The emerging role of immunotherapy in colorectal cancer

David Lynch[1], Adrian Murphy[2]

[1]Department of Internal Medicine, University of North Carolina Hospitals, Chapel Hill, North Carolina, USA; [2]Department Medical Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA

Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Adrian Murphy. Department Medical Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA. Email: amurph39@jhmi.edu.

Abstract: Modulation of the interaction between the immune system and the tumor microenvironment has long been a target of cancer research, including colorectal cancer (CRC). Approaches explored to date include vaccines (autologous, peptide, dendritic cell, viral and bacterial), cytokine therapy, toll-like receptors (TLRs), autologous cell therapy and checkpoint inhibition. Until recently these approaches have been shown to have only modest efficacy in reducing tumor burden. However, significant breakthroughs have been made, with the use of checkpoint inhibitors targeting programmed cell death protein-1 (PD-1), programmed cell death ligand-1 (PD-L1), and cytotoxic T lymphocyte antigen-4 (CTLA-4). Immunotherapy now represents a possible avenue of curative treatment for those with chemo-otherwise refractory tumors. Success with this approach to immunotherapy has largely been confined to tumors with high mutational burdens such as melanoma, renal cell carcinoma (RCC) and non-small cell lung cancer. This observation led to the exploration and successful use of checkpoint inhibitors in those with mismatch repair colorectal cancer which have a relatively high mutational burden. Ongoing trials are focused on further exploring the use of checkpoint inhibitors in addition to investigating the various combinations of immunotherapeutic drugs.

Keywords: Colorectal cancer (CRC); immunotherapy; vaccines cytokine therapy; toll-like receptors (TLRs); autologous cell therapy; checkpoint inhibition

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States with a 5-year survival rate for those with metastatic disease of 12% (1,2). Treatment modalities currently being used for metastatic CRC have been shown to have only modest efficacy and are also associated with significant toxicities (3). This unmet need for effective treatment of metastatic CRC has driven the search for novel strategies to improve survival while minimizing toxicities experienced by patients.

Immunotherapy has had promising results in solid tumors originally thought to be non-immunogenic, for example, lung cancer and melanoma (4). The immune system plays an intricate and complex role in all aspects of cancer from carcinogenesis to treatment (5). Over the past two decades in particular, great advances have been made in our understanding of the interplay between the immune system and cancer (*Figure 1*). This has led to the development of therapies such as cancer vaccines and adoptive cell therapy (ACT). These therapies have been shown to have an alternative side-effect profile in comparison to traditional chemotherapy yet, until recently, they had not been shown to be particularly effective (6). The FDA approval of immunotherapies such as the cancer vaccine sipuleucel-T and the immunomodulatory
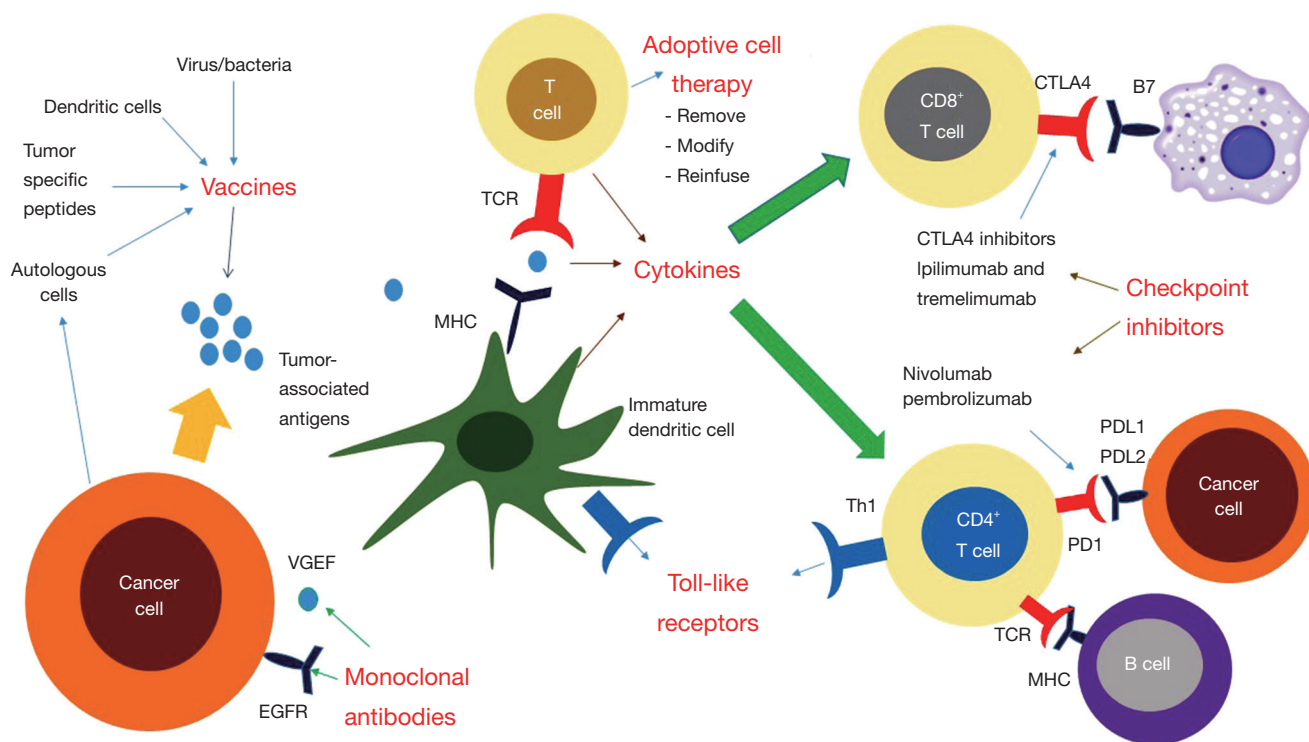
Figure 1 Components of immunotherapy in colorectal cancer. Tumor cells express specific antigens which can activate APCs by binding to MHC molecules on their cell surface which in turn bind to TCRs on T cells. Vaccines enhance and refine the immune system's attack on cancer cells by modulating the interaction between APCs and T-cells. ACT involves removing T-cells from patients, exposing the T-cells to specific TAAs and then reinfusing the activated T-cells. Cytokines mediate the interaction between APCs and T cells as well as the conversion of T cells into CD8+ cells. Cytokine therapy represents an avenue for further investigation in CRC (IL-2 and IFN-α have been FDA approved for use in other malignancies). Following tumor cell death, DAMPs are present on TLRs. Various TLR agonists (e.g., MGN1703) have been trialed in CRC. PD-L1 and PD-L2 are ligands on the surface of cancer cells which bind to PD-1 receptors on T cells to induce T-cell death. Anti-PD-1 antibodies bind to PD-1 receptors preventing interactions with its ligands. T-cells express CTLA-4 antigens, this represents another method by which the immune system is downregulated. Anti-CTLA-4 antibodies (e.g., ipilimumab) reverse the immunosuppressive effect when they bind CTLA-4 receptors on T cells. APCs, antigen presenting cells; MHC, major histocompatibility complexes; TCRs, T cell receptors; ACT, adoptive cell therapy; TAAs, tumor associated antigens; CRC, colorectal cancer; DAMPs, damage-associated molecular pattern molecules; TLRs, toll-like receptors; PD-L1, programmed cell death ligand-1; PD-1, programmed cell death protein-1; CTLA-4, cytotoxic T lymphocyte antigen-4.

monoclonal antibodies (Abs) ipilimumab, nivolumab and pembrolizumab, heralds an exciting change in the direction of cancer therapeutics. This review will focus on the use of immunotherapy in CRC.

Vaccines

Cancer vaccines have been used to facilitate the immune destruction of cancer cells in many different tumor types.

They work on the premise that the natural immune response of recognizing and destroying altered self-antigens is deficient in cancer. Some factors which contribute to this failure are the tumor's ability to prevent immune activation by hiding tumor-associated antigens and also its ability to suppress the immune system here instead once it has been activated. Cancer vaccines overcome this failure by helping to activate and maintain an anti-tumor immune response. The following categories of vaccines autologous, peptide,

dendritic cell and viral, are discussed.

Autologous vaccines

Autologous vaccines use cells which have been removed directly from a patient's own tumor. This approach guarantees that the vaccines will contain all tumor-associated antigens specific to each individual patient (7). However, whole cell vaccines contain antigens in isolated cells, the majority of which are also present on normal tissue. Therefore, the response generated by the vaccine is generally not specific enough to have a substantial impact on the tumor burden. For this reason autologous vaccines to date have demonstrated limited efficacy (8).

In an effort to improve immunogenicity, whole cell vaccines have been modified in various ways. One phase III trial combined an autologous whole cell vaccine and the BCG vaccine to determine whether surgical resection plus the vaccine was more beneficial than resection alone in 412 stage II and III colon cancer patients (9). Patients were randomized to observation or to receive three weekly intradermal vaccines consisting of 1×10^7 irradiated autologous tumor cells which had been digested from their primary tumor and stored as a cell suspension. Vaccines were administered with BCG organisms for 2/3 administrations of which the first two injections contained BCG organisms and overall these produced local reactions in 79% of patients. After a median of 7.6 years of follow-up, there were no statistically significant differences between the groups in terms of disease-free and overall survival. However, the 5-year survival rate was 84.6% for those with indurations greater than 10 mm, compared with 45% for those with indurations less than 5 mm suggesting that those who developed a greater local reaction may have benefited from the vaccine-induced immune response. No specific antibody responses were measured in this study so it is unclear whether a cutaneous reaction can serve as a surrogate marker of response.

Autologous cell vaccines have also been modified to secrete granulocyte macrophage colony-stimulating factor (GM-CSF) which can induce anti-tumor immunity. While the trials have not been shown to have any significant impact on overall survival, the vaccine has been shown to augment antitumor immunity in melanoma (10) and lung cancer (11). In a phase I study, nine patients with metastatic CRC were treated with a CRC vaccine composed of irradiated, allogeneic human colon cancer cells and GM-CSF-producing bystander cells in combination with a single dose of cyclophosphamide in order to deplete regulatory T (Treg) cells (12). GM-CSF acts as an important differentiation factor for dendritic cells thereby improving their capacity to present tumor antigens. The vaccine was found to be well tolerated and immunological correlates showed that this vaccine enhanced the production of anti-MUC1 Abs which suggests T cell targeting. At last follow-up, six patients had survived longer than 36 months and four of these patients were without disease recurrence.

Another way of modifying the immune response which has been explored in more detail in CRC uses Newcastle disease virus-infected (NDV), irradiated whole cell tumor vaccines. This method was adopted following the results of previous trials which demonstrated that infecting autologous cells with NDV results in potent up-regulation of both the innate and adaptive immune system (13). A phase II study administered this vaccine to 23 patients after R0 resection of CRC liver metastases (14). Patients received autologous, irradiated metastasis-derived tumor cells incubated with 32 hemagglutinin units of NDV 2 weeks post-operatively and the vaccine was administered at 14-day intervals followed by a single boost 3 months later. After a follow-up of 18 months, 61% of patients had developed tumor recurrence whereas 87% of matched control patients (had similar surgery but were not vaccinated) developed recurrences (P=0.05). Around 40% of patients experienced delayed-type hypersensitivity reactivity when challenged with autologous tumor cells but correlations between reactivity and survival were not performed.

The resultant phase III study randomized 51 patients with histologically confirmed liver metastases from CRC who underwent metastectomy to receive six doses of NDV-infected autologous tumor cell vaccine or a control group (15). There was no significant difference in terms of overall survival where 48% of patients who received vaccines died during the observation period compared to 64% of patients in the control group. A subgroup analysis showed that patients with colon primary tumors had improved survival rates in terms of overall survival (HR: 3.3; 95% CI, 1–10.4; P=0.042) and metastasis-free survival (HR: 2.7; 95% CI, 1–7.4; P=0.047).

The data from autologous cell vaccines in CRC to date have not altered clinical practice and further efforts to improve the immunogenicity of these vaccines are needed and to determine subgroups of patients most likely to respond to autologous cell vaccines.

Peptide-based vaccines

Peptide vaccines attempt to target more specific components of tumor cells by identifying peptides which are 8–11 amino acids long and are unique to cancer cells. Advantages of peptide vaccines include their low cost, ease of production and storage, their unlimited potential for modifications and their ability to target tumor-specific antigens (16). Trials to date have encountered problems which have limited the effectiveness of peptide vaccines such as: antigenic escape resulting in re-occurrence and HLA-restriction limiting the peptide vaccines to specific HLA haplotypes (16).

Some tumor-associated antigens have been targeted by peptide vaccines in CRC including: EpHA2 (17), β-hCG (18), carcinoembryonic antigen (CEA) (19), SART3 (20), p53 (21), mucin 1 (22), survivin 2B (23) and RNF43/TOMM34 (24). Many of these trials have succeeded in generating an antigen-specific immune response, however, few trials have shown a resultant increase in survival. In a phase II trial, 77 patients were treated with a β-hCG vaccine composed of the COOH terminal peptide of β-hCG conjugated to diphtheria toxoid, of which 56 patients (73%) developed anti-hCG Abs (18). Patients who were found to have β-hCG antibody levels higher than the median had a median survival of 45 weeks in comparison to 24 weeks in patients found to have antibody levels lower than the median (P=0.0002). However, survival did not correlate with the level of antibody mounted to diphtheria toxoid.

There has been an attempt to overcome the limitations of peptide vaccines by targeting multiple epitopes with longer amino acid sequences. A phase II trial was conducted in 96 chemotherapy-naive patients with metastatic CRC using five HLA*2402-restricted peptides (two from VEGF derived receptors and three from oncoantigens) (25). They were administered concurrently with oxaliplatin-based chemotherapy (FOLFOX or XELOX) and the primary objective was to compare the chemotherapy/peptide vaccine in patients with and without the HLA-A*2402 haplotype. However, there were no significant differences in terms of response rate, progression-free survival and overall survival. Given that Treg depletion was not a feature in this trial, it is possible that responses to this combination of peptide vaccines may be enhanced by combining it with checkpoint inhibitor therapies/cyclophosphamide. A similar phase II trial was conducted using IMA910, a vaccine consisting of 13 tumor-associated peptides (TUMAPs), which are naturally presented on major histocompatibility complexes (MHC) molecules of colorectal tumors (26). Patients with HLA-A*02 CRC (n=82) who were clinically stable after 12 weeks of oxaliplatin-based therapy underwent immunomodulation with a single low dose of cyclophosphamide (300 mg/m^2) and were immunized with IMA910 in combination with GM-CSF or a with IMA910, GM-CSF and topically applied imiquimod [toll-like receptor (TLR) 7 agonist] . Immune responses were noted in 71% and 72% of patients in both groups respectively. Patients who developed CD8 T-cell responses against numerous TUMAPs had an improved disease control rates compared to other in terms of disease control rate (18% vs. 2% at 6 months; P=0.012) and PFS (HR: 0.652; P=0.039). Further research efforts should be focused on identifying antigens specific to CRC in an attempt to improve clinical outcomes.

Dendritic cell vaccines

Dendritic cells play a key role in the key components of the immune system activation cascade and thus represent an opportunity for targeted immunotherapy (27). Antigens are presented by dendritic cells to T cells on MHC and co-stimulation is provided by a receptor-ligand pair. This combination then triggers the release of cytokines which mediate the immune response.

The original approach to dendritic cell vaccines involved infusing immature dendritic cells which would then pick up antigens in vivo. More novel vaccines have involved harvesting DCs from the patients, pulsing them ex vivo with tumor associated antigens (TAAs) (28), tumor cell lysates (29), apoptotic tumor cells (30), tumor RNA (31) or whole tumor cells (32), and allowing them to mature before re-infusing them with the aim of inducing a tumor-specific immune response. DC vaccines can also be modified to express co-stimulators such as CD40L (33).

CEA has commonly been the focus of clinical trials involving DC vaccines in CRC due to its known association with CRC. Four early phase trials showed that CEA vaccines were safe and effective in generating a CEA specific immune response (34-37). Following these positive results, there have not been any published results of any phase III trial looking at the efficacy of these vaccines in the CRC population. A phase II trial randomized patients pre-treated for metastatic CRC to receive an autologous tumor lysate dendritic cell vaccine plus best supportive care (BSC) or BSC (38). Although the vaccine was found to generate a tumor specific immune response, there were no

benefits seen in terms of PFS (2.7 vs. 2.3 months, P=0.628) and OS (6.2 vs. 4.7 months, P=0.41) compared to BSC, and the study was terminated early due to futility.

Viral/bacterial antigen vaccines

The common theme which is evident in the ultimate failure to date of peptide, autologous and dendritic cell vaccines is the inability to generate an immune response that is specific yet substantial enough to positively affect clinical outcomes. The primary function of the immune system is to protect against foreign pathogens and the human immune system has evolved over time in response to exposure to such antigens. Therefore, it is possible that a mechanism exists which can overcome the vaccine immunogenicity quandary by incorporating the use of pathogenic vectors. However, some disadvantages of vector vaccines are their limited immune responses against the vector, cost, potential for pathogenesis, and potential for insertional mutagenesis (6).

A phase I trial involving vaccinating 58 patients with advanced CEA-expressing cancers with fowlpox-expressing CEA and TRICOM (three costimulatory molecules B7-1, ICAM-1 and LFA-3) resulted in CEA-specific T-cell responses and stabilization of disease in 40% patients for at least 4 months (39). A similar phase II trial randomized 118 patients with metastatic CRC to receive ALVAC virus expressing CEA and costimulatory B7-1 vaccine either: prior to chemotherapy (IFL/FOLFIRI), to receive tetanus toxoid in addition to the viral vaccine or to receive the vaccine if clinically responding after four cycles of chemotherapy (40). All patients developed antibody responses to ALVAC but only three patients developed anti-CEA Abs.

Anti-CEA specific T cell responses were seen in 50% of those who received the vaccine and 40.4% of patients were found to have a clinical response. There were no differences in clinical outcomes between the groups with the majority of clinical responses reported as disease stability (37.5%).

Two other potential pathogen vector vaccine targets which have been targeted in preclinical trials are MUC1 (41,42) and GUCY2C (guanylyl cyclase C) (43). MUC1 is a glycoprotein expressed on the surface of normal glandular epithelia, it has been found to be overexpressed in >70% of human CRC. MUC1 as a target for cytotoxic T-cells has also been used in vaccination of breast, ovarian, and metastatic renal cancer patients (41). GUCY2C (guanylyl cyclase C) is a transmembrane receptor with canonical functions in maintaining intestinal homeostasis that is

usually only found on intestinal mucosa but is universally located on metastatic CRC (43). These studies provide good preclinical evidence that these targets merit further study in CRC and more research is needed to determine whether these epitopes can be successfully incorporated into vaccination strategies.

Cytokine therapy

Cytokines play a key role in all aspects of the innate and specific immune response. They function through extracellular signaling and operate in an autocrine and paracrine manner (44). Cytokine therapies which are currently being used in cancer treatment include IL-2 which has FDA approval for use in melanoma and renal cell carcinoma (RCC) and also IFN-α which is being studied in the treatment of multiple hematological malignancies as well as cervical cancer, carcinoid syndrome, medullary thyroid cancer, basal cell and squamous cell carcinoma. There have been limited studies involving cytokine therapies in CRC. A phase I study of PEGylated recombinant IL-10 (AM0010) administered daily subcutaneously for 4 months to 33 patients with advanced solid tumors (including four CRC patients) showed that this had a manageable safety profile and resulted in a sustained systemic Th1 immune stimulation (45). One patient with CRC had sustained disease stability greater than 40 weeks. Cytokine therapy represents an avenue that warrants further investigation in CRC and may be effective in combination with other immunotherapies although their toxicity profiles may likely limit their use in standard clinical practice.

TLR agonists

The vast majority of immunotherapeutic interventions in cancer have focused on adaptive immunity yet some recent approaches, including TLR agonists, target the innate immune system (46). Following tumor cell death, damage-associated molecular pattern molecules (DAMPs) are present on TLRs, which are found on innate immune cells (47). Currently ten human TLRs (TLR-1 to TLR-10) have been identified (48) and investigations in CRC have focused on TLR-9. TLR-9 has been shown to have a potential protective role against malignant transformation in colorectal mucosa (49). Early clinical trials implementing the use of CpG-oligodeoxynucleotides (CpG-ODN) as TLR agonists in various cancers were associated with both

narrow clinical efficacy and significant toxicity (50,51). Next generation TLR-9 agonists (such as MGN1703 and double stem loop immunomodulators, dSLIM) have shown more promise than their predecessors (52). A phase II trial evaluated the use of MGN1703 as maintenance therapy in patients with metastatic CRC. Fifty-nine patients with mCRC who had stable disease after standard first-line chemotherapy were randomized to MGN1703 60 mg (n=43) or placebo (n=16). MGN1703 was found to not only be well-tolerated but also to induce durable and prolonged PFS (HR: 0.50; 95% CI, 0.31–1.02; P=0.02) (53). Retrospectively, certain pretreatment characteristics were found to be predictive of improved PFS including normalized CEA, objective response, and the presence of activated NK T cells at the end of induction chemotherapy, were also found to be predictive of OS (HR: 0.63, median 24.5 vs. 15.1 months, not statistically significant) however these data were premature as only 35% patients who received MGN1703 had a survival event (compared to 50% of control patients) (54). A phase III trial, IMPALA trial of MGN1703 is being designed as maintenance therapy in patients with metastatic CRC after standard first-line therapy (ClinicalTrials.gov: NCT02077868).

ACT

Adoptive cell transfer is a form of passive immunotherapy that involves transferring immune cells or molecules to the host. The most successful approaches to ACT have involved harvesting autologous T cells from a tumor (TILs), then activating and expanding them to larger numbers *ex vivo* prior to reinfusing them back to the patient. Modifying T cells in this way allows T cells to overcome immune inhibition and tolerance which may have occurred *in vivo* (55). This method has had success in certain tumor types classically associated to be highly immunogenic, particularly melanoma (56). However, some of the difficulties encountered by ACT are the lack of immune memory, the time and cost associated with producing T cells and poor persistence *in vivo* (6).

Attempts to improve the efficacy of ACT include the genetic modification of T cells to express high avidity for T cell receptors (TCRs) (57). Antibody-based chimeric antigen receptors (CARs) combine intracellular signaling domains with the expression of a single chain variable fragment derived from a tumor-associated antigen capable of recognizing monoclonal antibody (58). There has been considerable interest in CAR T cells due to success with CD19-targeting T cells in hematologic malignancies (58).

Three patients with mCRC were treated with T cells which had been modified to express high avidity for CEA specific murine TCR in a phase I study (59). The murine TCR specifically mediates the recognition of peptide-loaded HLA-A*201+ T2 cells and CEA+ human CRC cell lines *in vitro* and genetically modified autologous T lymphocytes were adoptively transferred into patients with IL-2 after receiving lymphodepleting chemotherapy comprising cyclophosphamide and fludarabine. All three patients experienced reductions in CEA levels (74–99%) and one patient had an objective clinical response (partial) although they all developed severe transient inflammatory colitis constituting grade 3 diarrhea with subsequent halting of study accrual. It is plausible that colonic flora contribute to the development of colitis in the presence of lymphopenic conditions due to TLR stimulation. The potential toxicities of inadvertently targeting expression of antigens in normal tissues were also seen in a case report of a patient with mCRC who was treated with Her2-specific CAR T cells (60). In this case, a patient developed severe respiratory distress shortly after the infusion of 1×10^{10} autologous CAR T cells over-expressing *ERBB2* resulting in the death of this patient possibly due to a cytokine storm triggered by the recognition of native *ERBB2* expression in normal lung tissue. A phase I/II study of adjuvant immunotherapy involving sentinel lymph node (SLN) T lymphocytes in 55 patients with mCRC did not observe any treatment-related toxicity (61). At 24 months, 55.6% of the SLN T lymphocyte group were alive vs. 17.5% of the control group (P=0.02). Overall survival was significantly improved in those who received SLN-T lymphocyte compared to control (28 vs. 1 month, respectively). This study represents a significant breakthrough in CRC ACT however, further studies and a phase III study is needed to further assess efficacy.

Monoclonal antibody-based therapy

Monoclonal Abs have been shown to have clinical utility in the treatment of metastatic CRC (62). These Abs incur significant production costs and can cause hypersensitivity reactions in patients due to foreign (murine) protein recognition. Abs which have been approved include: cetuximab (63) or panitumumab (64) which targets EGFR and anti-VEGF Abs bevacizumab (65) and aflibercept (66).

Table 1 Ongoing trials involving novel monoclonal antibody targets

Target	Antibodies	Trial identifier
EGFR	SCT200	NCT02211443
	Nimotuzumab	NCT02508077
	Sym004	NCT00972465
	GC1118	NCT01899118, NCT02083653, NCT02352571
VEGF	Sevacizumab	NCT02453464
Tumor associated antigens	NPC-1	NCT01040000
CD137	Urelumab	NCT01471210, NCT02110082
4-1BB (CD137, TNFRSF9)	Pf-05082566	NCT01307267
CD27	CDX-1127 (varlilumab)	NCT01460134
Anti-oxidized macrophage migration inhibitory factor	Imalumab (Bax69)	NCT02448810
CEACAM1	CM-24	NCT02346955

Regorafenib, a tyrosine kinase inhibitor, has also been approved for use in refractory metastatic CRC (67). There are several ongoing clinical trials involving these targeted therapies in CRC (*Table 1*). An in-depth discussion of the use of monoclonal Abs is beyond the scope of this review.

Checkpoint inhibition

The most notable development in immunotherapy in the last decade has been the emergence of checkpoint inhibitors. Checkpoint inhibitors are monoclonal Abs which modify MHC-TCR signaling pathways by targeting co-inhibitory molecules such as PD-1, PD-L1/2, CTLA-4, lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin mucin-3 (TIM-3), and B and T lymphocyte attenuator (BTLA). Co-inhibitory molecules suppress the immune system by inducing T-cell apoptosis or dysfunction. Tumors harness these pathways in order to evade the human immune system (68).

CTLA-4

CTLA-4 is a receptor found on the surface of CD4 and CD8 T cells. It prevents immune stimulation by binding to B7 ligands on antigen presenting cells (APCs) and preventing immune stimulation which is normally provided by B7 binding to a CD28 receptor on T cells (69). Ipilimumab and tremelimumab are CTLA-4 inhibitors which have been developed for use in humans. Ipilimumab was approved by the FDA in 2011 for use in metastatic melanoma when two landmark phase III clinical trials showed that it was associated with improved survival (4). However these checkpoint inhibitors have failed to demonstrate similar success in CRC. A phase II trial administered with tremelimumab IV every 90 days until progression to 47 patients who had failed standard therapies (70). This treatment was relatively well tolerated in comparison to checkpoint inhibitor studies with 63.8% experiencing treated-related adverse events and 19.1% of patients had ≥ grade 3 toxicities. The vast majority of patients (n=44) did not receive a second dose due to progressive disease with only a single partial response recorded. Although this study included heavily pre-treated patients, it does not suggest further study of CTLA4 blockade as monotherapy in metastatic CRC.

PD-1

PD-1 belongs to the same CD28 receptor family as CTLA-4, while CTLA-4 prevents naïve T cell activation, PD-1 mainly induces exhaustion or anergy in effector T cells. PD-1 is particularly over-expressed in chronic inflammatory states and malignancy (71) and PD-1 inhibitors have been associated with impressive durable clinical responses in non-small cell lung cancer, RCC and bladder cancer (72-77).

Until recently however only a minority of patients with

CRC demonstrated significant responses to PD-1 blockade. A phase I study looked at 39 patients with multiple tumor types including metastatic melanoma, CRC, castrate-resistant prostate cancer, NSCLC, or RCC. One durable complete response (CRC) and two partial responses (melanoma, RCC) were observed in this study. A phase II study looked at 296 patients with similar tumor types and response rates for NSCLC, melanoma and RCC were 18%, 28% and 27% respectively while no objective response was seen in patients with CRC or prostate cancer (76). The results of these trials raised important questions regarding the low response rates in patients with CRC and what factors distinguished the single patient who did respond from other patients with CRC (78).

It was hypothesized that the response rates were directly related to the mutational burden of the tumor (78). CRC is usually associated with a low mutational burden unless the tumor is deficient in mismatch repair (dMMR) (also known as microsatellite instability). The solitary patient with CRC who had responded to the PD-1 inhibitor in the trial was subsequently found to have dMMR. A phase II trial was conducted in order to test this hypothesis which aimed to determine the clinical activity of pembrolizumab (PD-1 inhibitor) in 41 patients with mCRC with or without dMMR. Patients with dMMR had improved response rates compared to those without dMMR (78% *vs.* 40%) and immune-related progression free survival rates were also improved in those with dMMR CRC (11% *vs.* 0%). A significant difference in somatic mutations per tumor was noted following whole-exome sequencing with a mean of 1,782 somatic mutations in the dMMR cohort *vs.* 73 somatic mutations proficient mismatch repair (pMMR) tumors (P=0.007). This increase in mutational burden was in turn found to be associated with prolonged overall survival (P=0.02). More research is needed to determine the role of checkpoint inhibitors in those with proficient mismatch repair CRC, particularly finding strategies to overcome resistance to immune checkpoint blockade CRC.

Combination therapy

Due to the complex nature of tumorigenesis and modest levels of success of immunotherapies as monotherapy, recent trials have investigated whether clinical benefits can be improved using combinatorial therapies. Preclinical trials have had some success with various combinations of radiofrequency ablation, vaccines and checkpoint inhibitors. In patients with metastatic melanoma, a phase I study combined nivolumab and ipilimumab in 53 patients and found that the maximum tolerated dose induced an objective response in 53% of patients (80% or more reduction in tumor growth) (79). There are numerous ongoing trials examining the utility of combination immunotherapy for patients with advanced CRC (*Table 2*). The major challenge with these trials is to improve clinical benefit while developing regimens with acceptable toxicity profiles. As oncologists increasingly prescribe immunotherapeutic agents, it is imperative that they optimize patient safety by recognizing and promptly managing the autoimmune complications that can occur with these agents (80). In addition, the future landscape of immunotherapy in CRC will likely incorporate combinations of immunotherapeutic drugs with non-immunotherapeutic agents e.g., anti-angiogenic drugs (81).

Conclusions

Currently the prognosis for individuals with metastatic CRC remains poor. There has been limited success with traditional immunotherapeutic approaches in the past. However, immunotherapy now represents a possible avenue for improving survival for a subset of patients with CRC. Our understanding of the interaction between the immune system and the tumor microenvironment has progressed substantially in the last decade and this has resulted in the development of novel targeted therapies such as checkpoint inhibitors which have been shown significant promises. The success of these novel therapies has largely been confined to malignancies which are associated with a high mutational burden such as RCC, melanoma and non-small cell lung cancer. However, this success has been replicated in patients with deficient mismatch repair CRC. Ongoing trials in CRC are focused on expanding the use of these new immunotherapies and combining these therapies aiming to improve clinical outcomes. The challenge remains in identifying subsets of patients who are likely to respond to these therapies in addition to producing novel regimens with acceptable toxicity profiles.

Table 2 Future and ongoing/recently completed trials of immunotherapy in colorectal cancer

Trial identifier	Sponsor/collaborator	Title	Phase
Vaccines			
NCT01952730	Massachusetts General Hospital	A pilot safety study of vaccination with autologous, lethally irradiated colorectal cancer cells engineered by adenoviral mediated gene transfer to secrete human granulocyte-macrophage stimulating factor	I
NCT01461148	Oryx GmbH & Co. KG	Phase I/IIa study of immunization with frameshift peptides administered with Montanide® ISA-51 VG in patients with advanced MSI-H colorectal cancer	I/IIa
NCT01376505	Ohio State University Comprehensive Cancer Center	Phase I active immunotherapy trial with a combination of two chimeric (trastuzumab-like and pertuzumab-like) human epidermal growth factor receptor 2 (HER-2) B cell peptide vaccine emulsified in ISA 720 and nor-MDP adjuvant in patients with advanced solid tumors	I
NCT01734564	Universidad de Navarra	Phase II study with hiltonol and dendritic cells in solid tumors	II
NCT01348256	Universidad de Navarra	Randomized phase II study with dendritic cell immunotherapy in patients with resected hepatic metastasis of colorectal carcinoma	II
NCT01380600	Jennerex Biotherapeutics	A phase Ib dose escalation study of JX-594 (thymidine kinase-inactivated vaccinia virus plus GM-CSF) administered by biweekly (every 2 weeks) intravenous infusion in patients with metastatic, refractory colorectal carcinoma	Ib
NCT01890213	Duke University	A pilot study of active immunotherapy with CEA(6D) VRP vaccine (AVX701) in patients with stage III colorectal cancer	I
Adoptive cell therapy			
NCT02202928	Jingzhou Central Hospital	Phase II study of autologous tumor lysate-pulsed DC-CIK cell in colorectal cancer after surgery	II
Checkpoint inhibitors-CTLA4			
NCT00313794	AstraZeneca	Phase II, single arm study of ticilimumab in patients with refractory metastatic adenocarcinoma of the colon or rectum	II
NCT00378482	AstraZeneca	A rollover protocol for patients who received CP-675,206 in other protocols	II
Checkpoint inhibitors-PD1			
NCT02013804	MedImmune LLC	A phase I, multicenter, open-label study to evaluate the safety, tolerability, and pharmacokinetics of MEDI0680 (AMP-514) in subjects with advanced malignancies	I
NCT02404441	Novartis Pharmaceuticals	Open label multicenter phase I/II study of the safety and efficacy of PDR001 administered to patients with advanced malignancies	I/II
NCT02460198	Merck Sharp & Dohme Corp.	A phase II study of pembrolizumab (MK-3475) as monotherapy in subjects with previously treated locally advanced unresectable or metastatic (stage IV) mismatched repair deficient or microsatellite instability-high colorectal carcinoma (KEYNOTE-164)	II
NCT01375842	Genentech, Inc.	A phase I, open label, dose escalation study of the safety and pharmacokinetics of MPDL3280A administered intravenously as a single agent to patients with locally advanced or metastatic solid tumors or hematologic malignancies	I

Table 2 (continued)

Table 2 (*continued*)

Trial identifier	Sponsor/collaborator	Title	Phase
Checkpoint other			
NCT01943461	Merck KGaA	A phase I trial to investigate the tolerability, safety, pharmacokinetics, biological and clinical activity of avelumab (MSB0010718C) in Japanese subjects with metastatic or locally advanced solid tumors, with expansion part in Asian subjects with gastric cancer	I
NCT01772004	EMD Serono	A phase I, open-label, multiple-ascending dose trial to investigate the safety, tolerability, pharmacokinetics, biological and clinical activity of avelumab (MSB0010718C) in subjects with metastatic or locally advanced solid tumors and expansion to selected indications	I
NCT01115790	Eli Lilly and Company	A phase I study of LY2606368 in patients with advanced cancer	I
Combination therapy			
NCT02419677	The First People's Hospital of Changzhou	Phase III study of radiofrequency ablation combined with cytokine-induced killer cells for colorectal cancer liver metastases	III
NCT01714739	Bristol-Myers Squibb	A phase I dose escalation and cohort expansion study of the safety, tolerability and efficacy of anti-KIR (lirilumab) administered in combination with anti-PD-1 (nivolumab) in patients with advanced solid tumors	I
NCT02298946	National Cancer Institute (NCI)	A pilot study of AMP-224, a PD-1 inhibitor, in combination with stereotactic body radiation therapy (SBRT) in patients with metastatic colorectal cancer	I
NCT02335918	Celldex Therapeutics	A phase I/II dose escalation and cohort expansion study of the safety, tolerability and efficacy of anti-CD27 antibody (varlilumab) administered in combination with anti-PD-1 (nivolumab) in advanced refractory solid tumors	I/II
NCT02118337	MedImmune LLC	A phase I, open-label study to evaluate the safety and tolerability of MEDI0680 (AMP-514) in combination with MEDI4736 in subjects with advanced malignancies	I
NCT02298946	NCI	A pilot study of AMP-224, a PD-1 inhibitor, in combination with SBRT in patients with metastatic colorectal cancer	I
NCT01975831	Ludwig Institute for Cancer Research	A phase I study to evaluate the safety and tolerability of anti-PD-L1, MEDI4736, in combination with tremelimumab in subjects with advanced solid tumors	I
NCT01375842	Genentech, Inc.	A phase I, open label, dose escalation study of the safety and pharmacokinetics of MPDL3280A administered intravenously as a single agent to patients with locally advanced or metastatic solid tumors or hematologic malignances	I
NCT01966289	Sidney Kimmel Comprehensive Cancer Center	A pilot study of SGI-110 in combination with an allogeneic colon cancer cell vaccine (GVAX) and cyclophosphamide (CY) in metastatic colorectal cancer (mCRC) as maintenance therapy	I
NCT02460224	Novartis Pharmaceuticals	A phase I/II, open label, multicenter study of the safety and efficacy of LAG525 single agent and in combination with PDR001 administered to patients with advanced malignancies	I/II

GM-CSF, granulocyte macrophage colony-stimulating factor; CEA, carcinoembryonic antigen; PD-1, programmed cell death protein-1; PD-L1, programmed cell death ligand-1.

Acknowledgements

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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