46468-46479.
52. Loh, H.Y., et al., The Regulatory Role of MicroRNAs in Breast Cancer. Int J Mol Sci, 2019. **20**(19).
53. Di Leva, G., M. Garofalo, and C.M. Croce, MicroRNAs in cancer. Annu Rev Pathol, 2014. **9**: p. 287-314.
54. Falzone, L., et al., Integrated analysis of colorectal cancer microRNA datasets: identification of microRNAs associated with tumor development. Aging (Albany NY), 2018. **10**(5): p. 1000-1014.
55. Huang, Y., et al., MicroRNA-21 gene and cancer. Med Oncol, 2013. **30**(1): p. 376.
56. Peng, Q., et al., The clinical role of microRNA-21 as a promising biomarker in the diagnosis and prognosis of colorectal cancer: a systematic review and meta-analysis. Oncotarget, 2017. **8**(27): p. 44893-44909.
57. Sasaki, M., et al., The effectiveness of plasma miR-33a-5p as a predictive biomarker for the efficacy of colorectal cancer chemotherapy. Oncol Lett, 2021. **21**(6): p. 489.
58. Mou, Y., et al., MiR-1254 and MEGF6 regulates oxaliplatin resistance in human colorectal cancer cells. Am J Transl Res, 2021. **13**(1): p. 183-196.
59. Michael, J.V., J.G. Wurtzel, and L.E. Goldfinger, Regulation of H-Ras-driven MAPK signaling, transformation and tumorigenesis, but not PI3K signaling and tumor progression, by plasma membrane microdomains. Oncogenesis, 2016. **5**(5): p. e228.
60. Gray, R.T., et al., Evaluation of PTGS2 Expression, PIK3CA Mutation, Aspirin Use and Colon Cancer Survival in a Population-Based Cohort Study. Clin Transl Gastroenterol, 2017. **8**(4): p. e91.
61. Karakas, B., K.E. Bachman, and B.H. Park, Mutation of the PIK3CA oncogene in human cancers. Br J Cancer, 2006. **94**(4): p. 455-9.
62. Rosty, C., et al., PIK3CA activating mutation in colorectal carcinoma: associations with molecular features and survival. PLoS One, 2013. **8**(6): p. e65479.
63. Jehan, Z., et al., Frequent PIK3CA gene amplification and its clinical significance in colorectal cancer. J Pathol, 2009. **219**(3): p. 337-46.
64. Liao, X., et al., Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med, 2012. **367**(17): p. 1596-606.
65. Laurent-Puig, P., et al., Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol, 2009. **27**(35): p. 5924-30.
66. Scalise, J.R., et al., DNA Damage Is a Potential Marker for TP53 Mutation in Colorectal Carcinogenesis. J Gastrointest Cancer, 2016. **47**(4): p. 409-416.
67. Huh, J.W., H.R. Kim, and Y.J. Kim, Prognostic role of p53 messenger ribonucleic acid expression in patients after curative resection for stage I to III colorectal cancer: association with colon cancer stem cell markers. J Am Coll Surg, 2013. **216**(6): p. 1063-9.
68. Tzeng, S.T., et al., NDST4 is a novel candidate tumor suppressor gene at chromosome 4q26 and its genetic loss predicts adverse prognosis in colorectal cancer. PLoS One, 2013. **8**(6): p. e67040.

69. Annaval, T., et al., Heparan Sulfate Proteoglycans Biosynthesis and Post Synthesis Mechanisms Combine Few Enzymes and Few Core Proteins to Generate Extensive Structural and Functional Diversity. Molecules, 2020. **25**(18).
70. Riggins, G.J., et al., Mad-related genes in the human. Nat Genet, 1996. **13**(3): p. 347-9.
71. Armaghany, T., et al., Genetic alterations in colorectal cancer. Gastrointest Cancer Res, 2012. **5**(1): p. 19-27.
72. Sameer, A.S., Colorectal cancer: molecular mutations and polymorphisms. Front Oncol, 2013. **3**: p. 114.
73. Sarli, L., et al., Association between recurrence of sporadic colorectal cancer, high level of microsatellite instability, and loss of heterozygosity at chromosome 18q. Dis Colon Rectum, 2004. **47**(9): p. 1467-82.
74. Boulay, J.L., et al., SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer. Br J Cancer, 2002. **87**(6): p. 630-4.
75. Vishwamitra, D., et al., Type I insulin-like growth factor receptor signaling in hematological malignancies. Oncotarget, 2017. **8**(1): p. 1814-1844.
76. Codony-Servat, J., et al., Nuclear IGF-1R predicts chemotherapy and targeted therapy resistance in metastatic colorectal cancer. Br J Cancer, 2017. **117**(12): p. 1777-1786.
77. Zeinalian, M., et al., Clinical Aspects of Microsatellite Instability Testing in Colorectal Cancer. Adv Biomed Res, 2018. **7**: p. 28.
78. Boland, C.R. and A. Goel, Microsatellite instability in colorectal cancer. Gastroenterology, 2010. **138**(6): p. 2073-2087.e3.
79. Meyer, L.A., R.R. Broaddus, and K.H. Lu, Endometrial cancer and Lynch syndrome: clinical and pathologic considerations. Cancer Control, 2009. **16**(1): p. 14-22.
80. Sargent, D.J., et al., Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol, 2010. **28**(20): p. 3219-26.
81. Ribic, C.M., et al., Tumor Microsatellite-Instability Status as a Predictor of Benefit from Fluorouracil-Based Adjuvant Chemotherapy for Colon Cancer. 2003. **349**(3): p. 247-257.
82. Kheirelseid, E.A., et al., Mismatch repair protein expression in colorectal cancer. J Gastrointest Oncol, 2013. **4**(4): p. 397-408.
83. Pećina-Šlaus, N., et al., Mismatch Repair Pathway, Genome Stability and Cancer. Front Mol Biosci, 2020. **7**: p. 122.
84. Chen, W., B.J. Swanson, and W.L. Frankel, Molecular genetics of microsatellite-unstable colorectal cancer for pathologists. Diagn Pathol, 2017. **12**(1): p. 24.
85. Grady, W.M. and J.M. Carethers, Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology, 2008. **135**(4): p. 1079-99.
86. Lu, Y., et al., Silencing of the DNA mismatch repair gene MLH1 induced by hypoxic stress in a pathway dependent on the histone demethylase LSD1. Cell Rep, 2014. **8**(2): p. 501-13.
87. Kuppusamy, P., et al., Proteins are potent biomarkers to detect colon cancer progression. Saudi J Biol Sci, 2017. **24**(6): p. 1212-1221.
88. Tong, G., et al., The role of tissue and serum carcinoembryonic antigen in stages I to III of colorectal cancer-A retrospective cohort study. Cancer Med, 2018. **7**(11): p. 5327-5338.
89. Michor, F., et al., Dynamics of colorectal cancer. Semin Cancer Biol, 2005. **15**(6): p. 484-93.
90. Li, Y., et al., Carcinoembryonic antigen interacts with TGF-{beta} receptor and inhibits TGF-{beta} signaling in colorectal cancers. Cancer Res, 2010. **70**(20): p. 8159-68.
91. Araki, K., et al., High expression of HSP47 in ulcerative colitis-associated carcinomas: proteomic approach. Br J Cancer, 2009. **101**(3): p. 492-7.
92. Vidyasagar, A., N.A. Wilson, and A. Djamali, Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target. Fibrogenesis Tissue Repair, 2012. **5**(1): p. 7.
93. Dudeja, V., et al., Heat shock protein 70 inhibits apoptosis in cancer cells through simultaneous and independent mechanisms. Gastroenterology, 2009. **136**(5): p. 1772-82.
94. Morita, R., et al., Heat shock protein DNAJB8 is a novel target for immunotherapy of colon cancer-initiating cells. Cancer Sci, 2014. **105**(4): p. 389-95.
95. Bourboulia, D. and W.G. Stetler-Stevenson, Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. Semin Cancer Biol, 2010. **20**(3): p. 161-8.
96. Gong, Y., et al., TIMP-1 promotes accumulation of cancer associated fibroblasts and cancer progression. PLoS One, 2013. **8**(10): p. e77366.

97. Offenberg, H., et al., TIMP-1 expression in human colorectal cancer is associated with TGF-B1, LOXL2, INHBA1, TNF-AIP6 and TIMP-2 transcript profiles. Mol Oncol, 2008. **2**(3): p. 233-40.
98. Meng, C., et al., TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: A meta-analysis. PLoS One, 2018. **13**(11): p. e0207039.
99. Chand, M., et al., Novel biomarkers for patient stratification in colorectal cancer: A review of definitions, emerging concepts, and data. World J Gastrointest Oncol, 2018. **10**(7): p. 145-158.
100. Ko, C.C., et al., Imaging biomarkers for evaluating tumor response: RECIST and beyond. Biomark Res, 2021. **9**(1): p. 52.
101. Coy HJ, Douek ML, Ruchalski K, et al. Components of Radiologic Progressive Disease Defined by RECIST 1.1 in Patients with Metastatic Clear Cell Renal Cell Carcinoma. Radiology. 2019;292(1):103-109. doi:10.1148/radiol.2019182922.
102. Morgan, B., et al., Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: results from two phase I studies. J Clin Oncol, 2003. **21**(21): p. 3955-64.
103. Patel, U.B., et al., MRI after treatment of locally advanced rectal cancer: how to report tumor response--the MERCURY experience. AJR Am J Roentgenol, 2012. **199**(4): p. W486-95.
104. Das, K., et al., Role of (18)F-fluorodeoxyglucose Positron Emission Tomography scan in differentiating enhancing brain tumors. Indian J Nucl Med, 2011. **26**(4): p. 171-6.
105. Stone, W.Z., D.C. Wymer, and B.K. Canales, Fluorodeoxyglucose-positron-emission tomography/computed tomography imaging for adrenal masses in patients with lung cancer: review and diagnostic algorithm. J Endourol, 2014. **28**(1): p. 104-11.
106. Rowe, S.P. and M.G. Pomper, Molecular imaging in oncology: Current impact and future directions. CA Cancer J Clin, 2022. **72**(4): p. 333-352.
107. Ko CC, Yeh LR, Kuo YT, Chen JH. Imaging biomarkers for evaluating tumor response: RECIST and beyond. Biomark Res. 2021;9(1):52. doi:10.1186/s40364-021-00306-8.
108. Spada, C., et al., Clinical indications for computed tomographic colonography: European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastrointestinal and Abdominal Radiology (ESGAR) Guideline. Eur Radiol, 2015. **25**(2): p. 331-45.
109. Laghi, A., Computed tomography colonography in 2014: an update on technique and indications. World J Gastroenterol, 2014. **20**(45): p. 16858-67.
110. de Haan, M.C., P.J. Pickhardt, and J. Stoker, CT colonography: accuracy, acceptance, safety and position in organised population screening. Gut, 2015. **64**(2): p. 342-50.
111. Pickhardt, P.J., et al., Colorectal cancer: CT colonography and colonoscopy for detection--systematic review and meta-analysis. Radiology, 2011. **259**(2): p. 393-405.
112. Sato, K., et al., Usefulness of preoperative CT colonography for colon cancer. Asian J Surg, 2017. **40**(6): p. 438-443.
113. Simons, D., Kachelrieß, M. and Schlemmer, H.P., Recent developments of dual-energy CT in oncology. European radiology. 2014. 24: pp.930-939.
114. Fulwadhva, U.P., J.R. Wortman, and A.D.J.R. Sodickson, Use of dual-energy CT and iodine maps in evaluation of bowel disease. 2016. **36**(2): p. 393-406.
115. Gong, H.-x., et al., Dual energy spectral CT imaging for colorectal cancer grading: a preliminary study. 2016. **11**(2): p. e0147756.
116. Kato, T., et al., Clinical significance of dual-energy CT-derived iodine quantification in the diagnosis of metastatic LN in colorectal cancer. 2015. **41**(11): p. 1464-1470.
117. Kornprat, P., et al., Value of tumor size as a prognostic variable in colorectal cancer: a critical reappraisal. Am J Clin Oncol, 2011. **34**(1): p. 43-9.
118. Pham, T.T., et al., Functional MRI for quantitative treatment response prediction in locally advanced rectal cancer. 2017. **90**(1072): p. 20151078.
119. Martens, M.H., et al., Prospective, multicenter validation study of magnetic resonance volumetry for response assessment after preoperative chemoradiation in rectal cancer: can the results in the literature be reproduced? 2015. **93**(5): p. 1005-1014.
120. Petrillo, M., et al., MRI for assessing response to neoadjuvant therapy in locally advanced rectal cancer using DCE-MR and DW-MR data sets: a preliminary report. 2015. **2015**.

121. Lintoiu-Ursut, B., A. Tulin, and S. Constantinoiu, Recurrence after hepatic resection in colorectal cancer liver metastasis -Review article. J Med Life, 2015. **8 Spec Issue**(Spec Issue): p. 12-4.
122. Lim, M., et al., CT volumetry of the liver: where does it stand in clinical practice? 2014. **69**(9): p. 887-895.
123. Robinson, C., et al., CT colonography: computer-assisted detection of colorectal cancer. 2011. **84**(1001): p. 435-440.
124. Laurens, S.T. and W.J.J.P.c. Oyen, Impact of fluorodeoxyglucose PET/computed tomography on the management of patients with colorectal cancer. 2015. **10**(3): p. 345-360.
125. Brush, J., et al., The value of FDG positron emission tomography/computerised tomography (PET/CT) in pre-operative staging of colorectal cancer: a systematic review and economic evaluation. 2011. **15**(35): p. 1.
126. Maffione, A.M., et al., Value of 18F-FDG PET for predicting response to neoadjuvant therapy in rectal cancer: systematic review and meta-analysis. 2015. **204**(6): p. 1261-1268.
127. Culverwell, A., F. Chowdhury, and A.J.A.i. Scarsbrook, Optimizing the role of FDG PET–CT for potentially operable metastatic colorectal cancer. 2012. **37**: p. 1021-1031.
128. Davnall, F., et al., Assessment of tumor heterogeneity: an emerging imaging tool for clinical practice? Insights Imaging, 2012. **3**(6): p. 573-89.
129. Cui, C., et al., Quantitative analysis and prediction of regional lymph node status in rectal cancer based on computed tomography imaging. Eur Radiol, 2011. **21**(11): p. 2318-25.
130. Ng, F., et al., Assessment of primary colorectal cancer heterogeneity by using whole-tumor texture analysis: contrast-enhanced CT texture as a biomarker of 5-year survival. Radiology, 2013. **266**(1): p. 177-84.
131. Lubner, M.G., et al., CT textural analysis of hepatic metastatic colorectal cancer: pre-treatment tumor heterogeneity correlates with pathology and clinical outcomes. Abdom Imaging, 2015. **40**(7): p. 2331-7.
132. Baliyan, V., et al., Diffusion weighted imaging: Technique and applications. World J Radiol, 2016. **8**(9): p. 785-798.
133. Messina, C., et al., Diffusion-Weighted Imaging in Oncology: An Update. Cancers (Basel), 2020. **12**(6).
134. Jia, H., et al., Meta-analysis of diffusion-weighted magnetic resonance imaging in identification of colorectal cancer. 2015. **8**(10): p. 17333.
135. Curvo-Semedo, L., et al., Diffusion-weighted MRI in rectal cancer. **2012**: p. 35.
136. Lambregts, D.M., et al., Whole-body diffusion-weighted magnetic resonance imaging: current evidence in oncology and potential role in colorectal cancer staging. 2011. **47**(14): p. 2107-2116.
137. Ryan, J.E., et al., Assessing pathological complete response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer: a systematic review. 2015. **17**(10): p. 849-861.
138. Grosu, S., et al., Differentiating locally recurrent rectal cancer from scar tissue: Value of diffusion-weighted MRI. 2016. **85**(7): p. 1265-1270.
139. Yeo, D.-M., et al., Correlation of dynamic contrast-enhanced MRI perfusion parameters with angiogenesis and biologic aggressiveness of rectal cancer: Preliminary results. 2015. **41**(2): p. 474-480.
140. Lollert, A., et al., Rectal cancer: Dynamic contrast-enhanced MRI correlates with lymph node status and epidermal growth factor receptor expression. 2014. **39**(6): p. 1436-1442.
141. Hong, H.-S., et al., Correlations of dynamic contrast-enhanced magnetic resonance imaging with morphologic, angiogenic, and molecular prognostic factors in rectal cancer. 2013. **54**(1): p. 123.
142. Goh, V. and R.J.T.B.j.o.r. Glynne-Jones, Perfusion CT imaging of colorectal cancer. 2014. **87**(1034): p. 20130811.
143. Dighe, S., et al., Perfusion CT vascular parameters do not correlate with immunohistochemically derived microvessel density count in colorectal tumors. 2013. **268**(2): p. 400-410.
144. Xu, Y., et al., Predictive significance of tumor grade using 256-slice CT whole-tumor perfusion imaging in colorectal adenocarcinoma. 2015. **22**(12): p. 1529-1535.
145. Sun, H., et al., Assessment of tumor grade and angiogenesis in colorectal cancer: whole-volume perfusion CT. 2014. **21**(6): p. 750-757.
146. Petralia, G., et al., CT perfusion in oncology: how to do it. Cancer Imaging, 2010. **10**(1): p. 8-19.

147. Akhoon, N., Precision Medicine: A New Paradigm in Therapeutics. Int J Prev Med, 2021. **12**: p. 12.
148. Augestad, K.M., M.A. Merok, and D. Ignatovic, Tailored Treatment of Colorectal Cancer: Surgical, Molecular, and Genetic Considerations. Clin Med Insights Oncol, 2017. **11**: p. 1179554917690766.
149. Morris, V.K., et al., Treatment of Metastatic Colorectal Cancer: ASCO Guideline. 2023. **41**(3): p. 678-700.
150. Beniwal, S.S., et al., Current Status and Emerging Trends in Colorectal Cancer Screening and Diagnostics. Biosensors (Basel), 2023. **13**(10).
151. Valentini, A.M., et al., RAS-expanded Mutations and HER2 Expression in Metastatic Colorectal Cancer: A New Step of Precision Medicine. Appl Immunohistochem Mol Morphol, 2018. **26**(8): p. 539-544.
152. Grothey A, Fakih M, Tabernero J. Management of BRAF-mutant metastatic colorectal cancer: a review of treatment options and evidence-based guidelines. Ann Oncol. 2021;32(8):959-967. doi:10.1016/j.annonc.2021.03.206.
153. Zhang X, Wu T, Cai X, et al. Neoadjuvant Immunotherapy for MSI-H/dMMR Locally Advanced Colorectal Cancer: New Strategies and Unveiled Opportunities. Front Immunol. 2022;13:795972. doi:10.3389/fimmu.2022.795972.
154. Ivanova, M., et al., HER2 in Metastatic Colorectal Cancer: Pathology, Somatic Alterations, and Perspectives for Novel Therapeutic Schemes. Life (Basel), 2022. **12**(9).
155. Carlsen, L., K.E. Huntington, and W.S. El-Deiry, Immunotherapy for Colorectal Cancer: Mechanisms and Predictive Biomarkers. Cancers (Basel), 2022. **14**(4).
156. Cai, X., et al., Current Progress and Future Perspectives of Immune Checkpoint in Cancer and Infectious Diseases. Front Genet, 2021. **12**: p. 785153.
157. Han, Y., D. Liu, and L. Li, PD-1/PD-L1 pathway: current researches in cancer. Am J Cancer Res, 2020. **10**(3): p. 727-742.
158. Sobhani, N., et al., CTLA-4 in Regulatory T Cells for Cancer Immunotherapy. Cancers (Basel), 2021. **13**(6).
159. Buchbinder, E.I. and A. Desai, CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol, 2016. **39**(1): p. 98-106.
160. Jeelani, S., et al., Theranostics: A treasured tailor for tomorrow. J Pharm Bioallied Sci, 2014. **6**(Suppl 1): p. S6-8.
161. Perera, T.R.W., et al., The Future of Biomarkers in Veterinary Medicine: Emerging Approaches and Associated Challenges. Animals (Basel), 2022. **12**(17).
162. Davis, K.D., et al., Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nat Rev Neurol, 2020. **16**(7): p. 381-400.
163. Galldiks N, Langen KJ. Anwendung der Aminosäure-PET in der Diagnostik und Therapie von zerebralen Gliomen [Use of amino acid PET in the Diagnostic and Treatment Management of cerebral gliomas]. Fortschr Neurol Psychiatr. 2012;80(1):17-23. doi:10.1055/s-0031-1281851.
164. Herrmann, K., et al., Radiotheranostics: a roadmap for future development. Lancet Oncol, 2020. **21**(3): p. e146-e156.
165. Rutstein, S.E., et al., Hidden costs: The ethics of cost-effectiveness analyses for health interventions in resource-limited settings. Glob Public Health, 2017. **12**(10): p. 1269-1281.
166. Wang K, Shen R, Meng T, Hu F, Yuan H. Nano-Drug Delivery Systems Based on Different Targeting Mechanisms in the Targeted Therapy of Colorectal Cancer. Molecules. 2022;27(9):2981. doi:10.3390/molecules27092981.
167. Yao, Y., et al., Nanoparticle-Based Drug Delivery in Cancer Therapy and Its Role in Overcoming Drug Resistance. Front Mol Biosci, 2020. **7**: p. 193.
168. Golshani, G. and Y. Zhang, Advances in immunotherapy for colorectal cancer: a review. Therap Adv Gastroenterol, 2020. **13**: p. 1756284820917527.
169. Bohr, A. and K. Memarzadeh, The rise of artificial intelligence in healthcare applications. Artificial Intelligence in Healthcare. 2020:25-60. doi: 10.1016/B978-0-12-818438-7.00002-2. Epub 2020 Jun 26.