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COLORECTAL CANCER: GENERALITIES, DIAGNOSIS, AND BIOLOGICAL THERAPIES AS NEW TREATMENT OPTIONS

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ABSTRACT

The development of a malignant adenocarcinoma in the colon's inner layer or rectum wall is known as colorectal cancer. It is attributed to genetic information but also can be acquired due to lifestyle or environmental risk factors. The pathology is usually diagnosed through a colonoscopy. One promising alternative for its treatment is biological therapies. They are designed to improve, focus, or restore immune system function. These can be used to complement traditional treatment (e.g., surgery, radiotherapy, and chemotherapy). Immunotherapy is a classification of biological therapy, and it stimulates the immune system by generating a more effective therapeutic response in cancer treatment. It is possible to identify monoclonal antibodies, vaccines, oncological viruses, and novel methods such as chemoimmunotherapy, in which a cytotoxic agent and an immune response modifier are combined in a single drug. These options have brought promising results through several preclinical and clinical investigations.

KEYWORDS: colorectal cancer, biological therapies, monoclonal antibodies, vaccines, oncologic viruses, chemoimmunotherapy.

INTRODUCTION

Cancer is abnormal tissue growth originating by mutations in the genes that control different cellular processes, including growth, duplication, apoptosis, and cell-cell interactions. Also, it is associated with defects in cell signaling pathways, angiogenesis stimulation, aneuploidy[1]

(i.e., a mutation in which the number of chromosomes is abnormal),[2] and changes in cellular metabolism and microenvironment. It can be developed by humans of all ages, affecting various organs.[1]

Colorectal or large bowel cancer involves the colon, the rectosigmoid junction, and the rectum. Multiple factors are associated with its development, including genetic susceptibility, specific host characteristics (e.g., sex, age, ethnicity), environmental and lifestyle factors such as diet and smoking, the employment of some medications, and specific status of diseases.[3]

According to the World Health Organization (WHO), colorectal cancer was in 2018 the fifth and fourth type of cancer with a major worldwide incidence rate in men and women, respectively. On the other hand, it ranked third for Latin America and fourth for the Caribbean and represented the fifth leading cause of cancer death in both regions.[4]

When a patient is diagnosed with colorectal cancer, surgery, radiation therapy, and chemotherapy are the most frequent treatment possibilities. The choice depends on the cancer type, the stage, the age, and the individual's health status.[5] However, many adverse effects are associated with the administration of these therapies.[6]

The surgical treatment produces pain and tenderness in the operation area, constipation, or diarrhea. In addition, it requires hospitalization.[5, 6] Moreover, chemotherapy's effects (e.g., vomiting, nausea, diarrhea, neuropathy, tiredness,[6] hair loss, anorexia, bleeding) depend on the type and dose of the drug and the intake period.[5] Similar effects are caused by radiation therapy treatment, which includes fatigue, mild skin reactions, stomach upset and pain, loose bowel movements, bloody stools, sexual problems, and infertility.[5, 6]

Due to the previous, biological therapy has become a new weapon. Although this type of treatment has specific side effects (e.g., fatigue, diarrhea, nausea, fever, pain, cough, and decreased appetite),[6] are less frequent than those mentioned for traditional therapies. Some advantages include lower administration frequency, better treatment selectivity, and the consideration of techniques to work effectively with traditional treatments, improving its activity and selectivity. For example, antibodies can selectively carry radioisotopes, toxins, chemotherapy, and other cytostatic or cytotoxic molecules to the tumor site.[7]

Within biological therapy is immunotherapy. It is the immune system's stimulation to generate a more effective cancer treatment response.[8, 9] It is divided into passive and active. Each one has adverse effects, such as evolving resistance (passive immunotherapy) or unwanted autoimmunity activation (active immunotherapy). Even so, they are not as expected, and some of them have been associated with a clinical benefit.[8]

Therefore, this review aims to show the therapeutic options at the biological and biotechnological levels in treating colorectal cancer.

OVERVIEW OF COLORECTAL CANCER

As an organ of the digestive system, the colon has a length of almost one and a half meters. It is divided into four parts: ascending, transverse, descending, and sigmoid. Its wall has four main layers: the mucosa (innermost), the submucosa, the muscularis propria, and the serosa or adventitia layer (outermost). Cancer starts in the inner layer and grows toward the outer one. It usually begins as a non-malignant growth in the glandular cells of the inner lining of the colon or rectum because of genetic, lifestyle, or environmental risk factors, among which the microbiota is mentioned.[10, 11, 12, 13]

Normal cells are transformed into adenomas or polyps that grow slowly over 10 to 20 years. Thus, as these grow, the probability of becoming a cancerous tumor increases (i.e., the development of a malignant adenocarcinoma). Once formed in the large inner intestinal lining, it can grow into the colon or rectum wall and enter nearby blood or lymphatic vessels. The development is related to its stage,[11, 12, 13] which indicates the tumor extent.

The stage is used to plan the required tests and later assign the most appropriate treatments for each patient. These exams include complete blood count, chemistry profile, carcinoembryonic antigen, computed tomography, magnetic resonance imaging, and specific tumor markers (e.g., mismatch repair or MMR deficiency, mutations in the K-RAS, N-RAS, and B-RAF genes). The two most common staging systems are the Surveillance, Epidemiology, and End Results (SEER) summary staging system, utilized for descriptive and statistical analysis of tumor registry data, and the tumor, node, metastasis (TNM) system, a classification of the American Joint Committee on Cancer (AJCC), employed in clinical scenarios.[11, 13] The SEER system is divided into *in situ*, local, regional, and remote. Its characteristics are shown in **Table 1**.[11]

Table 1: Cancer characteristics according to stage classification of SEER system.[11]

Stage	Cancer characteristics
In situ	It has not yet begun to invade the colon or rectum wall.
Local	It has grown into the colon or rectum wall but has not invaded nearby tissues.
Regional	It has spread through the colon or rectum wall, invading nearby tissue o extending to nearby lymph nodes.
Distant	It has spread to other body parts (liver, lung, among others).

In addition, the TNM classification evaluates three aspects of cancer growth: tumor penetration (T), lymph node involvement (N), and the presence or absence of metastasis (M). Besides, it establishes subdivisions within each category, helpful for determining the disease stage. This system is summarized in **Table 2**. Additionally, **Table 3** shows the classification to assign colon cancer-specific stages based on this classification.[14]

Table 2: Definitions of the TNM system for colorectal cancer.[14]

Primary tumor (T)	
TX	Primary tumor could not be evaluated.
T0	No evidence of primary tumor.
Tis	Carcinoma *in situ*: intraepithelial tumor or invasion of the lamina propia.
T1	Tumor invades the submucosa.
T2	Tumor invades the muscularis propria.
T3	Tumor invades through the muscularis propria into pericolorectal tissues.
T4a	Tumor penetrates to the surface of the visceral peritoneum.
T4b	Tumor invades directly or adheres to other organs or structures.
Regional lymph nodes (N)	
NX	Regional lymph nodes could not be evaluated.
N0	No metastasis on regional lymph nodes.
N1	Metastasis in 1 to 3 lymph nodes.
N1a	Metastasis in 1 lymph node.
N1b	Metastasis in 2 to 3 lymph nodes.
N1c	Tissue tumor deposits, without metastasis on lymph nodes.
N2	Metastasis in more than 4 lymph nodes.
N2a	Metastasis in 4 to 6 more lymph nodes.
N2b	Metastasis in 7 or more lymph nodes.
Distant metastasis (M)	
M0	No distant metastasis.
M1	Distant metastasis.
M1a	Metastasis confined to an organ or a site.
M1b	Metastasis in more than one organ or site, or in peritoneum.

Table 3: Cancer stage assignment according to the TNM classification.[13]

Stage	T	N	M
0	Tis	N0	M0
I	T1	N0	M0
	T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0
IIIA	T1-T2	N1/N1c	M0
	T1	N2a	M0
IIIB	T3-T4a	N1/N1c	M0
	T2-T3	N2a	M0
	T1-T2	N2b	M0
IIIC	T4a	N2a	M0
	T3-T4a	N2b	M0
	T4b	N1-N2	M0
IVA	Any T	Any N	M1a
IVB	Any T	Any N	M1b

It has been reported that 15 to 20 % of cases are associated with DNA MMR deficiency, characterized by microsatellites or repeated sequences associated with MMR genes. These mutations and epigenetic alterations occur in the MLH1, MSH2, MSH6, and PMS2 genes. They are related to protein complexes responsible for DNA repair through sequence recognition (MSH2/MSH6 or MLH1/PMS2 complexes), linkage and correction of mutations (MSH2 and MSH6), and chromosomal arrangements (PMS2 and MLH1).[15, 16, 17, 18, 19] Likewise, they have been related to a variation of Lynch syndrome, a type of colon cancer of autosomal genetic inheritance with incomplete penetrance,[20] associated with mutated MHL1, MSH2, MSH6, and PMS2 genes. Such a situation increases the risk of suffering from this and other types of cancer.[15, 20, 21] Affected individuals have a lifetime risk of nearly 80 % developing colorectal cancer without any intervention. Hence the importance of timely identification of individuals carrying these genes. Morbidity and mortality benefit from intensive endoscopic surveillance, and prophylactic surgery has been found.[22]

Physiopathology of colorectal cancer

The determining factors in its progression are inflammation and immunity. An increase in the activity of interleukin 4 (IL-4) has been found in the initial stages, which activates the maturation of T cells to T helper 2 (Th2) lymphocytes, whose interleukins (IL-10, IL-5, and IL- 4) are linked to tumor growth or metastasis.[23, 24, 25] IL-6 (stimulates B lymphocytes and mediates acute phase reactions) is correlated with the forming, invasion, and metastasis of

colorectal cancer. Studies have shown that its absence in the tumor microenvironment increases immunotherapy efficacy against it.[25, 26, 27]

Furthermore, IL-8 and vascular endothelial growth factor (VEGF), responsible for chemotaxis and neutrophil activation and stimulating angiogenesis in normal cells, respectively, are involved in tumor angiogenesis, favoring pathology recurrence. They affect tumor progress, metastasis, and chemical resistance. IL-8 also contributes to a rise in the concentration of the αvβ3 integrin (i.e., a proangiogenic protein under standard conditions). Said integrin is secreted by mesenchymal stem cells, promoting angiogenesis and tumor growth. Moreover, it is associated with a receptor with a possible therapeutic role: CXRC2. Among its effects are a possible antitumor activity and sensitization of the tumor towards chemotherapy. Due to these properties, IL-8 could become a target for treating colorectal cancer in the future.[25, 26]

As a complement, patients show lower serum concentrations of IL-9 in serum and intestinal tissues. It is associated with cancer progression. Likewise, in the progression, there is a relationship between an augmented IL-10 (inhibits the production of interferon gamma or IFN-γ and IL-12, both pro-inflammatory cytokines) in serum and a survival decrease.[25, 26]

At the genetic level, the involved genes in colorectal cancer can be divided into tumor suppressor genes, proto-oncogenes, and genes for DNA repair. The firsts are a regulatory pathway to decrease the mechanisms that favor cell growth.[22] In this disorder, the most frequently inactivated genes are APC, which regulate division, migration, and maintenance of genomic stability,[28] tp53, whose final protein is crucial for genomic integrity, recognizing DNA damage and inducing apoptosis, and p16, which restricts proliferation at the G1 checkpoint of the cell cycle.[29] Consistent with Knudson's two-hit hypothesis, acquired (or somatic) mutations are needed in the suppressor gene's two alleles to disable their function altogether and promote cancer development. In autosomal dominant syndromes, a pre-existing germline mutation is inherited in one allele (first hit), and an acquired mutation deactivates the other (second hit).[22]

Besides, proto-oncogenes are components of the signaling pathways that stimulate normal cell growth and proliferation. A mutation triggers an active gene product with a resulting tumor effect. RAS is a family of genes, including H-RAS, N-RAS, and K-RAS. The last two

are relevant to colorectal cancer. When mutated, they produce overactive proteins that increase tumor growth. Although they have been considered a pharmacological target treatment, these genes do not work if they are mutated.[13, 22] Another proto-oncogene is B-RAF. Its mutation is common in colorectal cancer. It generates cancer cells to grow and spread more quickly. If the mutation exists, it is possible to add a B-RAF inhibitor to the treatment regimen.[13]

Regarding DNA repair genes, they maintain the integrity of the genome. If errors in modifying a nucleotide occur, they are corrected by a refined process known as base excision repair. Another way the errors are introduced into the genome is with the mismatch of nucleotides during normal DNA replication. A MMR system corrects these. When the repair processes are dysfunctional, damaging mutations can accumulate in genes, directly controlling cell growth and proliferation.[22]

Figure 1 exemplifies the staging of mutations related to this cancer. The process begins with a point mutation in a critical gene that regulates cell growth, such as APC. Later they accumulate in other genes, such as KRAS, 18q, and tp53. Plus, cell proliferation has other genes, allowing cancer cells to migrate to distant tissues.[22]

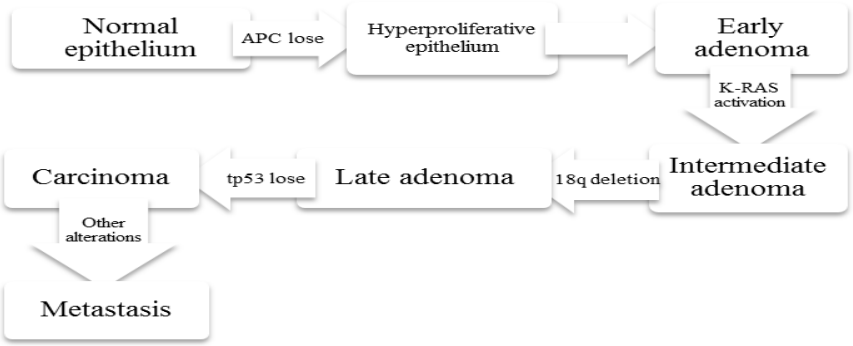

Figure 1: Genetic model of colorectal tumorigenesis (modified from 22).

Finally, heat shock proteins (HSPs) block programmed cell death and promote the activation of factors that degrade the extracellular matrix. The increase in its expression seems to be involved in most of these tumor development stages and the acquisition of drug-resistant phenotypes, especially when the neoplasms are subjected to cytotoxic therapies (selection of resistant cells).[30] In a study done, it was discovered that a type of HSP (DNAJB8) is expressed and has a vital task in a type of colorectal cancer cells.[31]

DIAGNOSIS

Diagnosis is made through a colonoscopy, and a biopsy is requested due to recurring symptoms that may reflect intestinal problems, such as bleeding, abdominal and rectal pain, nausea, and vomiting. Colonoscopy allows one to view the entire colon and remove polyps. Sometimes a complete procedure cannot be performed as there may be a bowel obstruction or other barriers. A postoperative colonoscopy is recommended to rule out any synchronous tumors in these cases. If it is not possible, a radiographic diagnosis may be required. The biopsy is usually taken during the colonoscopy, confirming the diagnosis. Another way is barium enemas with flexible sigmoidoscopy (FSIG), capable of diagnosing tumors in the sigmoid colon.[12, 32]

A solid tumor can be categorized according to TNM staging (**Table 2**). Pathologic evaluation also includes tumor type, histologic grade, venous and lymphatic invasion, and whether the resected margins are tumor-free.[14] Other factors contemplated are histological categorization (e.g., signet ring cell, mucinous, micropapillary, medullary, cribriform, serrated, adeno-squamous, comedo-type, spindle cell, and undifferentiated), their genetic content and mutational analyzes, and the molecular pathways involved.[33] Contemplating these factors is essential in determining optimal strategies for treatment and adequate follow-up.

Besides TNM staging, Immunoscore is a quantitative assay that allows, employing formalin-fixed, paraffin-embedded tumor tissue slides, the determination of total (CD3+) and cytotoxic (CD8+) T lymphocyte populations. They measure T cell infiltration in the tumor, functioning as prognostic markers. It is carried out by measuring the population density in the tumor slides, analyzed by software or biopsy, categorizing 0 as low immune cell densities and 4 as high ones. This way, it is possible to diagnose and improve patient survival prognosis.[33]

Colon cancer has essential characteristics in defining the prognosis at the histological level. Tumors with poor differentiation, extracellular mucin, intraepithelial lymphocytosis, and an immune reaction similar to Crohn's disorder in lymphoid nodules and germinal centers have a better prognosis in the early stages. However, these characteristics do not provide prognostic benefits in metastatic disease.[19]

TRADITIONAL THERAPIES

Conventional therapies for colorectal cancer refer to surgery, radiation therapy, and chemotherapy.[34] Surgery is the first-line of treatment. It is estimated that about 40 % of patients who undergo it develop relapses in a near period.[35, 36, 37]

When surgical treatment is not possible, or the cancer is metastatic, systemic options (chemotherapy) are suggested. During the early stages, sensitivity is high for drugs such as irinotecan and oxaliplatin in MMR deficiency colon cancers.[18] Standard chemotherapy is utilized as an adjuvant when diagnosed in stage III (without distant metastasis). It consists of fluoropyrimidines (5-fluorouracil or 5-FU plus leucovorin or capecitabine), acting on the folate-homocysteine cycle, and the synthesis of pyrimidines and purines[38, 39, 40] and oxaliplatin, whose therapeutic action is linked to the disruption of DNA replication and transcription, triggering the activation of the apoptosis cascade.[41, 42] This combination is administered as a XELOX (capecitabine plus oxaliplatin) or FOLFOX (5-FU plus oxaliplatin) regimen.[40, 43] In Costa Rica, the Social Security System (CCSS, for its Spanish acronym) implements FOLFOX as an adjuvant treatment and in advanced illness cases.[44]

Moreover, radiotherapy is essential in the multidisciplinary management of most non-metastatic tumors. It is second in a curative contribution after surgery and essential for improving symptoms in incurable cancer patients. It delivers lethal doses of ionizing radiation (X-rays, ɣ-rays, electrons, or protons) accurately and precisely in the tumor tissue, resulting in DNA damage and the consequent cell death. Its impact is highly dependent on tumor radiosensitivity. Still, it is infrequent in colorectal cancer and is more closely linked to specific characteristics of the patient and the tumor.[45, 46]

Although these therapeutic strategies are available, the results have not been satisfactory. For example, while 70 to 80 % of patients are eligible for a given surgery, the five year overall survival is only 50 to 60 %. Additionally, relapse develops within the first five years of the surgical procedure in 85 % of cases.[47] Plus, while post-surgery adjuvant chemotherapy reduces the recurrence risk, it occurs in most patients within the first three years after the procedure. Adjuvant therapy based on 5-FU has reduced the risk of recurrence and death by 30 and 26 %, respectively, but these numbers are still low.[48]

As a complement, traditional therapy has high toxicity. Neoadjuvant radiotherapy originates functional sensitivity to structures like the anal sphincter and the urinary tract.[49] Other adverse effects are classified as early toxicity (cystitis, diarrhea, and perineal dermatitis) and late toxicity (fecal incontinence, bowel dysfunction, genitourinary dysfunction, bleeding and perforation, and pelvic fractures).[50] Thus, the chemotherapeutic possibilities cause serious adverse effects, including neutropenia, anemia, diarrhea, nausea, vomiting, and neurological toxicities (both in the FOLFOX and XELOX regimens).[51] Studies show an increase in grade 2 or 3 diarrhea in patients with preoperative 5-FU and pelvic radiation[52] and increased gastrointestinal toxicity and high neurotoxicity rates with the FOLFOX regimen.[53]

Given the above, there is a need for new and innovative therapies. Therefore, efforts have been directed to biological therapy.

BIOLOGICAL THERAPY

Biological therapy is directed against cancer to attack specific cells. Certain drugs stop tumor angiogenesis, some prevent the growth signals from reaching the cells, and others work by several mechanisms simultaneously. It is less likely to damage normal cells than conventional treatments, resulting in fewer side effects.[13]

One of these therapies is immunotherapy. It includes using biological drugs to help the immune system (natural defense against infections and pathologies) recognize and kill cancer cells. There are satisfactory investigations of monoclonal antibodies such as ipilimumab, nivolumab, and pembrolizumab, showing an advance in therapy for colorectal cancer.[13, 25]

Besides, there are autologous vaccines containing tumor-associated antigens specific to the patient.[25] Each of these pharmacological groups is detailed below.

Monoclonal antibodies

Antibodies are a defense tool for cells. They are proteins composed of a Y-shaped monomer composed of two light chains, two heavy chains, and a hypervariable region at one end. The hypervariable region changes from one antibody to another, allowing great diversity to respond to distinct antigens. The heavy and light chains pair to create three structural domains, two antigen-binding fragments (Fab) and one crystallizable fragment (Fc), joined by a flexible region called the hinge. The Fc fragment is a heavy chain dimer of the constant heavy 2 and constant heavy 3 segments, while the Fab fragment is a mixed light-heavy chain

dimer of variable light-constant light paired with variable heavy-constant heavy 1 segments. Heavy chains have constant and variable regions. The latter defines the classes or isotypes of immunoglobulins (Ig). These are IgA, IgD, IgE, IgG, and IgM. IgA has two subclasses, IgA1 and IgA2, while IgG (found in a higher proportion in humans) is divided into four isotypes: IgG1, IgG2, IgG3, and IgG4. The Ig properties differ in each class and subclass.[54, 55, 56]

As with monoclonal antibodies, they are produced in laboratories with high specificity and elevated affinity for a given therapeutic target, acting similarly to human antibodies. They are used to treat several disorders, including cancer. There are different ways for their production, classified into four types:[55, 56]

- **Murine**: proteins are derived from rodent species (antibody name ends in -omab).
- **Chimeric**: the variable portion is of murine origin and the rest of human origin (-ximab).
- **Humanized**: the hypervariable regions are murine, while the rest correspond to human portions (-zumab).
- **Fully human**: made entirely of human proteins (-umab).

The employment of murine antibodies dragged the immunogenicity problem, with the patients' consequent production of human antibodies against said treatments, reducing their effectiveness. Various alternatives were explored to overcome this difficulty, including chimerization and humanization.[56]

Referring to cancer treatments, there are two therapeutic lines. Passive immunotherapy (i.e., therapeutic administration of live cellular immune cells to a patient) is achieved by blocking angiogenesis mechanisms and increasing cellular cytotoxicity. As for active immunotherapy, it involves the stimulation of the immune system.[57, 58, 59] In colorectal cancer, the therapeutic agents of passive immunotherapy include bevacizumab (humanized IgG1 antibody)[58, 60, 61] and cetuximab (chimeric IgG1 antibody).[62, 63, 64]

For passive immunity, it has been reported that there was a decrease in the colorectal tumor volume after the administration of bevacizumab. Its mechanism of action includes blocking the VEGF activation cascade. This protein is produced as an inflammatory response to oxidative stress and hypoxia. Furthermore, its administration to persons with the metastatic colorectal disease has been shown to have a major positive effect on their survival as an adjuvant to the FOLFOX and XELOX regimens.[25, 40, 65]

As a complement, cetuximab binds to the epidermal growth factor receptor (EGFR) with high specificity and better affinity than endogenous ligands, blocking the cascade of effects triggered by activating this receptor.[59] The gene that encodes for EGFR is up-regulated between 60 and 80 % of all cases. The signaling pathway triggered by this receptor's activation regulates cell differentiation, proliferation, migration, angiogenesis, and apoptosis.[66] These scenarios explain why cetuximab and bevacizumab are relevant in colorectal cancer.[40, 67]

Recent studies show that therapies with antibodies and chemotherapeutic agents, such as oxaliplatin, have not shown efficacy in the early stages.[40] There is an associated problem, specifically the resistance of colorectal cancer against these chemotherapeutic and immunotherapeutic agents.[66, 68]

Regarding active immunity, the body expresses distinct proteins targeted by monoclonal antibodies. To properly function cytotoxic T lymphocytes, the major histocompatibility complex (MHC) activates through antigen-presenting cells (APCs). This mechanism produces T lymphocytes' activation, followed by the binding of CD86/CD80 ligands for the co-stimulation of the CD28 receptor, a cell surface protein that culminates the activation.[69,70] There is a checkpoint mediated by cytotoxic T lymphocyte-associated-protein 4 (CTLA-4) as an inactivation mechanism. It was the first control point studied, and its expression is increased in many types of cancer. This protein, homologous to CD28, binds to CD86 and CD80 ligands with much higher affinity, preventing the activation of cytotoxic T cells.[71, 72] Ipilimumab is used as an anti-CTLA-4 monoclonal antibody.[71]

Subsequently, programmed cell death protein 1 (PD-1) was studied. PD-1 has been one of the most exhaustively studied regulators because of its crucial role in fine-tuning T cell function and maintaining immune system homeostasis. Fruitful clinical trials with PD-1/PD-L1 monoclonal antibodies have unlocked new expectancies in cancer immunology. This checkpoint blockade treatment is part of the standard therapy for diverse malignancies.[73]

Colorectal cancer cells express PD-L1 (PD-1 ligand) at high levels, and both epithelial growth factor (EGF) and insulin promote its expression and membrane transport. This high expression proposes that PD-1/PD-L1-based blocking therapy may be more effective and long-lasting in this cancer.[74, 75]

Pembrolizumab, a humanized monoclonal antibody, is a PD-1 receptor blocker. Under one trial, it was given to patients with mutations in MMR genes. The results indicated an increase in the progression-free survival and objective response rate, reaching 78 and 40 %, respectively, in deficiency MMR (dMMR). These rates were also evaluated for various mutations in MMR genes, such as proficient MMR (pMMR) colorectal cancer.[18, 19]

In addition, by dosing pembrolizumab at 10 mg/kg every 2 weeks or 200 mg every 3 weeks until unacceptable toxicity, a duration of response greater than 6 months was found in 78 % of dMMR cases. Therefore, the United States Food and Drug Administration (FDA) granted accelerated approval for its utilization in metastatic or unresectable, MSI-H/dMMR (high-frequency microsatellite instability) colorectal cancer that progressed after treatment with oxaliplatin, fluoropyrimidine, and irinotecan, and for patients with MSI-H/solid dMMR tumors which progressed after prior therapy and did not have suitable treatment options.[18, 19] Participants with MSI-H or dMMR advanced colorectal carcinoma in phase III studies randomly received pembrolizumab or the investigator's election of one of six standard chemotherapy regimens for this carcinoma. The study's central hypothesis is that the monoclonal antibody prolongs overall survival or progression-free survival compared to existing chemotherapy.[76]

Regarding nivolumab, a fully human IgG4 monoclonal antibody, it works by selectively blocking the activation of PD-L1 and PD-L2.[77] In phase II clinical trial results, with doses of 3 mg/kg every two weeks until reaching unacceptable toxicity, illness progression, or withdrawal, a control of 69 % of cases were observed for at least 12 weeks. These findings suggest that disease control and a durable response can be established with the drug in patients with dMMR metastatic colorectal cancer who received prior treatment. This study was essential in its FDA approval after previous treatment with fluoropyrimidine, irinotecan, and oxaliplatin.[19] In other research, emerging data on its efficacy when employed in dMMR/MSI-H metastatic cancer in conjunction with ipilimumab suggest the possibility of elevated response rates, with 55 % of persons reporting a response to treatment and overall survival of 12 months of 85 %.[77]

Another fully human monoclonal antibody is durvalumab. Phase II studies have been conducted as a PD-L1 blocking agent. Progression-free survival of 5.5 months was obtained, although only 40 % of patients completed 12 months of treatment.[77]

Atezolizumab is a humanized IgG1 monoclonal antibody that binds directly to PD-L1, blocking its interaction with the PD-1 receptor. It is the first monoclonal antibody approved by the FDA to block this ligand, indicated for metastatic non-small cell lung cancer and advanced or urothelial carcinoma. It currently has early-stage clinical studies supporting its efficacy in treating metastatic colorectal cancer and ongoing clinical trials with dMMR patients. Phase III studies aimed at combining atezolizumab and other cancer treatment lines have failed to demonstrate significant survival. Nonetheless, they continue to be elaborated with distinct approaches.[78, 79]

Avelumab is another monoclonal antibody that inhibits PD-L1. It is an IgG1 isotype of human origin,[80] approved in 2017 by the FDA for metastatic Merkel cell carcinoma.[81] The phase II studies showed antitumor activity and manageable toxicity in patients with previously treated dMMR/MSI-H metastatic colorectal cancer.[82] Phase III studies are presently being performed to know the benefit of its utilization after adjuvant chemotherapy in patients with dMMR colorectal cancer.[83]

Vaccines

Vaccines are immunoreactive therapies. An antigen is administered to the subject or patient in a controlled manner to elicit an immune response, generating memory towards pathogens.[84] Specific antigens for cancer treatment can produce apoptosis activation as a mechanism of action, either *in situ* in the tumor or by a systemic effect. At the systemic level, this reaction is stimulated by releasing inflammatory mediators such as ATP and HMGB1 (i.e., a chaperone protein involved in gene transcription, DNA binding, DNA repair, and development of pro-inflammatory effects), generating a cascade that activates apoptosis. This method is managed as immunogenic cell death (ICD) treatments, in conjunction with other traditional first-line therapeutic options such as doxorubicin or oxaliplatin.[85, 86]

Autologous (prepared with isolated tumor cells from the patient and later administered to the same one) and allogeneic vaccines (prepared with isolated tumor cells from one patient and later administered to another)[87] have the potential for their treatment. Nevertheless, they must overcome obstacles due to the tumor progress, such as tolerance, where the immune system does not distinguish the own from the strange. This problem includes lymphocyte anergy and lymphocytes' inability to respond (very low antigen concentrations). Besides, there are issues related to the weak nature of antigens and the activation of evasion

mechanisms, allowing tumor cells to bypass immune checkpoints and inhibiting T cell responses.[88, 89, 90]

In one approach, the PolyPEPI1018 CRC vaccine underwent a phase I/II, open-label, single-arm, multicenter investigation to evaluate the tolerability, safety, efficacy, and immunogenicity in a subcutaneous injection. The vaccine contained six synthetic peptides mixed with Montanide™, a mineral oil-based adjuvant used in immunotherapeutic trials of prophylactic vaccines, capable of increasing humoral and Th1 responses. The peptides were selected to induce T lymphocyte responses against 12 dominant epitopes from seven cancer testis antigens, the most observed in this illness. They were enhanced to produce specific T cell responses against long-lasting colorectal cancer.[91, 92, 93]

Another vaccine established consists of locally injecting a cytotoxic drug-inducing ICD, generating immunogenic apoptotic fragments and cells. This drug is co-administered with CpG (toll-like receptor 9 or TLR9 receptor agonist) adjuvant, which forms a complex with apoptotic bodies. These complexes have shown *in vitro* transfers to macrophages. When CpG was administered in conjunction with doxorubicin in a preclinical investigation with animals, there was a significant effect in decreasing tumor size and increasing the recruitment of helper (CD4+) and CD8+ T cells.[86]

In addition, there is evidence that some parasitic infections induce antitumor activity in humans and experimental animals (negative correlation between certain parasitic infections prevalence and cancer). Furthermore, the survival rate of cancer patients infected with parasites was higher than that of uninfected ones.[88]

However, its employment to induce anticancer activity is restricted by live parasites' virulence and their morbidity, and the mechanisms by which this happens are controversial. They are supported by some theories proposing an increase in acquired or innate immunity, angiogenesis induction, and an improvement in cell apoptosis and common antigens' presentation.[88]

A preclinical study was conducted to investigate the efficacy of autoclaved parasitic antigens (*Schistosoma mansoni* and *Trichinella spiralis*) through an animal model. The results indicated that *S. mansoni* antigens had a protective effect, while those of *T. spiralis* did not. It was also speculated that glycosylated antigens from *S. mansoni* could elicit an adaptive

immune response with cross-reactivity, effective in complicated colon tumorigenesis processes.[88]

In another approach, OncoVax, an autologous tumor-cell vaccine with Bacillus Calmette-Guérin (BCG) adjuvant, improved progression-free survival in colon cancer patients in a phase III study. The clinical benefit was limited to patients with less advanced illnesses (stage II disease).[94]

Oncologic virus

Viruses are immunogenic. Their genetic materials can be designed to incorporate any gene and express it in the host. Oncolytic viruses include a broad group with the potential for cancer treatment. Several recombinant types can infect and express a gene implanted in immune cells, such as dendritic cells. This way, there is a more effective tumor antigens presentation to the immune system, giving a directed response of CD8+ T lymphocytes to tumor cells.[95, 96] Many have been modified to improve their selectivity for tumor cells and potency, amplifying the dose administered by viral replication.[95, 97] Preclinical and clinical studies have indicated the synergy of this therapy with chemotherapy and radiotherapy.[97]

Reoviruses are used for colorectal cancer. They have a double-stranded RNA genome that specifically targets and replicates in transformed cells with an activated Ras signaling pathway[96] Ras proteins function as GTPases, shifting among the guanosine triphosphate (GTP)-bound active and the guanosine diphosphate (GDP)-bound inactive form. They are regulated by guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively.[98]

Many proteins of Ras-activated pathways possess serine and threonine kinase activity. Raf-1 and mitogen-activated protein kinases (MAPKs) are indispensable in cellular processes, for example, cell cycle regulation, apoptosis, proliferation, migration, and differentiation. Likewise, phosphatidylinositol-3-kinase (PI3K) is essential for suppressing apoptosis. Ras activation in the MAPK and PI3K signaling pathways regulates diverse cellular processes, including survival, cell cycle progression, transcription, and migration. Activated Ras oncogenes have been found in different forms of human cancer, such as of the lung, pancreas, and colon epithelial carcinomas.[98, 99]

A phase I clinical study is currently being carried out using reovirus as a vector in combination with other drugs to induce a lytic immune response on solid tumors. The product is registered as Reolysin®. The reovirus specificity for Ras-transformed cells (exhibiting morphological changes, abnormal growth, and alterations in cell adhesions) and the relatively non-pathogenic nature of the virus in humans make it an attractive candidate in colorectal cancer therapy.[99, 100]

Other oncolytic viruses (e.g., the vaccinia virus) can spread systemically in the bloodstream, facilitating metastases treatment, as proved in preclinical experiments. Oncolytic adenoviruses were among the first viruses to have clinical studies. An adenovirus, Δ24RGD(DNX2401), whose target is an integrin, has been investigated in these studies.[95] Integrins are transmembrane glycoprotein receptors that mediate the attachment and migration of cells by recognizing variable extracellular matrix molecules. They can play a relevant role in tumor angiogenesis by mediating tumor interaction with endothelial cells.[101] Yet, despite demonstrating safety in these studies, they gave limited clinical efficacy as monotherapy. Consequently, the possibility of increasing its potency is explored, defined as the virus's ability to replicate, cause cell lysis, and spread.[97]

Chemoimmunotherapy

It consists of immune response modifiers with chemotherapy to treat cancers resistant to standard first-line therapies.[102, 103] IL-2 is responsible for the growth and activation of B, T, and Natural Killer (NK) cells. Thus, it can promote activation-induced cell death and self-reactive T cell elimination.[104] For its part, INF-α has antitumor effects in many malignant tumors, showing antiproliferative capacity and immunomodulatory activity to a certain extent. Distinct B cell malignancies are sensitive to this interferon.[105] Furthermore, current data support its ability to prolong remission in patients with a low tumor burden and even improve survival.

Traditionally, cytotoxic chemotherapeutic agents have immunosuppressive side effects, diminishing antitumor immunity. Nonetheless, there is evidence that they can change the tumor and enhance the immune response against the tumor.[102, 103]

Doxorubicin is a drug from the anthracyclines group commonly utilized to treat patients with solid neoplasms.[106, 107] Positive results have been obtained when ICD immunostimulants are

used in conjunction with doxorubicin, decreasing tumor size and increasing CD8+ T lymphocyte count.[86]

Tumor-associated macrophages (TAMs) can adopt an M1 (classically activated) and M2 (alternatively activated) phenotype. M2 secrete pro-tumor cytokines that induce chemoresistance, such as tumor growth factor beta (TGF-β) and IL-10. They promote an anti-inflammatory state, being inhibitors of pro-inflammatory cytokines. Likewise, they are involved in cellular processes such as hematopoiesis, proliferation, angiogenesis, differentiation, migration, and cellular apoptosis. Moreover, macrophages associated with releasing crucial mediators of the M1 phenotype, such as IL1-β, IFN-γ, and tumor necrosis factor alpha (TNF-α), are related to cytotoxicity and promotion of a pro-inflammatory state. Along with this, they produce more cytokines that improve the efficacy of chemotherapeutic agents and induce tumor regression.[70, 108, 109, 110]

Another treatment investigated through *in vitro* assays involves the combination of attenuated microorganisms such as *Salmonella*-laden temperature-sensitive liposomes (thermobots) with high intensity focused ultrasound (HIFU) heating (between 40 and 42 °C). It can provoke macrophage-related immune changes, synergistically enhancing colon cancer chemotherapy.[111]

Salmonella, particularly *S. typhimurium* (YS-1646), shows high chemotaxis towards serine, ribose, and aspartate, produced by quiescent cancer cells and hypoxic cells within benign and metastatic tumors.[111, 112, 113] In addition, *S. typhimurium* presents membrane lipopolysaccharides (LPS), capable of inducing the secretion of pro-inflammatory cytokines, nitric oxide, and eicosanoids. As a complement, it is loaded with low-temperature sensitive liposomes (LTSLs).[111] LTSLs contain lysophosphatidylcholines, which undergo a structural phase change and release doxorubicin in tumors by heating with HIFU.[111, 114, 115, 116] This way, tumor targeting, localization, and the maximum tolerated dose of doxorubicin are improved without inducing severe systemic toxicity. The LPS membrane is a classic activator of M1 macrophages, helping to overwhelm chemoresistance and the tumor's immunosuppressive microenvironment to enhance this therapy.[111, 117]

CONCLUSIONS

Colorectal cancer is a pathology with an elevated incidence and mortality worldwide. Traditional treatments (surgery, chemotherapy, and radiotherapy) have been widely used, despite many adverse effects associated with their utilization. Plus, depending on the tumor stage, the results are not promising. From this situation, different biological therapies have been made to increase the possible options for its treatment. Monoclonal antibodies, specifically bevacizumab and cetuximab (passive immunotherapy), ipilimumab, pembrolizumab, nivolumab, durvalumab, atezolizumab, and avelumab (active immunotherapy), vaccines, and oncologic viruses are considered relevant therapies for this type of cancer since they have demonstrated a better outcome from the subjects involved in diverse preclinical and clinical trials. Conjointly, strategies have been developed through chemoimmunotherapy, where specific drugs are combined to improve the therapeutic benefits.

Though, there is still much work to be done. Efforts should be directed towards elucidating the mechanisms associated with the therapeutic targets of this disorder. This way, augmenting the response against this cancer will be possible, improving the patient's quality of life.

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