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Integrating *in situ* vaccination with checkpoint blockade therapy: a novel approach to enhance anti-tumour immunity in colorectal cancer

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Highlights:

- Investigates the use of peptide-functionalized PLGA nanoparticles in the treatment of colorectal cancer.
- Examines the potential of in-situ vaccination using dendritic cell therapy in colorectal cancer.
- Evaluates the synergy between nanomedicine and immunotherapy in colorectal cancer treatment.

Abstract: Colorectal cancer (CRC) remains a challenging disease due to its high diversity and complex immune escape mechanisms. The anticipated incidence of colorectal cancer is expected to reach 3,200,000 new cases per year, indicating a 63% increase, with an estimated 1,600,000 deaths occurring annually, which signifies a 73% rise by the year 2040. In contrast to current treatment modalities, such as chemotherapy, radiotherapy, and surgical interventions, immunotherapy has significantly enhanced both survival rates and overall well-being for patients diagnosed with CRC. One of the new immunotherapeutic options that shows promise in colorectal cancer treatment is immune checkpoint inhibitors (ICIs). By removing the immune system's inhibition, ICIs allow functioning cytotoxic T cells to identify and eradicate cancerous cells. The primary issue with checkpoint inhibition is the rise in autoimmune adverse events that limit treatment. In situ vaccination (ISV) also offers a promising strategy to enhance anti-tumour immunity by delivering tumour-specific antigens directly to the tumour microenvironment. In situ vaccination, facilitated by dendritic cells, promotes robust T-cell priming and memory formation within the tumour microenvironment. This review discusses the prospects of combining ISV with checkpoint inhibitors,

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which can enhance immune cell function. This dual approach may lead to a more potent and durable anti-tumour effect with minimal adverse events, ultimately contributing to significant improvements in tumour regression, overall survival, and the overall well-being of individuals diagnosed with CRC.

Keywords: colorectal cancer; immunotherapy; immune checkpoint inhibitors; *in situ* vaccination; combination therapy; therapeutic peptides; PD-1/PD-L1 inhibitors; dendritic cells

1. Introduction

Colorectal cancer (CRC) is a malignancy that affects the colon (large intestine) or rectum and is one of the most prevalent cancerous tumors, influenced by multiple contributing factors [1]. Globally, CRC ranks as the second leading cause of cancer-related deaths (9.2%) and the third most commonly diagnosed cancer (6.1%) [2]. Often, patients with CRC exhibit no conventional clinical symptoms or present only nonspecific signs in the early stages. This lack of early indicators contributes to delayed diagnoses, even though early detection can significantly improve patient outcomes [3]. CRC is characterized by its aggressive behaviour and high lethality, with metastasis being the primary cause of mortality. Each year, the incidence and mortality associated with CRC continue to rise [4]. Notably, CRC is emerging as a significant health challenge in sub-Saharan African (SSA) nations [5]. It is the fifth most commonly diagnosed cancer in SSA and the third in South Africa (SA). Many low- and middle-income countries (LMICs) lack awareness, screening programs, and diagnostic infrastructure, leading to delayed presentations—especially among SSA and SA populations. Late diagnosis is associated with poor prognosis and increased mortality rates [5].

The global burden of CRC is projected to escalate significantly. By 2030, CRC-related deaths are expected to increase by 60%, with over 2.2 million new cases and 1.1 million deaths. By 2040, the incidence is anticipated to reach 3.2 million new cases (Figure 1). Mortality rates from colon and rectal cancer are projected to rise by 60.0% and 71.5%, respectively, by 2035 [6]. Notable global disparities exist in CRC incidence and mortality, influenced by gender, age, ethnicity, and socioeconomic factors [7]. In South Africa, CRC rates are rising rapidly, with mortality rates reported at 7.9 per 100,000 population. The prevalence of hereditary colorectal cancer in this region is reportedly 3 to 5 times higher than in high-income countries [8]. In SSA, the incidence rate is 4.04 cases per 100,000, with higher prevalence in men (4.38) than in women (3.69) [9].

Colorectal tumours generally develop gradually through genetic, morphological, and histological changes. These malignancies differ significantly in clinical presentation and genetic and molecular profiles, with diverse etiologies and risk factors [10]. CRC development is influenced by behavioural, environmental, and genetic factors. Age is a critical determinant, with the risk increasing sharply after age 45 and peaking in individuals over 80, though a growing number of cases are being diagnosed in adolescents [11]. Early-onset CRC (EOCRC) is increasing among young Black Africans in Southern Africa. While the exact etiology of CRC remains incompletely understood, genetic predisposition plays a role in approximately 20% of cases, particularly among first-degree relatives of affected individuals [12]. Syndromes such as Familial Adenomatous Polyposis (FAP) and mutations in Mismatch Repair Genes (MMR) are known to predispose individuals to inherited CRC. Diets high in fat and animal protein and low in fibre have also been associated with increased CRC risk due to enhanced production of carcinogens in the gut [13]. Non-cancerous conditions such as colorectal polyps, adenomas, and

inflammatory bowel diseases like ulcerative colitis also increase cancer risk. Additional factors include exposure to carcinogens, physical inactivity, obesity, and prior pelvic radiation therapy [14].

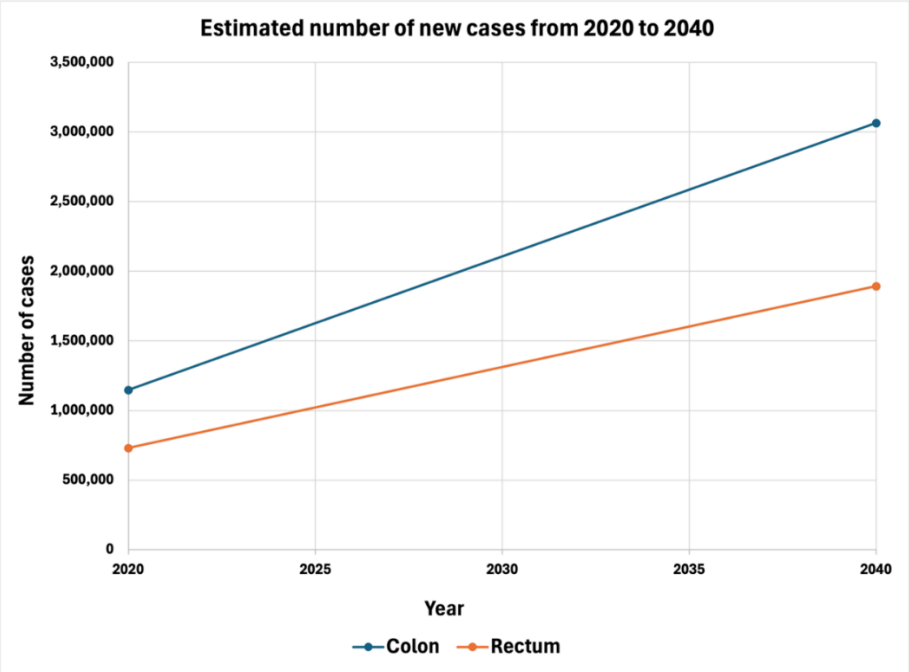

Figure 1. A graph illustrating the projected global trend of new colorectal cancer cases from 2020 to 2040. The blue line represents colon cancer, and the orange line represents rectum cancer [2].

Conventional treatment modalities—surgery, chemotherapy, and radiotherapy—remain the mainstay of CRC management. More recently, immunotherapy has emerged as a transformative approach, significantly improving outcomes for some CRC patients [15]. However, these therapies show variable success, with limited efficacy in many individuals [15]. The immune system plays a crucial role in CRC pathogenesis. Within the CRC tumor microenvironment, innate immune cells such as macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), and bone marrow-derived suppressor cells orchestrate tumor progression by releasing cytokines and chemokines [16]. To initiate a robust adaptive immune response, tumor cells must present immunogenic antigens ("signal 1") via the major histocompatibility complex (MHC) directly or through DCs. DCs, activated by tumor-associated damage ("signal 0"), enable cross-presentation of tumor antigens. Co-stimulatory molecules ("signal 2") further enhance cytotoxic T-cell activation [16].

Although monotherapy is commonly used, combinatorial treatments that target different tumor pathways are proving more effective [17]. Among these, combining Checkpoint Blockade Therapy (CBT) with In Situ Vaccination (ISV) has shown promise. CBT requires functional T cells to overcome immunosuppressive tumor signals, but its effectiveness may be limited by insufficient tumor-specific T cells [18]. ISV enhances the quantity and quality of these T cells by stimulating antigen presentation and immune priming [19]. Studies in murine models suggest that the synergy between CBT and ISV could lead to more effective antitumor responses while minimizing autoimmune side effects [18].

Recent advancements in nanotechnology have led to the development of theranostic systems—nanoparticulate platforms such as liposomes, micelles, dendrimers, and nanocapsules that integrate therapeutic and diagnostic functions. These systems are designed to improve diagnostic

accuracy and enable precise drug delivery while minimizing side effects. Theranostic nanoparticles are particularly useful in cancer management for real-time monitoring and early detection of therapeutic responses. Their modifiable physicochemical properties make them ideal for multifunctional applications in oncology [20]. Nanomedicine enables the creation of delivery systems that are safer, more efficient, and capable of enhancing the specificity of drug accumulation in tumor tissues through passive and active targeting mechanisms [21].

Innovations in drug delivery focus on optimizing efficacy through controlled and targeted multi-drug release. These platforms improve bioavailability, reduce degradation, and minimize toxicity. While synthetic polymers like PLGA (FDA-approved) offer favorable pharmacokinetic properties, natural polymers such as alginate, chitosan, and gelatin are gaining preference for their superior biocompatibility [22]. The architecture of delivery systems—from films to nanoparticles—plays a pivotal role in determining clinical success. Biodegradable nanoparticles are now widely used in imaging, drug delivery, cancer therapy, and diagnostic applications due to their high surface area-to-volume ratio and customizable characteristics [23]. Polymeric nanoparticles, in particular, have shown immense promise in enhancing therapeutic outcomes and drug stability. Their ability to penetrate tissues, accumulate in target sites, and be tailored with ligands for specific cell targeting makes them ideal for overcoming drug resistance and enabling combination therapy.

Silk fibroin (SF), a natural protein polymer, possesses excellent properties for drug delivery applications. It effectively transports a wide range of therapeutic agents, including small molecules and biologics [22]. Furthermore, quantum dots (QDs), such as cadmium telluride (CdTe), have demonstrated precise imaging and drug delivery capabilities. For instance, QDs conjugated with AS1411 aptamers successfully targeted glioblastoma cells and facilitated simultaneous imaging and therapy [24]. Similarly, Doxorubicin-Chondroitin Sulfate–Chitosan (Dox-ChS-CS) nanoparticles have demonstrated improved tumour localisation and therapeutic efficacy, underscoring the utility of engineered nanoparticles with surface ligands for targeted cancer therapy and image-guided drug delivery [25].

This review explores the potential of combining *in situ* vaccination (ISV) with immune checkpoint inhibitors to amplify immune cell activation. This synergistic strategy holds promise for producing a more robust and sustained anti-tumour response with reduced adverse effects, ultimately leading to improved tumour regression, extended overall survival, and enhanced quality of life for individuals affected by colorectal cancer (CRC).

2. Current colorectal cancer treatment approaches

Various treatment options exist based on the patient's attributes and the tumour's stage. These include, among other treatments, radiation, chemotherapy, immunotherapy, molecular targeted therapy, and surgery [26]. The selection of a treatment or a combination of treatments is influenced by multiple factors, such as the tumour's location, classification, stage, grade, and the overall well-being of the individual [26]. These treatment modalities are not tumour-specific, they can also affect healthy cells near the tumour or in other parts of the body.

Generally, surgical treatments often require extended hospital stays and may result in the permanent loss of essential structures, leading to decreased quality of life. Meanwhile, chemotherapy resistance is a common challenge, as some tumour cells may evade the drugs or become resistant over time, making them more aggressive and prone to spreading to distant areas. Radiation therapy can effectively target

cancerous cells; however, it may also damage the adjacent healthy tissues located within the treatment zone [27]. These widely utilized therapies are frequently linked to causing resistance to medicines and the formation of distant metastasis and may present several difficulties with drug delivery and their adverse events [28]. More than 20% of CRC patients are ineligible for surgery at the time of diagnosis because of liver metastases, even though surgery is the sole curative treatment for localized disease [29].

The goal for patients with unresectable tumours or those who are not surgically tolerant is to minimize tumour size and prevent additional tumour growth and spread. The most effective methods for managing these patients' conditions are chemotherapy and radiation therapy. Notably, to minimize and stabilize the tumour as much as possible, chemotherapy or radiation therapy may occasionally be administered either before or following surgery as a preoperative or postoperative treatment [30,31]. About 50% of CRC patients eventually develop liver metastases, which are a major factor contributing to the disease's lethality even though adjuvant chemotherapy, neoadjuvant radiation therapy, and surgery have improved patient outcomes thus far [32]. To overcome these limitations, additional therapy modalities and conventional treatments to enhance therapeutic results are required.

2.1. Surgery

Currently, surgery is the primary approach to treatment for the preliminary phase of CRC, followed by radiotherapy and chemotherapy. The tumour's location and stage determine the type of resection, degree of lymphadenectomy, and particular procedures. According to studies, individuals diagnosed with EOCRC who undergo surgical intervention exhibit a five-year survival rate exceeding 90% [33]. The primary aim of surgical intervention for colon cancer is to remove the entire tumour while maintaining ideal intestinal function and reducing side effects. Palliative and radical surgery are the two primary surgical techniques. The shortfalls of CRC surgery include metastases at the port site, the formation of adhesions leading to small bowel obstruction, anastomotic leakage, thrombosis, ileus, colonic ischemia, infections, as well as dysfunctions related to the cardiovascular and urinary systems [32].

2.2. Radiotherapy

Radiotherapy is primarily used to treat rectal cancer, contributing to improved local control, enhanced quality of life, and increased survival rates. The main radiotherapy approaches for rectal cancer include pre-operative radiotherapy, pre-operative concurrent chemoradiotherapy, and post-operative concurrent chemoradiotherapy combined with adjuvant chemotherapy [34]. The side effects of radiation therapy vary depending on the area of the body being treated. Different types of cells and tissues respond to radiation in various ways, with rapidly dividing cells—such as those in the skin, the lining of the mouth and gastrointestinal tract, and bone marrow blood cells is the most affected [35]. These side effects typically occur during treatment and tend to improve within a few weeks after it ends. However, some side effects may persist even after treatment is completed. If radiation doses are high enough, some cells may be unable to repair themselves, leading to long-lasting or permanent late effects. Overall, the adverse effects associated with radiation therapy are impacted by a variety of variables, such as the type of radiation administered, the particular region of the body undergoing treatment, the dosage and radiation timing, including the overall well-being of the patient [27].

2.3. Chemotherapy

Chemotherapy for CRC includes neoadjuvant chemotherapy, adjuvant chemotherapy after surgery, and palliative chemotherapy. Neoadjuvant chemotherapy, usually in combination with radiotherapy, shrinks the tumour, makes surgery possible, improves quality of life, and reduces the risk of recurrence. Adjuvant chemotherapy targets any remaining tumour cells after surgery to enhance the procedure's effectiveness. Palliative chemotherapy focuses on improving the overall well-being and extending the survival period in individuals with advanced CRC [36]. Chemotherapeutic medicines' toxicity and ability to harm nearby healthy cells are their main disadvantage. The most frequently observed adverse effects include bleeding, gastritis, diminished appetite resulting from the impairment of gastrointestinal tract cells, discomfort, and challenges in swallowing caused by sore throat and mouth ulcers (Table 1). Additionally, loss of hair and microbial infections may arise as a consequence of compromised immune function. Furthermore, the main cause of chemotherapy failure is cancer cells' potential to develop resistance to antitumour medications over time [37].

Table 1. List of approved chemotherapeutic agents for the treatment of CRC and their side effects.

S/N	Chemotherapeutic agents	Side effects	References
1	5-Fluorouracil (5-FU)	Hand-foot syndrome, Diarrhoea, Myelosuppression, Oral mucositis, Emesis/Nausea, Erythema, Ocular toxicity, Cardiotoxicity, Small bowel toxicity, alopecia.	[37]
2	Raltitrexed	Myelosuppression, Oral mucositis, Diarrhoea, Emesis/Nausea,	[37]
3	Capecitabine (Xeloda)	Diarrhoea, hand-foot syndrome	[38]
4	Irinotecan (Camptosar)	Acute diarrhoea/cholinergic syndrome Emesis/Nausea, Myelosuppression, delayed diarrhoea.	[37]
5	Oxaliplatin (Eloxatin)	Myelosuppression, diarrhoea, Nausea/Emesis, Neurotoxicity.	[37]

2.4. Immunotherapy

Immunotherapy has become a pivotal development in the therapeutic landscape of cancer care, quickly gaining recognition as a primary therapeutic approach for various solid tumours, including certain forms of colorectal cancer [39]. Immunotherapy has generated a lot of optimism over the last 10 years, partly because it has been shown to reliably increase survival rate in a small number of individuals with historically refractory tumours that would otherwise have a dismal prognosis [40]. Human CRC cells express many different tumour-associated antigens (TAAs), including carcinoembryonic antigen (CEA), Wilms tumour gene 1 (WT1), mucin 1 (MUC1), melanoma-associated antigen gene (MAGE), ring finger protein 43 (RNF43), outer mitochondrial membrane (TOMM34) [41]. In recent years, it appears that patients, particularly those with high mutation rates in CRC, benefit from therapeutic approaches that use the host immune system against the disease. Immunotherapy has emerged as a validated clinical strategy in the management of cancer. By harnessing the patient's immune system to fight malignancies, this approach has led to the development of innovative therapeutic modalities, including chimeric antigen receptor T-cell therapy, ICIs, cytokines, immunomodulators, oncolytic viruses, and cancer vaccines, which have yielded unprecedented clinical outcomes [15]. As of now, tiny molecules and

antibody treatment are a significant portion of cancer immunotherapies under clinical research, including all authorized cancer immunotherapies.

One important factor in stopping the growth of tumour cells is the immune system. Colorectal cancer can be linked to several pathogens that contribute to the onset and advancement of the disease. These microorganisms foster conditions conducive to carcinogenesis by mechanisms such as inducing chronic inflammation, directly damaging DNA, or altering the host's immune system and gut microbiota. *Clostridioides difficile* is identified as a possible infectious carcinogen for colorectal cancer because it can lead to intestinal dysbiosis and persistent infections, which may create an environment that promotes carcinogenesis [42].

2.4.1. Cancer immunity cycle

A key element of the immune system is its ability to avoid attacking the body's own healthy cells. The immune system is made up of both innate and adaptive immunity, which function together to regulate the host's immune response. Innate immunity serves as the first line of defence against external invaders, acting rapidly and in a non-specific way. In contrast, adaptive immunity activates a secondary defence system that generates a prolonged immune response tailored to specific antigens. Through tumour immune surveillance, the immune system can also mount an attack against altered or damaged host cells, such as cancer and virus cells. Dendritic cells (DCs) and macrophages, classified as professional antigen-presenting cells (APCs), are specialized innate immune cells that are specifically adapted to present antigens. To optimally initiate the adaptive immune response mediated by T cells, APCs are capable of presenting tumour-associated antigens (TAAs) in conjunction with major histocompatibility complexes (MHCs). Additionally, they provide necessary activation signals via co-stimulatory receptors, such as Toll-like receptors [43].

Human cancers possess numerous somatic gene mutations and epigenetically modified genes, which can lead to the production of proteins that may be identified as external antigens. Although an intrinsic immune response to cancer has been documented in both preclinical studies and clinical patients, such responses frequently lack efficacy. This inefficiency arises because tumours promote tolerance among tumour-specific T cells and express ligands that engage inhibitory receptors, thereby suppressing the functions of T-cells inside the tumour microenvironment. Several actions must be taken and allowed to continue and grow for an anticancer immune response to effectively kill the cancer cells. These phases are called the Cancer Immunity Cycle (CIC) (Figure 2). The CIC is comprised of seven distinct phases. Initially, the process begins with the release of antigens resulting from the demise of tumour cells. The second phase involves the recognition and processing of these antigens. In the third phase, APCs activate and prime naive T-cells. The fourth phase sees the migration of these primed T-cells towards the tumour site. Subsequently, in the fifth phase, T-cells infiltrate the tumour tissue. The sixth phase is characterized by the recruitment of T-cells, which then identify the tumour cells. Finally, in the seventh phase, cytotoxic cells execute the destruction of the cancer cells [44].

Immune cells can function as potent inhibitors of tumour activation and progression. Immune cells play a dual role in the context of cancer, acting both as effective suppressors of tumour initiation and progression and as facilitators of tumour growth, invasion into adjacent tissues, and distant metastasis. Despite their potential, the immune system has inherent limitations in its ability to eliminate cancer. This challenge arises from several factors, notably the similarity between TAAs and normal host tissues,

which can lead to immune evasion. Previous research has indicated that tumour antigens characterized by high specificity can provoke a more robust immune response aimed at tumour rejection [45].

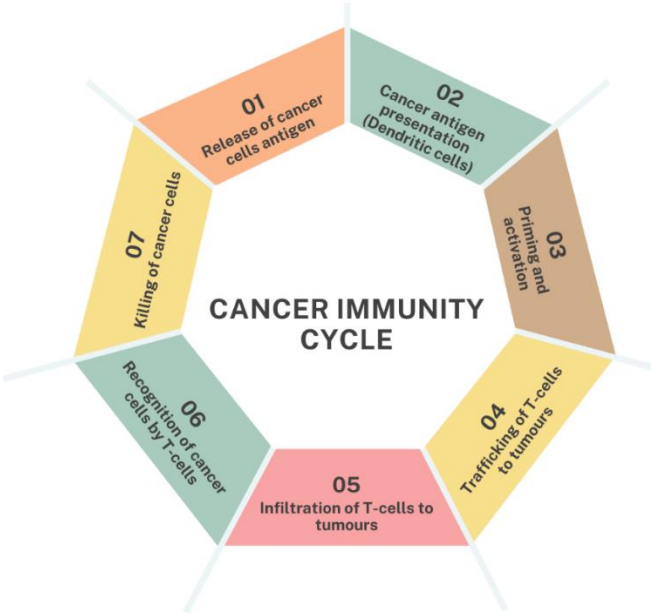

Figure 2. Cancer immunity cycle [46].

3. Checkpoint blockade therapy: overview and status in colorectal cancer treatment

A highly promising approach to enhance therapeutic anti-tumour immunity involves the inhibition of immune checkpoints. Immune checkpoint mechanisms are used by tumour cells to reduce T cell activation and prevent tumour-specific T lymphocytes from attacking them [47]. The surface receptors of immune checkpoint molecules are vital for regulating the immune system. The main role of these checkpoints is to carefully uphold self-tolerance and modulate both the strength and duration of immune responses in peripheral tissues, all while minimizing unintended damage to surrounding tissues.

ICIs are among the emerging therapeutic alternatives promising in cancer treatment. ICIs competitively attach to checkpoint molecules to prevent the immune system from being suppressed by checkpoints [48]. Immune checkpoints are immune system-negative regulators that function as a buffer for self-defence, maintaining self-tolerance, averting autoimmune reactions, and protecting tissues from immune-related harm [27]. ICIs function by alleviating the suppression of the immune system enabling functional cytotoxic T-cells to target and destroy malignant cells. ICIs have demonstrated effectiveness in treating various cancers that are otherwise difficult to manage [15]. They have effectively treated lung cancer, melanoma, cancer of the bladder, renal cell carcinoma (RCC), and CRC [49]. When treating CRC, ICIs have achieved unmatched effectiveness. Cancer metastases, resistance to medicines, undesirable adverse reactions, and inadequate rate of response continue to impede the continued use of ICI therapy for CRC [50]. Contrary to other traditional treatments, immune cells are usually the focus of ICIs rather than tumour cells. The primary difficulty associated with checkpoint inhibition lies in the heightened frequency of autoimmune adverse events that can limit the effectiveness of therapy.

The majority of patients receiving treatment with ICIs have experienced immune-related adverse events (IAEs) to varied degrees, despite the ICI's encouraging effectiveness. The adverse events that are frequently reported include gastrointestinal issues, endocrine abnormalities, and rash or pruritus [51]. Myocarditis

linked to ICIs typically presents with arrhythmias and may occur alongside conditions like myocarditis and myasthenia gravis. This combination is often associated with severe disease and a poor prognosis [52].

To dampen the immune system and trigger energy, several immunotherapeutic agents use tumour cells to take advantage of major histocompatibility complex (MHC)-T-cell receptor (TCR)-dependent signaling pathways. These signaling pathways include programmed cell death 1 (PD-1), PD-1 ligand (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), indoleamine 2, 3-dioxygenase, and lymphocyte-activation gene [53]. Immune checkpoint receptors serve an essential function in regulating the immune response. while preventing autoimmunity. These receptors facilitate the release of cytokines by T-cells, functioning as both co-inhibitory and co-stimulatory agents. The activation of a co-inhibitory immune checkpoint against tumour antigens serves to diminish the immune response directed at cancer cells [41]. The most effective and widely used checkpoint inhibitors in clinical practice are those for CTLA-4, PD-1, and PD-L1 receptors [54].

3.1. PD-1/PD-L1 inhibitors

In 1992, PD-1 was discovered as a potential modulator of apoptosis; however, additional research revealed that it may also serve a comparable function to CTLA-4 in regulating excessive activation of the immune system [55]. These targeting checkpoints have garnered much attention lately and can produce a long-lasting immune response in various cancer types [47]. Two significant negative immune regulators are PD-L1 and PD-1. PD-1 is a checkpoint protein that is a member of the CD28 family and functions as a suppressive receptor on T cells. While it is typically not present on T cells, its expression increases in response to antigen stimulation and the release of cytokines during T cell activation. Additionally, PD-1 is expressed in B cells, monocytes, and dendritic cells, where it plays a role in modulating various immune responses. PD-L1, a type 1 transmembrane glycoprotein belonging to the B7 ligand family, is found on activated T and B cells as well as certain non-hematopoietic cells. Its presence is crucial for regulating T cell activity, particularly in dendritic cells and other APCs. When tumour cells recognize the PD-1 protein on T cells, they respond by upregulating PD-L1. The binding of PD-1 to PD-L1 on tumour cells induces apoptosis in T cells, thereby suppressing the response of the immune system against the tumour [56]. PD-1, a type 1 transmembrane glycoprotein belonging to the immunoglobulin superfamily, shares 20% amino acid identity with CTLA4 and 15% with CD28 [57]. PD-1, a homolog of CD28, has since been shown to be an essential controller of adaptive immune responses and is mainly associated with suppressive immune signals [58]. The U.S. Food and Drug Administration (FDA) has approved several PD-1/PD-L1 inhibitors for treating various cancers (Table 2).

In many malignancies, the expression of PD-1 is notably elevated in tumour-infiltrating lymphocytes (TILs), which encompass both CD4+ and CD8+ T cells. This heightened expression of the protein is linked with tumour progression and a lower overall rate of survival among individuals with cancer [59]. Research on the inhibition mediated by PD-1/PD-L1 has demonstrated that this inhibitory pathway is a vital component of the tumour immune evasion mechanism. This mechanism hinders the growth and maturation of naive T cells and adds to the fatigue and anergy of T cells [59]. By binding to the PD-1 receptor on the surface of T cells in a transmembrane form, PD-L1 expressed on the surface of tumour cells mediates immune escape of tumour cells and resistance to traditional chemotherapeutic and radiation treatment by delivering regulatory signals to the cells and preventing the initiation and multiplication of T cell. Numerous studies have shown that the suppression of the PD-1/PD-L1 pathway represents an effective

approach in treating malignancy. This evidence provides a solid scientific basis for the exploration of ICIs in clinical oncology. Targeting the PD-L1/PD-1 checkpoint as a cancer immunotherapy approach has demonstrated long-lasting clinical efficacy across a range of cancer types, emphasizing the important role that PD-L1/PD-1 plays in suppressing T cell-mediated anticancer immune responses [60].

Table 2. The benefits and drawbacks of various therapies for colorectal cancer.

Treatment option	Benefits	Drawbacks	References
Surgery	Laparoscopic surgery is both secure and efficient. Laparoscopic surgery costs much less than open surgery and has a reduced likelihood of infection of the surgical site. Robotic surgery offers better skill, accuracy, and visual acuity, more accurate dissection of lymph node as well as intracorporeal anastomoses. Navigation surgery facilitates continuous surveillance of blood circulation in the colon and rectum. Navigation surgery is beneficial for the mapping of lymph node.	Laparoscopic surgery is associated with reduced survival rates in some subgroups. Robotic surgery is associated with extended surgical durations, increased expenses, and a more challenging learning process. Transanal total mesorectal excision employs a technique that lacks standardization and carries various risks. These risks include the potential for bacterial or cancer cell contamination of adjacent tissues, injury to the urethra, damage to the bowel, anastomotic leaks, urinary complications, and bleeding.	[61]
Radiotherapy	As neoadjuvant therapy, it reduces tumour size, thereby making surgical excision more manageable, particularly in cases of rectal cancer. As adjuvant therapy, it eliminates cancer cells to lower the likelihood of recurrence. It reduces associated symptoms and control malignancy in patients who are not suitable candidates for surgery. Can be used to stop or alleviate symptoms associated with advanced colorectal cancer. It is likely to also treat metastatic sites such as the lungs or bones, by temporarily reducing tumour size, although it is unlikely to result in a complete cure of the cancer.	Skin irritation manifesting as redness, blistering, and peeling. Gastrointestinal issues including nausea, diarrhea, irritable bowel movements, and the presence of blood in the stool. Incontinence of the bowel. Irritation of the bowel characterized by burning sensations, discomfort and blood in the urine. Exhaustion. Erectile dysfunction and vaginal itchiness. Tissue damage resulting in scarring, fibrosis, and adhesions.	[61]
Chemotherapy	Can be used in diverse applications. Has the potential to inhibit or reduce the progression of cancer. A wide range of available treatment molecules. Can be utilized in conjunction with other treatments.	Risk of toxicity affecting both cancerous and healthy cells. Multiple adverse effects. Impaired immune function due to damage to immune cells. Variability among individuals. Requires regular monitoring and hospital appointments.	[61]
Immunotherapy	Possesses the capability to treat a variety of cancer types. May lack specificity for cancer types. Could offer enduring protection against cancer	It is influenced by the patient's immune status. A range of side effects may occur. Potential resistance development. Risk of negative immunological reactions.	[61]

The binding of PD-1 to PD-L1 diminishes the immune surveillance conducted by T cells, which can result in T cell apoptosis and a subsequent failure to mount an immune response. This interaction provides cancer cells with a mechanism to escape immune detection by inhibiting the activity of

tumour-infiltrating CD4+/CD8+ T cells (CD4+/CD8+ TILs) and leads to a reduction in key cytokines, including tumour necrosis factor (TNF), interferon-gamma (IFN-γ), and interleukin-2 (IL-2) [62]. Many studies have lately concentrated on the use of nonpeptidic small compounds and peptide-based inhibitors that target PD-1/PDL-1.

3.1.1. Therapeutic peptides for PD-1/PDL-1 blockade

Peptide-based drugs have gained attention in cancer therapy for their ability to target specific cells, penetrate tumour tissues, and stimulate immune responses that boost conventional treatments [63]. The anti-cancer peptide leuprolide, approved by the FDA in 1985 and later in Canada and Europe, has been used to treat cancers like prostate and breast cancer. Leuprolide's applications have expanded over the years, reaching global sales of $2 billion in 2019. Around 80 peptide drugs are approved for clinical use, with over 150 in clinical trials and 400–600 in preclinical stages, signalling continued growth in the peptide therapeutics market [64]. Numerous peptides that act as inhibitors of the PD-L1/PD-1 pathway have been explored for their potential applications in cancer immunotherapy (Table 3).

Table 3. List of approved FDA-approved PD-1/PD-L1 inhibitors.

Drug	Target checkpoint/ligand	Date of Approval	Approved indications	References
Nivolumab	PD-1	March 2015	Stage III-B or IV Squamous NSCLC	[65]
Durvalumab	PD-L1	February 2016	Stage III non-small-cell lung cancer (NSCLC)	[66]
Pembrolizumab	PD-1	October 2016	Stage IV non-squamous and squamous canacer	[67]
Atezolizumab	PD-L1	October 2016	Stage III-B or IV non-squamous and squamous NSCLC	[68]
Avelumab	PD-L1	February 2016	Stage III non-small-cell lung cancer (NSCLC)	[65]
Cemiplimab	PD-1	September 2018	Metastatic cutaneous squamous cell carcinoma	[65]

Peptides are now regarded as one of the primary treatments for tumour suppression, and their use in cancer treatment has increased significantly [69]. Therapeutic peptides overcome the drawbacks of small-molecule medications and antibodies in several ways. Peptides exhibit great specificity and affinity for their intended target, just like antibodies do, making them useful for interfering with protein-protein interactions [54]. Peptides have traditionally been polypeptides with 2 to 50 amino acid residues. They play important functions in human physiology, acting as growth factors, hormones, neurotransmitters, ion channel ligands, and antibacterial agents. Peptides are the middle ground between the small molecules and protein treatment groups. They have advantages over small molecules and proteins in terms of affordability, security, ease of production, less immunogenicity, and improved organ or tumour penetration [70]. Peptides show potential as new anti-cancer drugs in the treatment of colorectal cancer. Peptides can directly target tumour cells without harming healthy cells, which is the basis of targeted therapy [71].

Peptides are valuable in drug delivery systems (DDS) for directing other drugs to specific tissues or cells. Their biocompatibility allows them to be paired with drug carriers to create cancer-targeted delivery vehicles [72]. Peptide-based DDS advancements include carrier systems, targeting methods, controlled release, co-delivery systems, and personalized therapies, all aimed at improving delivery efficiency, targeting accuracy, and reducing side effects. In the field of cancer immunotherapy, peptides are utilized in a variety

of functions (Figure 3). Common carrier systems like nanoparticles, liposomes, and polymer microspheres encapsulate and transport peptide drugs [72]. Peptides confer a targeted approach to cancer treatment within the realm of nanomedicine, thereby minimizing adverse drug reactions and enhancing therapeutic efficacy. Furthermore, these peptides possess the capability to self-assemble, functioning not only as carriers that transport therapeutic agents to cancer cells with precision but also as active agents in cancer treatment through their self-assembled structures. Additionally, peptides play a crucial role in enabling the controlled release of drugs in nanomedicine by responding to specific stimuli [73]. There is no build-up of therapeutic peptides in particular organs, which causes significant organ damage in chemotherapy. The benefits of peptides, in contrast, encompass their ability to penetrate deep tissues, facilitate effective internalization within cells, exhibit reduced toxicity and immune response towards the bone marrow and liver, and allow for straightforward chemical modifications when compared to antibodies [74].

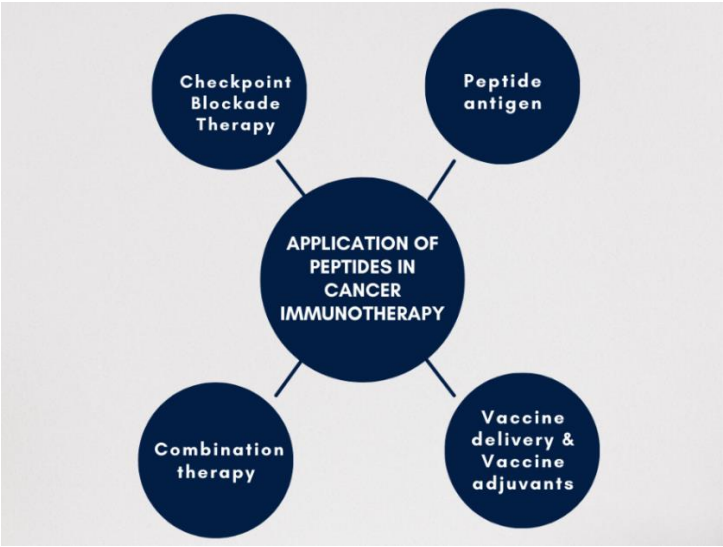

Figure 3. A diagram illustrating the use of peptides in cancer immunotherapy [75]. Adapted with permission from Luthra *et al*. 2021. Reprinted with permission [75]. Copyright 2021 Springer.

Anti-cancer peptides (ACPs) work through mechanisms like directly killing cancer cells, disrupting tumour-supportive environments, inhibiting metastasis, and activating the immune system. Immunotherapy, which harnesses the body's immune system to combat cancer, provides lasting anti-cancer effects, prevents metastasis and recurrence, and has shown significant success. ACPs address challenges in immunotherapy, such as tumour immune evasion, immune tolerance, and resistance, while minimizing side effects like autoimmune issues [76]. Peptide-based vaccines, a promising cancer treatment, offer advantages over chemotherapy, including stability, precise targeting, low toxicity, and ease of preparation, enhancing immune responses and extending patient survival [77].

Altering naturally occurring peptides or their variations to enhance their characteristics, stability, or functionality is known as “peptide engineering”. It can be used to optimize current peptide medications to improve their pharmacokinetic characteristics, boost their bioactivity, or lessen their adverse effects. Peptides can be designed to specifically target oncogenic elements, as demonstrated by the development of proteolysis targeting chimaeras (PROTACs) that focus on inhibiting Forkhead box protein M1 (FOXM1). This strategy seeks to diminish the levels of glucose transporter 1 (GLUT1) and PD-L1 within the framework of cancer treatment [78]. The first reported inhibitor, AUNP-12, which comprises 29

amino acids, was patented in the year 2014. In studies involving animals, AUNP-12 has been proven to significantly impede the growth and migration of tumour cells, exhibiting only a limited range of undesirable effects. Beyond AUNP-12, various peptide-based inhibitors targeting the PD1/PDL1 pathway have been developed and utilized in cancer immunotherapy (Table 4). For instance, a small peptide composed of 7 to 8 amino acids exhibited significant bioactivity in murine models bearing melanoma B16F10 cells, leading to a 64% reduction in lung metastases. Additionally, a cyclopeptide consisting of 7 to 9 amino acids, distinguished by a circular conformation due to an amide bond linking the C and N terminals, was evaluated. In tests measuring Crystal Field Stabilization Energies (CFSE), this cyclic peptide derivative was found to enhance the proliferation of spleen cells in mice with increased PD-L1 expression in human breast cancer cells (MDA-MB-231) and resulted in a 54% decrease in lung metastasis in mice with melanoma B16F10 [79].

Table 4. A compilation of diverse peptides that inhibit PD-L1/PD-1 and are utilized in the field of cancer immunotherapy.

Name of peptide	Sequence	Target	References
PDL1Pep-1, PDL1Pep-2	CLQKTPKQC, CVRARTR	PD-L1	[88]
CLP001, CLP002	HYPERPHANQAS, WHRSYYTWNLNT	PD-L1	[89]
Peptide-57	F(NMeAla)NPHLSWSW(NMeNle)(NMeNle)RCG	PD-L1	[74]
AUNP-12	SNTSESFKFRVTQLAPKAQIKE SNTSESF*	PD-1	[90]
YT-16	YRCMISYGGADYKCIT (cyclic)	PD-1	[91]
nABP284	SRLKEIANSPTQFWRMVARNTLGNGAKQSLNIEHARL	PD-L1	[92]

Tumour-homing peptides facilitate cell damage and apoptosis by binding to specific receptors on tumour cells and delivering therapeutic payloads. Antagonistic peptides inhibit the biological functions of tumour cells by engaging with cell surface receptors, such as PD-L1 and various hormone receptors. Interference peptides, regardless of their incorporation of tumour-homing peptides, are capable of infiltrating cells, binding to intracellular targets, and obstructing the interaction between the target and its associated binding partner [74]. Diverse carriers, including nanoparticles, exosomes, and cells, along with cargoes like cytotoxic peptides, radionuclides, and medications are utilized for the purpose of targeted delivery, leveraging peptides that specifically home in on tumours.

Peptide fragments exhibiting anti-tumour properties, such as IMB-P1 and IMB-P6, which are derived from hPRDXN monoclonal antibodies, have shown a capacity to obstruct the PD-1/PD-L1 interaction. This mechanism results in enhanced efficacy relative to conventional antibody-based immunotherapeutic approaches. IMB-P1-7 are peptides derived from peroxiredoxin-5 (PRDX5), PRDX5's primary function is that of an antioxidant enzyme that keeps peroxide from building up in cells [54]. Peroxiredoxin 5 (PRDX5) is found extensively in the cytoplasm and mitochondria and was discovered by 2D Nano-LC-ESI-LTQ-Orbitrap MS/MS as one key element of goat anti-cancer bioactive peptides (ACBPs). It was further enhanced by diverse expression, and its tumour-fighting activity has been confirmed in both *in vitro* and *in vivo* [70].

The IMB-P1 peptide, with the amino acid sequence AFTPGCSKTHLPGFVEQAEAL (residues 42–62), is located at the α2 position and is the active site. Comparable to PRDX5 *in vivo*, the peptide IMB-P1 with key residue C47 displayed anticancer efficacy. According to a transcriptome study, Peptide IMB-P1 may affect the expression of several genes implicated in cancer and degeneration. In addition, the down-regulation

of genes linked to oxidation is a significant finding. IMB-P1 exhibited comparable anticancer efficacy to intact PRDX5 in CT26 cells, with increased Reactive Oxygen Species (ROS) levels [70].

Functionalizing nanoparticles to increase their targetability and potential in cancer treatment is a common use for peptides. It is not just one kind of nanocarrier that can functionalize nanostructures with peptides; many types of nanoparticles have been altered and functionalized with peptides specifically for applications in cancer therapies [71]. Peptide-functionalized nanostructures are increasingly pursued for biomedical applications due to their remarkable specificity, excellent biocompatibility, and ease of accessibility. These nanostructures are being explored for various purposes, including drug delivery, biological imaging, liquid biopsy, and targeted therapies. Furthermore, they are recognized as promising candidates for novel therapeutic interventions [80]. Since tumour-homing peptides engage directly with cell surface receptors or binding partners, they have been utilized to drive nanoparticles to cancer cells.

Research has indicated that LyP-1-functionalized sodium alginate nanoparticles demonstrated good stability *in vitro* and enabled sustained drug release for cancer treatment. Compared to free nanoparticles, the peptide-functionalized alginate nanoparticles showed elevated plasma concentrations and enhanced tumour suppression due to their targeted delivery capabilities [81].

4. *In-situ* vaccination: overview and current status in colorectal cancer treatment

In situ vaccination (ISV) is one tactic to promote the cancer immunity cycle. *In situ* vaccination is a method that uses tumour antigens found at a tumour location to stimulate tumour-specific adaptive immune responses [82]. ISV aims to enhance dendritic cell activity within the tumour microenvironment to capture and process tumour-derived antigens prior to their transport to immune cells en-route to the draining lymph node [83]. In ISV treatment, an immune-stimulatory agent is introduced directly into one or multiple tumours within a patient. This method counteracts the local immunosuppression caused by the tumours and is intended to provoke a strong reaction of antitumour T-cells. Subsequently, these T-cells disseminate throughout the body, targeting and eliminating cancer cells, including those present in metastases, irrespective of their clinical detectability [84]. The process entails the administration of immunomodulators either directly into the tumour or systemically, which enhances the display of TAAs by APCs. This mechanism facilitates the stimulation of cytotoxic T cells and the formation of both effector and memory CD8+ T cells. For effective antitumour immune responses, several critical elements must be present: i) the presence of TAAs within an immunogenic milieu to stimulate phagocytosis and activate dendritic cells (DCs), ii) proficient antigen presentation accompanied by co-stimulatory signals to engage CD8+ cytotoxic T cells, and iii) a cytotoxic T cell response that can effectively counteract the inhibitory signals emanating from the tumour and its surrounding microenvironment. These components are essential for driving adaptive antitumour responses that exhibit both local and systemic effects [85].

ISV presents a promising therapeutic strategy for various phases of CRC. Research suggests that the activation of this method through oral nanomedicine has the potential to trigger immunogenic cell death in colorectal cancer cells, thereby generating robust vaccine neoantigens at the tumour site [86]. In particular, ISV using K3-SPG has demonstrated strong effectiveness in inhibiting colorectal cancer growth and boosting the antitumour impact of checkpoint inhibitors, suggesting a positive response for different grades of colorectal cancer [87]. The utilization of ISV presents several advantages, including targeted immune-mediated efficacy, a broad systemic immune response, straightforward implementation, swift delivery, safety, cost-effectiveness, and the potential for integration with other immunotherapeutic

approaches, in addition to conventional treatments like radiation, chemotherapy, and surgical interventions. However, the limitations associated with ISV encompass tumour immune evasion, restricted infiltration of immune cells, and the presence of an immunosuppressive microenvironment, among other factors.

Research conducted in preclinical settings revealed that K3-SPG-ISV, a second-generation nanoparticle designed as a Toll-like receptor 9 (TLR9) ligand and administered through intratumoural injection, demonstrated notable anticancer effects in pancreatic ductal adenocarcinoma (PDAC) and colorectal cancer (CRC) models. The tumour growth inhibition achieved with K3-SPG-ISV was more pronounced compared to that observed with K3-ISV or K3-SPG administered intravenously, increased time of survival, and improved the anti-cancer effects of checkpoint inhibitors. In the PDAC model, it is noteworthy that K3-SPG-ISV demonstrated systemic anticancer effects and facilitated the development of immunological memory. Additionally, the integration of K3-SPG with an agonistic CD40 antibody within an ISV framework enhanced the antitumour response. The substantial effectiveness observed in preclinical models offers a robust basis for the clinical application of K3-SPG-based ISV in treatable pancreaticobiliary and gastrointestinal tumours [87]. Numerous pre-clinical investigations have been conducted utilizing animal experimental models that are representative of PD-1/PD-L1 inhibitors, as well as various cancer cell lines (Table 5).

Table 5. Cellular cultures and animal experimental models representative of PD-1/PD-L1 pre-clinical studies.

Type of cancer	Cancer cell line	Strain of mouse	Procedure for creating a murine tumour model	Treatment approach	References
Cancer of the colon	CT26, SK-MEL-28, B16-F10	BALB/c mice, NSG mice	Xenograft models	Anti-PD-1Ab	[93]
Cancer of the skin, Cancer of the colon	B16-F10, CT26	C57BL/6 mice, BALB/c mice	Xenograft models	Anti-PD-L1 mAb, anti-TRP-1 mAb	[93]
Cancer of the skin, Cancer of the colon	B16, CT26	C57BL/6 mice, BALB/c mice	Genetically modified mouse model, xenograft models, intravenous (i.v.) injection	Anti-PD-1Ab	[93]
Cancer of the skin, Cancer of the colon	MC38-hPD-L1, A375	NOG mice, human PD-L1/LAG-3 double knock-in mice	Xenograft models	Anti-PD-L1 × LAG-3 bispecific Ab, anti-PD-L1Ab	[94]
Cancer of the skin, Cervical cancer, Colorectal cancer	HeLa, HCC827, PC3, NCI-H2023, 4T1, B16.F10, CT26	BALB/c	Xenograft models	Anti-PD-1Ab, anti-PD-L1Ab, PD1-Fc-OX40L	[93]

4.1. Adoption of dendritic cells in ISV

Dendritic cells (DCs) are the most powerful antigen-presenting cells and are essential for cancer immunotherapy because they can trigger, control, and sustain certain anticancer immune responses [95]. DCs are present in various components of the body, including the skin, peripheral blood, mucosal

surfaces, interstitial tissues, and both lymphoid and non-lymphoid tissue regions, where they function as guardian cells within the immune system [96]. DCs can establish immunological tolerance by stimulating the growth of regulatory T cells and causing effector T cells to become anergic and absent [97]. Furthermore, they are integral to the process of developing antigen-specific immunity and tolerance. Consequently, DC modification has a great deal of promise for producing effective antitumour immunity and can also deliver immunomodulatory signals through cytokines and cell-cell interactions. DCs are recognized as crucial modulators that orchestrate the response to ICIs and various cancer immunotherapies. This significance arises from their ability to induce new T-cell immunity and their function in activating antigen-specific T-cells [95]. Numerous lines of evidence now support the association of intratumoural DCs or the manifestation of transcriptional signatures specific to DCs, characterized by a T-cell inflamed phenotype and the presence of CD8 T-cell infiltration, favourable prognosis in cancer patients, and responsiveness to various cancer immunotherapies [98,99].

DCs are essential in immunity as powerful antigen-presenting cells that prime T-cells, activate immunological memory, and induce B-cell responses. They also promote immune tolerance by presenting self-antigens to T-cells, maintaining immune balance. Functioning as an intermediary between innate and adaptive immune responses, DCs are central to the immune system and crucial for effective immune responses. Given their pivotal role, further study of DCs is needed to fully understand their mechanisms and to harness their potential in immunotherapy and vaccine development. Conventional DCs have the primary ability to stimulate T-cells, that forms the basis of the “cancer immunity cycle”. There is substantial phenotypic variation and functional plasticity in the subpopulations of dendritic cells. The main subsets of DC—conventional DCs (cDCs), monocyte-derived DCs (moDCs), plasmacytoid DCs (pDCs), and Langerhans cells (LCs)—all have diverse migratory pathways and characteristics, which lead to a range of immunological and inflammatory responses [100].

Analyzing the relationships between DCs and CRC cells may help discover important mediators in the progression of tumour formation and possible therapeutic targets for intervention [101]. For DC vaccination, tumour blood vessel-derived antigens offer an intriguing avenue to elicit cytotoxic CD8+ T cell responses directed against the CRC’s underlying blood vessel network [102]. To activate the host's immune response towards tumour antigens, dendritic cells are considered an attractive choice for cancer vaccines for colorectal tumour patients, and positive outcomes have been observed. The interest in utilizing DCs compartments to enhance the stimulation and mobilization of T-cells within the tumour microenvironment is on the rise. This strategy seeks to improve the response rates to immune checkpoint blockade among patients, especially those with tumours that exhibit low immunogenicity and a non-inflamed phenotype. Recent research indicates that DCs are more pivotal in the mechanisms that govern immune checkpoint blockade and various cancer therapies than previously recognized. These strategies encompass the stimulation of inherent pathways for the detection of DNA or RNA, the engagement of immunological checkpoints and co-stimulatory molecules expressed by DCs, and the modulation of adaptive immunity through the process of immunogenic cell death (ICD) [103,104]. DC vaccines must, however, undergo additional research before they may be considered a clinically effective treatment for advanced colorectal cancer.

A phase III trial (MIND-DC, NCT02993315) evaluated adjuvant DC therapy in stage IIIB/C melanoma using autologous natural DCs loaded with tumour antigens. The therapy induced functional antigen-specific T-cell responses in 67.1% of patients but there was no enhancement of either

recurrence-free or overall rate of survival compared to placebo. The treatment exhibited good tolerability, indicating its feasibility and potential for immunological benefits; however, additional optimization is required to enhance clinical efficacy [105].

DC-based immunotherapy was assessed in a phase 1/2 clinical study in individuals with resected pancreatic cancer after standard-of-care treatment. Allogeneic tumour cell lysate was loaded into autologous DCs for the patients. The treatment showed fewer adverse events and a 2-year recurrence-free survival (RFS) rate of 64%, higher than the anticipated 40%. Increased frequencies of central memory T cells and activated CD4+ T cells were among the vaccine-specific T-cell activations detected by immune monitoring. These results underline DC-based immunotherapy's promise as an adjuvant treatment and encourage more research in randomized trials [106].

Although DC vaccination has shown promise as a cancer treatment, several obstacles restrict its efficacy. These include challenges in activating NK cells or γδ T cells, boosting certain cytotoxic T lymphocytes (CTLs), and overcoming the immunosuppressive effects of MDSCs and Tregs. Its success is further hampered by problems such as low tumour antigen expression, overexpression of checkpoint proteins, low T lymphocyte avidity, and a lack of efficient adjuvants. ICIs and peptide-loaded autologous DC vaccines are suggested combined treatments to improve clinical results [107].

5. Synergistic potential of CBT and ISV for the management of colorectal cancer

The response of cancers to ICIs is heterogeneous, with certain cancer types showing limited therapeutic benefit. Despite the transformative impact of ICIs on cancer treatment, their effectiveness varies considerably among different malignancies. Colorectal cancer (CRC) presents a range of responses to ICIs, which are significantly influenced by molecular attributes such as microsatellite instability (MSI) and mismatch repair (MMR) proficiency. The application of checkpoint-blocking antibodies has demonstrated favorable outcomes in patients with CRC by targeting immune inhibitory pathways, thus opening avenues for new therapeutic interventions [49]. The effectiveness of immunotherapy is characterized by significant individual variability. It has been reported that only a minority of patients exhibit a clinical response to this treatment, with around 20% demonstrating a positive reaction to anti CTLA-4 or PD-L1/PD-1 therapies [108]. Additionally, cancer treatment with ICI causes immune-related side effects in a subset of cancer patients.

ISV is a unique immunotherapy approach recently developed in response to the challenge of finding and delivering tumour antigens. This method covers the whole range of cellular immunogenicity by using dying or dead tumour cells to produce endogenous antigens right at the tumour site. This stimulates systemic anti-tumour actions by inducing a strong local immune response against malignancy. Its main benefit is that antigen identification, manufacturing, and targeted delivery are avoided, making it a customised immunotherapy for various solid tumours [109] . *In situ* cancer vaccinations have significant benefits, but they also have drawbacks. A strong anti-tumour immune response cannot be induced when APCs do not adequately absorb antigens. The majority of antigens acquired by APCs face significant difficulties in accessing the endoplasmic reticulum for presentation by MHC I molecules, primarily due to their uptake occurring within lysosomes. This hinders CD8+ T cell activation, which is essential for the anti-tumour response. Furthermore, effector T cells' efficacy is restricted by the immuno-suppressive tumour microenvironment, which encourages tumour immune escape. APCs first collect tumour antigens

during the vaccine process, but DCs absorb fewer antigens as a result of the tumour antigens' poor release and specificity, which makes it difficult to start an efficient anti-tumour immune response [109].

Combinatorial therapy is necessary to achieve greater effectiveness with fewer side effects. Exploring novel and developing immunotherapeutic approaches beyond ICIs has the potential to create new prospects for individuals whose cancers have not responded to these therapies alone. Various vaccination strategies can be integrated with combination-based therapies to enhance therapeutic outcomes, potentially mitigating autoimmunity by specifically directing the immune response towards tumours rather than healthy tissues [110].

Combination therapy, which involves using two or more therapeutic agents, is a fundamental approach in cancer treatment. The utilization of combination therapies that address various immune mechanisms is anticipated to improve the modulation of the immune system, leading to a stronger anti-tumour response [111]. Combination therapy can significantly enhance the effectiveness of cancer treatment, so identifying the best drug combinations and their interactions can speed up the advancement of this approach [112]. The use of combination anticancer therapies is clinically advantageous for several reasons. First, when the mutually beneficial anticancer effect is attained, combination therapy produces more excellent therapeutic benefits and enhances the therapy results. Second, clonal heterogeneity is eliminated by the combinational strategy, which is also linked to higher response rates. Third, combined drug regimens can minimise toxicity by allowing individual drugs to be used at lower doses while maintaining therapeutic efficacy. Additionally, combination therapies help reduce the development of resistance to drugs. Combination therapy facilitates the concurrent targeting of various molecular pathways essential for the survival of cancer cells, thereby interfering with the cellular processes associated with adaptive resistance [113]. Combining cancer immunotherapy with radiation, chemotherapy, and surgery would make the treatment of CRC incredibly effective [41]. One combination involves *In-situ* Vaccination (ISV) and Checkpoint Blockade Therapy (CBT) (Figure 4).

Combination therapy is particularly applicable to immunotherapies, aiming to optimize treatment responses for a range of tumour types while simultaneously decreasing the incidence of side effects. The integration of CBT with ISV is grounded in a mechanistic immunological framework that seeks to enhance clinical outcomes. Although T cell activity is commonly associated with the PD-L1/PD-1 pathway, studies have demonstrated that DCs represent a crucial target for antibodies that inhibit PD-L1 [60]. CBT is predicated on the existence of T cells that can identify and target tumour cells, which must not be rendered unresponsive by factors such as "exhaustion" or immunosuppressive signals; one of the many reasons why CBT fails is if the number of such cells is inadequate. ISV can generate elevated numbers of the tumour-identifying effector T cells needed for the CBT response [19].

A pre-clinical trial using an ISV method involving intratumoural Flt3L injections, local irradiation (XRT), and poly-ICLC demonstrated significant potential in treating low-grade B-cell lymphomas. In a mouse model, this combination greatly boosted DC accumulation, tumour antigen absorption, and IFN-γ-producing T cell responses, leading to 40% tumour reduction. Adding anti-PD-1 therapy boosted efficacy, leading to complete tumour regression at treated sites and improved survival [114].

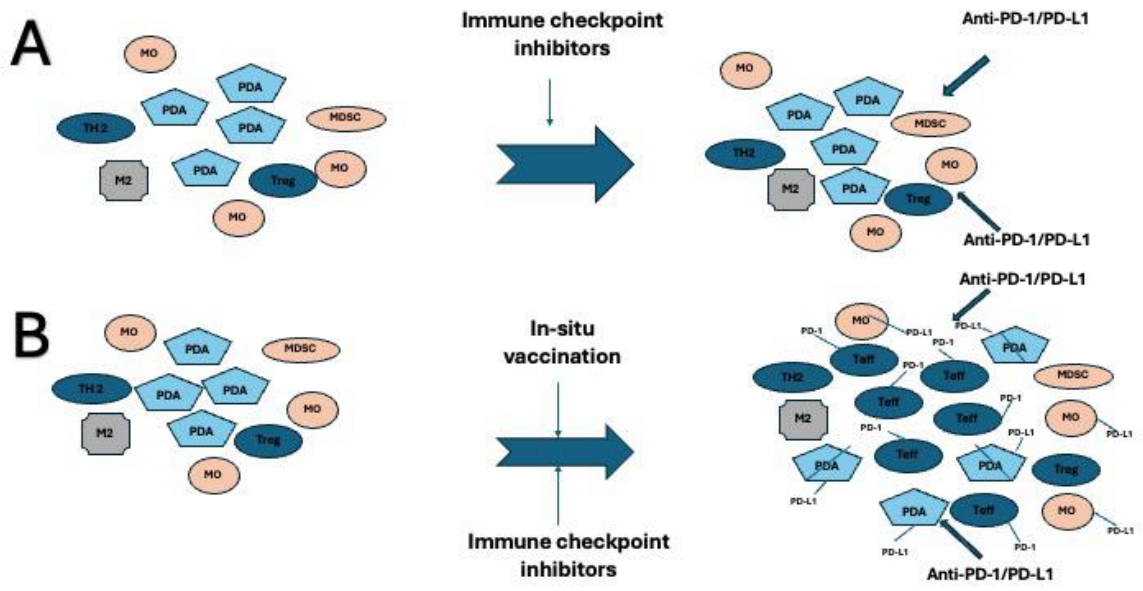

Figure 4. Illustration of the combined effect of vaccine-based therapy and immune checkpoint inhibitors; A illustrates the effect of ICIs, whilst B illustrates the effect of the combination of ICIs and vaccine-based therapy [115].

According to preclinical research, immune checkpoint inhibitors and *in-situ* vaccination therapy may work in combination to boost anticancer immune responses. Multi-peptide vaccinations combined with anti-PD-1 antibodies have demonstrated that there is an improvement in progression-free survival in models of breast cancer. Furthermore, in colon and pancreatic cancer models, GM-CSF-secreting cancer vaccines (GVAX) demonstrated increased anticancer activity in conjunction with antibodies that target PD-L1 and PD-1. In cases where PD-1 inhibition alone was ineffective, tumour shrinkage was accomplished using STINGVAX, a modified GVAX formulation. In several preclinical models, dual inhibition of PD-1 and CTLA-4 in conjunction with vaccinations has demonstrated superior tumour eradication efficacy [115].

A clinical trial for resectable pancreatic cancer looked at the combination of GVAX vaccination, low-dose cyclophosphamide, the anti-PD-1 monoclonal antibody nivolumab, along with the anti-CD137 agonistic monoclonal antibody urelumab. The triple therapy greatly increased activated intratumoural cytotoxic T cells and was tolerated well. Despite the lack of statistical significance attributed to the limited sample size, the combination therapy exhibited enhanced disease-free and overall rate of survival when compared to alternative treatment groups, indicating a potential effectiveness that merits additional investigation [116].

The potential synergistic effects of combining CBT with ISV in the treatment of CRC remain to be substantiated. However, it is hypothesized that such a combination could enhance antitumour efficacy while simultaneously decreasing the occurrence of adverse events and mitigating autoimmunity. This is predicated on the assumption that ISV may increase the population of antitumour effector T cells, leading to a more rapid and effective response to CBT, potentially resulting in shorter treatment duration and lower dosages of CBT. To fully elucidate the implications of integrating ISV with CBT for CRC treatment, further extensive preclinical and clinical investigations are essential. Significant challenges

must be addressed, particularly in identifying the most effective ISV strategies tailored to individual tumours and determining the optimal reagents for these strategies.

5.1. Application of nanotechnology for combining CBT & ISV in the treatment of colorectal cancer

Nanotechnology is a rapidly emerging field that is predicted to expand exponentially. Extensive research and development investigations into nanotechnology have revealed that this technology holds boundless promise across a diverse variety of technological domains, as well as risks [117]. Pharmaceutics has made promising strides in applying nanotechnology to medications to counteract the shortcomings of traditional colon-targeting formulations. Nanotechnology leverages the unique properties of materials at the nanoscale, typically between 1–100 nm, to develop or modify innovative products. It is rooted in nanoscience, which explores the distinctive behaviours and characteristics of materials at this scale [118]. Nanotechnologies hold immense promise in medicine, with applications spanning imaging techniques, diagnostic instruments, drug delivery mechanisms, tissue-engineered constructs, implants, and pharmaceutical therapies have markedly enhanced the management of a wide array of medical conditions. These advancements have been particularly impactful in the treatment of cardiovascular diseases, cancer, musculoskeletal ailments, psychiatric and neurodegenerative disorders, as well as bacterial and viral infections, and diabetes [119].

Nanotechnology is revolutionizing oncology by advancing nano agents, molecular diagnostics, fluorescent materials, and targeted therapies for more effective cancer diagnosis and treatment. Researchers are leveraging nanoparticle conjugation to enhance drug specificity and precision, enabling the direct delivery of anticancer agents to tumours. Additionally, nanomedicine supports tumour imaging, immunotherapy, and heat-based ablation therapies, while optimizing the pharmacokinetics and pharmacodynamics of chemotherapy drugs [120]. Nanotechnology can greatly improve cancer immunotherapy by targeting immunosuppression in the tumour microenvironment (TME). While the immune system recognizes and destroys some tumours, its antitumour response is frequently weak and hampered by immunosuppression, allowing malignancies to escape detection and spread [18].

Drug delivery to the colon is essential in ensuring the medications have the desired therapeutic effect. The drug delivery approach should be formulated to ensure the targeted release of the medication specifically within the colonic environment, thereby preventing premature drug release in the upper gastrointestinal tract [10]. Improving drug delivery is a primary motivation for investigating nanomedicine in the battle against colorectal cancer. This has led to a flourishing research area in nanocarrier-loaded repurposed pharmaceuticals for colorectal cancer. Nanomedicine can potentially improve therapeutic outcomes and minimize adverse effects by better-targeting tumour cells while sparing healthy ones [121]. Nanomedicine uses sensors and tiny robots to diagnose diseases, deliver medicine directly where needed, and even interact with living cells.

5.1.1. Nanoparticles

According to the International Organization for Standardization (ISO), nanoparticles (NPs) are defined as small-scale entities with dimensions that fall within the nanoscale, characterized by the fact that the lengths of their longest and shortest axes do not significantly differ [122]. NPs can impact immune and cancer cell activity within the tumour microenvironment (TME) using a range of medications and other

substances, thereby improving antitumour immunity [123]. NPs are created through various top-down and bottom-up methods. One such approach is Microfluidics, which offers an appealing alternative for synthesis and has advantages over traditional bulk methods [124]. The microfluidic method is an advanced technique that generates NPs of controlled size with a high level of reproducibility [125]. There are several benefits to using NPs as biological systems for drug delivery. Nanoparticle-mediated drug delivery systems are gaining popularity in combinatorial therapy because they allow for targeted drug delivery, delayed drug release, and improved drug stability, which helps to prevent fast clearance at infection sites [17]. NP-based combination therapy has solved the problem of pharmacokinetic variations between different medications by assembling numerous drugs within the same carrier, hence combating drug resistance and boosting the therapeutic efficacy of cancer treatment [126].

Numerous types of cargo, including small molecules as well as larger proteins and nucleic acids, can be effectively adsorbed onto the surface and encapsulated between particles, owing to the substantial surface area and favorable surface-to-volume ratio [127]. NPs are selected for the treatment of colorectal cancer owing to their distinct physical, chemical, and biological properties, which facilitate targeted drug delivery, the regulated release of therapeutic compounds, and enhanced infiltration and retention within tumour tissues. Nanoparticles can potentially improve ICI therapies, improve tumouricidal activity, and have fewer adverse effects than traditional chemotherapeutics [128]. Nanoparticles have the capability to convey ICIs directly to the tumour microenvironment, hence hindering the mechanisms that tumours utilize to evade immune surveillance. The utilization of nanoparticles allows for the precise targeting of the tumour vasculature or the extracellular matrix, which in turn enhances the infiltration of immune cells into cancerous environments. Furthermore, these nanoparticles can transport enzymes or therapeutic drugs that disrupt or transform the extracellular matrix, thereby diminishing the barriers that impede immune cell access.

Polymeric nanoparticles are tiny particles with sizes varying from 1 to 1000 nm that can contain active substances embedded within or attached to the surface of the polymeric core [129]. Polymeric nanoparticles are among the most utilised carriers for therapeutic delivery. The primary structural configurations of polymeric nanoparticles are categorized into two types: nanospheres are characterized by the uniform dispersion of the drug within a polymeric matrix, in contrast to nanocapsules, which contain the drug in a core that is surrounded by a polymer membrane (Figure 5) [130]. Industrial polymeric materials like poly(lactic-co-glycolic acid) (PLGA), poly(glutamic acid) (PGA), and nanoparticles made from naturally occurring polymers like chitosan, gelatin, and collagen are extensively researched and employed in the biomedical field [131]. The FDA-approved synthetic polymer PLGA can create NPs directed to a particular location to secure and efficiently deliver medications. Numerous cancer treatments, such as tumour-targeted delivery of drugs, gene therapy, hyperthermia, and photodynamic therapy, can be employed with PLGA-based NPs [125].

For many years, research into employing nanoparticles in the delivery of medications to treat cancer has been a promising field. However, their inadequate use as therapies has resulted from their damaging impact on cells, low absorption performance, and treatment tolerance [132]. Newly synthesized nanoparticles encounter obstacles such as swift agglomeration and oxidation, which impede their wider application. These issues can be addressed through surface functionalization, a process that entails the attachment of chemical functional groups to the external surface of nanocomposites. This modification facilitates self-organization, enhances compatibility, and increases the potential for various applications

of the improved functionalization [133]. Surface functionalisation of nanoparticles is essential for the advancement of precision drug delivery systems and improving clinical outcomes by promoting passive accumulation, facilitating active targeting and shielding sensitive antigens and adjuvants from degradation. This strategy facilitates substantial drug incorporation, improved bioavailability, and diminished cytotoxic effects. Peptide-functionalized NPs are among the best biomolecules for increasing the effectiveness of drug administration. The functionalisation of NPs with peptides has resulted in the emergence of therapeutics that can target and track tumours, potentially replacing cancer treatments [134]. A key strategy for cancer treatment is nanoparticles functionalized with peptides, which tackle the issues of selectivity and effectiveness in the delivery of the drugs. Peptide-modified nanoparticles enhanced medication delivery, reduced harm to healthy tissues, and enhanced cancer cell targeting. Self-assembled peptide structures' adaptability and varied nano-architecture and biocompatibility make them a novel drug delivery platform [73].

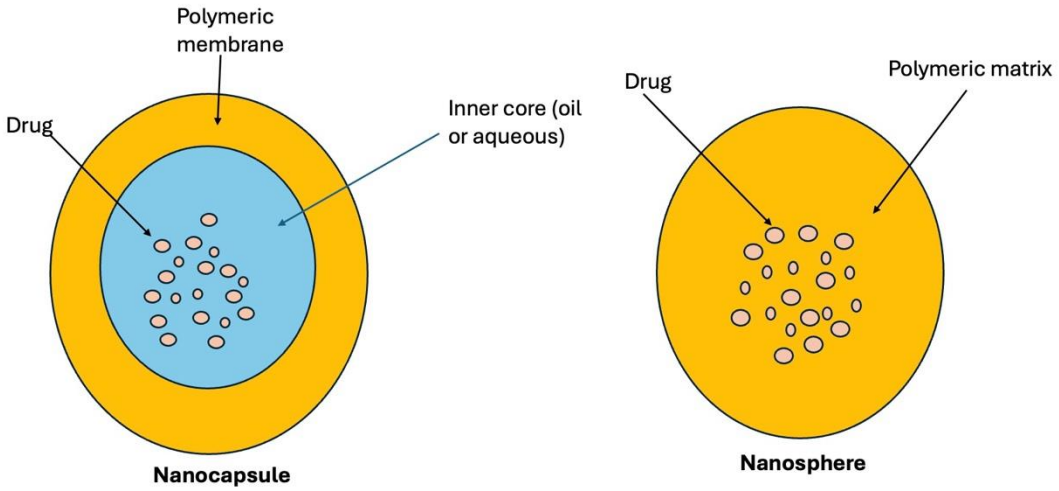

Figure 5. A diagram showing the two structural forms of polymeric nanoparticles [130].

5.1.2. Proposed nano-based combinatorial therapy for integrating CBT with ISV

A new study area called nano-immunotherapy has emerged due to advancements in immunotherapy and nanomedicine. Its goal is to use nanomedicine to fully exploit immunotherapy's therapeutic potential. Nanocarriers present a promising opportunity in the field of immuno-oncology by improving immunostimulatory activity and positively modifying pharmacological characteristics. This development holds the promise of improving the effectiveness and precision of therapeutic interventions while simultaneously minimizing toxicity and preserving the anticancer effectiveness of immunotherapeutic agents [135]. Numerous preclinical studies have shown that nanoparticles safely and effectively increase the effectiveness of immunostimulatory drugs, indicating their significance in the field of immuno-oncology. The use of NPs combined with ISV (NP-ISVs) has demonstrated enhanced outcomes in immunotherapeutic treatments, which include those that incorporate malignant heat and checkpoint blockade treatment [136].

Essentially, checkpoint blockage can be directly improved using nanoparticles by facilitating the transport of ICIs to CRC tumour locations. Integrating immuno-oncology and nanotechnology has led to several nanomaterials designed to boost ICI effectiveness in colorectal cancer (CRC) [50]. Particles of both micro and nano size can either directly attach to receptors on the surface of tumour cells or passively target tumour tissue through the leaky vasculature. NPs can increase the effectiveness of ICIs by converting immunologic tolerant cell carcasses into immunogenic tumour vaccines and controlling cell death pathways [137]. NPs can restore the functioning of ICIs by re-programming immune-suppressive tumour microenvironments (TMEs). Enough tumouricidal immune cell infiltration is required to react to ICIs [138]. During carcinogenesis, CRC tissues continuously produce exosomes, chemokines, and cytokines as systemic agents to recruit immunosuppressive cells and reshape the extracellular matrix (ECM). This results in an immunological desert milieu commonly called a "cold" tumour. Cold tumours refer to a tumour that is unlikely to elicit a robust immune response. Cold tumours are typically encased by cells that can inhibit the immune response, thereby preventing T cells, which are a specific type of immune cell, from targeting and destroying the tumour cells. In TME, malignant characteristics can be reversed, and tumour-resident immunosuppressive cells can be reduced using delivery agents called NPs that block immune suppressive pathways. NPs can strongly elicit antigen-specific immunity against colorectal cancer by promoting the immune response against tumour-exclusive antigen pulses. NPs serve a dual function by simultaneously delivering antigens and immune-enhancing adjuvants to the host, acting as both preventive and therapeutic measures. This approach pre-sensitizes the immune system and promotes the sustained generation of cytotoxic CD8+ T lymphocytes [139].

Nanoparticle-mediated delivery of antibodies has shown significant efficacy in selectively targeting and activating antigen-presenting cells and T cells, demonstrating therapeutic advantages in several malignancies, such as cancer of the skin, non-small cell lung cancer, cancer of the kidney, and Hodgkin's lymphoma. Historically, the design of nanocarriers has focused on the direct targeting and destruction of tumours. However, the effectiveness of these approaches has frequently been hindered by systemic and local obstacles that impede nanoparticle penetration and cellular uptake within the tumour microenvironment. In contrast, within the realm of immunotherapy, these nanocarriers can be strategically directed towards immune cells located in lymphoid organs can be purposefully guided to immune cells situated in lymphoid organs or other immune-associated locations, thereby overcoming the challenges linked to targeted delivery to tumours. Immune cells possess a distinctive ability to traverse natural barriers, including tumour vasculature and the blood-brain barrier, which are generally resistant to penetration by NPs. This characteristic enables nanocarriers to achieve therapeutic effects indirectly by modulating immune cells, potentially allowing for a more effective antitumour response.

6. Novel pre-clinical models

Novel preclinical models, such as 3D cultures (spheroids and organoids), transform biomedical research by more closely imitating *in vivo* tissues. These models outperform typical 2D cultures by improving disease understanding, medication screening, and individualized treatment, making them critical for translational research [140].

Tumour organoids have surfaced as a valuable resource in cancer research [141]. Cancer-like organoids can be derived from mouse or human tumour tissues, and they effectively preserve the structural integrity, genetic characteristics, and heterogeneity of the original tumour tissue. These

organoids provide reliable insights into the drug responses of individual patients, facilitating the development of tailored treatment strategies for personalised therapy. Notably, organoids generated from CRC and metastatic tissues exhibited both genetic diversity and consistent morphological features [142].

Spheroids are used to study the molecular and genetic characteristics of activation and progression of CRC. CRC is highly heterogeneous due to genetic and epigenetic mutations, which can affect treatment response and patient survival. 3D cultures from CRC patients contain diverse spheroid-forming cells with varying rates of growth and levels of drug sensitivity [143]. CRC and metastatic tissue-derived organoids retain genetic diversity and structural stability. Beyond CRC, organoids have been developed for other cancers, cancers of the breast, bladder, lung, pancreas, and prostate [144]. Studies have frequently examined CRC models, where several 3D spheroid models were developed using patient-derived xenografts, patient-derived cells for assessment of drugs, and CRC cell lines such as DLD-1 and HCT-116 [145,146]. The dissociation of cancer cells or tumour tissues creates a three-dimensional tumour spheroid. The 3D spheroid model provides superior insights into tumour characteristics, drug development, cellular interactions, and the metabolic profiles of cancer cells when compared to traditional 2D systems and animal models [147]. Spheroids are promising *in vitro* models for anticancer drug screening because they provide more clinically translatable outcomes than traditional methods. However, they are not yet ready for widespread clinical application in precision medicine. Advances in spheroid development could improve reproducibility by incorporating various cell types from the colorectal tumour microenvironment and addressing CRC heterogeneity. Enhanced 3D culture systems would strengthen CRC drug testing and open new possibilities for designing targeted treatments [144].

7. Conclusion

CRC constitutes a major public health concern globally, being the second highest cause of cancer mortality and the third most frequently identified cancer. Innovative and potent treatment techniques are urgently required, with incidence and mortality rates expected to increase dramatically by 2040. While traditional approaches such as surgical intervention, chemotherapy, and radiotherapy have served as the bedrock of CRC treatment, their limitations, including toxicity and uneven efficacy, highlight the need for improvements in approaches to therapy. Immunotherapy is emerging as a transformational paradigm, with immune checkpoint drugs targeting PD-1, PD-L1, and CTLA-4 demonstrating encouraging results in subsets of patients. Despite these advances, the heterogeneity of patient responses needs multifaceted approaches to improving treatment efficacy. A revolutionary development in immunotherapy is the incorporation of nanomedicine through nanoparticle-based drug delivery systems, which allow for exact drug targeting, controlled release, and enhanced stability, which greatly increase immunotherapies' therapeutic potential.

The combination of CBT and ISV may exhibit greater effectiveness and a reduced occurrence of adverse events associated with combination treatment when compared to monotherapy in the management of colorectal cancer. While this hypothesis has not been completely substantiated, the amalgamation of CBT and ISV holds the promise of improving antitumour effectiveness and minimizing adverse effects, which could consequently decrease the likelihood of autoimmunity [18]. An increase in the quantity of antitumour effector T cells by ISV may enhance the speed and efficacy of CBT, potentially resulting in shorter treatment periods and/or lower dosages [18]. An extensive investigation is required to enhance the understanding of the outcomes associated with the integration of ISV and CBT

in the treatment of colorectal cancer. Developing an effective therapeutic approach that minimizes adverse effects is crucial for alleviating this disease's global burden and mortality.

Combining checkpoint blockade therapy with *in-situ* vaccination, utilizing therapeutic peptides as checkpoint inhibitors and dendritic cells for in-situ vaccination, presents a novel strategy to amplify anti-tumour immunity in colorectal cancer. Therapeutic peptides, serving as checkpoint inhibitors, can precisely target immune checkpoint pathways, reducing immune suppression while minimizing off-target effects. Concurrently, *in situ* vaccination with dendritic cells primes and activates the local immune response directly at the tumour site, enhancing T-cell recognition and memory against cancer cells. This combined approach addresses key challenges in colorectal cancer therapy, such as immune resistance, autoimmune side effects and tumour heterogeneity, offering a promising pathway to more effective and durable treatment outcomes. Further research will be crucial to optimise and validate this strategy in clinical settings, paving the way for improved patient outcomes in colorectal cancer.

8. Prospects on the combination of CBT & ISV

The integration of ISV with ICIs presents a promising approach to augmenting the effectiveness of therapeutic cancer vaccines. This combination has shown safety, immunogenicity, and efficacy in traditional vaccines aimed at infectious diseases. Nonetheless, comprehensive investigations are necessary to elucidate the immunological mechanisms underlying ICI therapies, especially regarding their potential to enhance the efficacy of cancer vaccines. Critical areas for exploration include the optimal timing for the administration of therapeutic vaccines alongside ICIs and the potential for this combination to expand the spectrum of cancer types that may benefit from ICI treatment. Furthermore, long-term monitoring is crucial to evaluate the sustainability of immune responses, alongside the need for larger and more heterogeneous sample populations to account for individual differences in immune reactions to both ISV and ICIs.

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Authors' contribution

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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