Am J Digest Dis 2015;2(1):11-28
www.ajdd.us /ISSN:2329-6992/AJDD0011566

Review Article

Immunotherapy for colorectal cancer

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Received June 17, 2015; Accepted July 5, 2015; Epub August 7, 2015; Published August 15, 2015

Abstract: Immunotherapy for colorectal cancer has been under investigation for many years. Many recent scientific advances contribute to the success of cancer immunotherapy, including our better understanding of anti-tumor immune response, tolerance and regulation, and the advent of newer therapies including agonist antibodies to the co-stimulatory molecules targeting T cells. Immunotherapy may represent a novel effective treatment for colorectal cancer patients. Many preclinical data have provided the basis of immunotherapy of colorectal cancer, demonstrating the potential benefit for patients. With the success of immunotherapy such as ipilimumab and programmed death-1 (PD-1) pathway-targeted agents, activating the immune system for the treatment of colorectal cancer has become more promising. In this article, we review the immune responses against tumors and summarize the preclinical and early clinical data on colorectal cancer immunotherapy. We will also discuss possible strategies and recent progress in immunotherapy for colorectal cancer, in particular, vaccines, passive transfer of anti-cancer monoclonal antibodies and donor T cells. Continued advances in antibody and T cell engineering should further help improve treatment outcomes of colorectal cancer.

Keywords: Colorectal cancer, immunotherapy, review, PD-1

Introduction

Colorectal cancer (CRC) is one of the 3 most commonly diagnosed cancers among both men and women worldwide. With 140,000 new cases in US every year and account for 50,000 deaths in 2014 [1]. Innovative surgical approaches and effective chemotherapy are the mainstream therapeutic modalities for colorectal cancer to optimize patient's outcome. Local tumor ablative therapies, such as microwave coagulation and radiofrequency ablation therapy are provided as adjunct therapy for resection. However, in patients with distant metastasis, survival decreases [1]. The chemotherapy has shown modest efficacy against regionally metastatic colorectal cancer and are not effective against distant metastases [2, 3].

Besides, surgery and chemotherapy produce side effects limiting their use in CRC [4, 5]. Recently, evidence showed that the antibody, ipilimumab enhanced the ability of endogenous T cells to eliminate a variety of malignancies and improved patient survival. Moreover, clinical trials of antibodies against another T cell checkpoint molecule PD-1 have improve clinical survival in many tumor types such as melanoma, lung cancer, stomach cancer [6].

Successes in immunotherapy of melanoma and prostate cancer have resulted in the interest of immunotherapy for colorectal cancer, with some promising results. The Sipuleucel-T, an autologous active cellular immunotherapy, has shown evidence of efficacy in reducing the risk of death in prostate cancer. This success has also contributed to revitalization of the immunotherapy [7, 8]. Incorporation of various immunotherapeutic strategies is imperative to potentiate the immune system to allow effective recognition and killing of tumors because cancers possess a variety of strategies to evade immune surveillance and elimination.

Immunotherapies for CRC include the following strategies: monoclonal antibody against cancer antigens, cancer vaccines, and adoptive cell therapy. Recently antibodies against immune check point of T cell immunity and TNF family have demonstrated encouraging effect in other tumor and thus are under investigation against CRC. Immunotherapy has become an interesting adjuvant therapy for the treatment and eradication of colorectal cancer.

Colorectal cancer-associated antigens and antibody

Cancer related antigens are the targets of CRC immunotherapy, including carcinoembryonic antigen (CEA), MUC1 and Guanylyl cyclase C (GUCY2C, GCC). Theoretically, in tumors that have a mutational load above 10 somatic mutations per megabase (Mb) of coding DNA, it is sufficient to produce neoantigens that can be seen by T cells. Formation of neoantigens that can potentially be recognized by autologous T cells is expected to be common for colorectal cancer [9]. However, in CRC, mutated peptides predicted to bind to autologous MHC class I molecules are less frequent than expected, suggesting cancer immunoediting, and immune-mediated elimination [10]. CEA is a cell membrane-associated protein and is involved in cell adhesion. CEA promotes tumor cell aggregation and spread [11]. Its widespread expression in many other tissues may contribute to its immune tolerance. Thus, CEA-based vaccine elicited poor immune responses. MUC1 is another transmembrane glycoprotein on the surface of secretory epithelial cells. Its overexpression on adenocarcinomas, including cancers of the breast, lung, colon, pancreas, stomach, prostate, and ovary, results in a loss of polarization and altered glycosylation, making it a potential target for multiple types of cancer [12]. The overexpression and abnormal glycosylation of MUC1 is seen in many colorectal adenomas and is associated with a poor prognosis [13]. Recently, in patients without cancer but with premalignant lesions (advanced colonic adenomas, precursors to colon cancer), a vaccine based on the MUC1 has elicited MUC1-specific immune response without autoimmunity and induced long term memory that is important for cancer prevention [14]. Guanylyl cyclase C (GUCY2C, GCC) is a receptor for the endogenous hormones- guanylyl and uroguanylin [15]. GUCY2C regulates the intestinal epithelial-mesenchymal homeostasis. Oral GUCY2C is suggested for treatment for gastrointestinal disorders [16]. One study showed the ability of adenovirus expressing GUCY2C to generate protection against colon cancer metastases in mice [17]. Moreover, other antigens, such as Her2/neu, Sialyl-Tn, survivin and mutated p53 and K-ras, have been investigated in colorectal cancer, but have no significant effect.

Monoantibody (mAb) therapy is the most common immunotherapy for GI cancers. Compared with conventional chemotherapy, which eliminates not only tumor cells but also mitotically active normal cells, mAb therapy only targets tumor-antigen and therefore has less severe adverse effects [18]. Four mAb therapies targeting GI malignancies have been approved by FDA: bevacizumab, cetuximab, panitumumab, and trastuzumab [19]. Antibodies against tumor associated antigens therapy has shown efficacy for the treatment of colon cancer for many years [20]. Currently, tumor-targeting mAbs used in CRC include: (1) cetuximab, targeting the epidermal growth factor receptor (EGFR), can inhibit cancer cell-intrinsic vital signal transduction pathways [21]. Cetuximab not only inhibits EGFR signaling, but also triggers complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) [22] and it has a direct immunostimulatory activity [23]. Cetuximab has modest single-agent efficacy in the treatment of patients with metastatic CRC whose tumors do not harbor a KRAS mutation. In combination with topoisomerase I inhibitor irinotecan, it is associated with an overall survival (OS) and progression-free survival (PFS) advantage in first-line therapy in patients with KRAS non mutant metastatic CRC; it can be combined with irinotecan to overcome resistance in patients with KRAS non mutant CRC who have previously progressed on prior irinotecan chemotherapy. Other substance like low molecular weight tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib can also be used in combination therapy targeting EGFR. The coadministration of cetuximab and erlotinib synergistically suppressed the growth of colon cancer cell lines, achieved promising efficacy in patients with KRAS non-mutant metastatic CRC, and merits further evaluation in randomized studies [24].

(2) Panitumumab is another antibody targeting EGFR that is approved for first-line therapy for

EGFR-expressing metastatic colorectal carcinoma [25]. Although when compared with irinotecan alone, addition of panitumumab to irinotecan in pretreated advanced colorectal cancer did not improve the overall survival of patients with wild-type KRAS tumors [26], recent study proved that panitumumab-FOLFOX4 significantly improved progression-free survival versus FOLFOX4 in wild type KRAS patient with metastatic colon cancer and support the clinical use of panitumumab-FOLFOX4 for patients with previously untreated wild type KRAS metastatic colon cancer. Also, KRAS testing is critical to the selection of appropriate patients for treatment with panitumumab [27]. The Kirsten rat sarcoma viral oncogene (KRAS) has been proven to be a crucial factor in predicting of the outcome of anti-EGFR treatment in colorectal cancer [28].

(3) Bevacizumab, targeting vascular endothelial growth factor (VEGF) and interfering with the trophic interaction between neoplastic cells and the tumor stroma, is approved for use in patients affected by CRC. Bevacizumab has been approved for use in combination with fluoropyrimidine/irinotecan- or fluoropyrimidine/oxaliplatin-based chemotherapy for the treatment of patients with metastatic CRC that is resistant to first-line bevacizumab-based therapy [29].

Bevacizumab, cetuximab and panitumumab have demonstrated efficacy in combination with chemotherapy in the first-line therapy of advanced colorectal cancer. Anti-EGFR therapy showed a superior effect in both KRAS-WT and all RAS-WT patients with advanced CRC [30]. A recent study showed that bevacizumab improves pathological response of colorectal cancer liver metastasis and pathological response may represent a strong predictor of survival. New prospective trials are needed to confirm the prognostic role of pathological response. This will help identify a population of patients who could benefit from further bevacizumab-based treatment [31].

Immunomodulatory monoantibody therapies for CRC (Table 1)

*Anti-CTLA4 (**Figure 1**)*

Immunotherapies enhancing the ability of endogenous T cells to eliminate cancer cells have shown clinical efficacy in a variety of human malignancies. Preclinical mouse tumor models and to human melanoma has demonstrated that endogenous T cell compartment could help control tumor growth [32]. A clinical trial utilizing ipilimumab, an antibody that targets the T cell checkpoint protein-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), improved clinical survival of patients with melanoma [33] and at least part of this clinical activity could be attributable to the function of cytotoxic T cells [34], indicating that in a substantial fraction of patients, the endogenous T cell compartment is able to recognize antigens presented on major histocompatibility complexes (MHCs) on the surface of the tumor cells [9] CTLA4 is a negative regulator expressed on the surface of all T cells. CTLA4 normally binds to B7 (CD80/CD860 on the surface of antigen-presenting cells, providing a powerful brake or checkpoint that limits the activation and proliferation of T cells. Ipilimumab blocks this CTLA4/B7 interaction, thereby release the checkpoint and amplify T cell responses. The mechanism of how anti-CTLA4 changes the immunosuppressive tumor microenvironment is still being characterized [35, 36]. Anti CTLA4 monoantibody (mAb) therapies are currently being explored for GI malignancies.

Another CTLA-4-blocking antibody, tremelimumab, was also investigated in clinical trials. Tremelimumab has also been studied in Phase II trials of patients with metastatic colorectal, gastric, esophageal cancers, alone or in combination with other anticancer therapies [37, 38]. In patients with metastatic gastric and esophageal cancer, enhanced proliferative responses to CEA and 5T4 antigen have been demonstrated, indicating the need of further investigation that combines CTLA4 blockade with antigen-targeting therapy [37]. However in colorectal cancer, no obvious clinical benefit has been demonstrated by tremelimumab treatment [38]. In preclinical research, combination of anti-CTLA4 and anti CD25 improved the potency of DC-based vaccine, evidenced by augmented tumor-free survival and the development of long-lasting immune responses [39]. Also it has been indicated that radiotherapy could increase the presentation of antigen by myeloid cells within the tumor stroma and thereby enhance T-cell killing of tumor cells. Combination of anti CTLA4 and radiotherapy improved survival of patients with melanoma.

Table 1. Studies applying immunomodulatory therapy for the treatment of colorectal cancer

Study	Phase	Target	Subject	Therapy	Results	Conclusions
Chung KY, 2010	II	CTLA4	47 patients	Patients who are resistant to other therapies received tremelimumab intravenously every 3 months until progression.	Most evaluable patients only received one dose. Half patients (45%) lived ≥ 6 months after enrollment. One patient had a stable pelvic mass and substantial regression in an adrenal mass (partial response).	Tremelimumab did not show significant effects.
Saha A, 2010	Preclinical	CTLA4 and CD25	Mouse	Antibodies combined with dendritic cell-based vaccine.	This antibodies-plus-vaccination strategy dramatically improved the tumor-free survival and allowed the development of long-lasting immune responses.	Simultaneous blockade of T-cell regulatory pathways is a promising approach for the induction of antitumor immunity against CRC.
Topalian SL, 2012	II	PD-1	19 CRC out of 296 cancer patients.	Anti-PD-1 antibody were given every 2 weeks. Patients received up to 12 cycles.	Anti PD-1 mAb has not demonstrated objective responses in colorectal cancer but yielded clinical benefit in other cancers. There was one drug-related death due to pneumonitis in patients with colorectal cancer.	Anti-PD-1 antibody produced objective responses in approximately 20% patients of all cancer patients. Its efficacy is correlated with PD-L1 expression on tumor cells.
Brahmer JR, 2012	II	PD-L1	18 CRC out of 207 of all cancer patients.	Anti PD-L1 antibody was administered every 14 days in 6-week cycles for up to 16 cycles or until the patient had a complete response or confirmed disease progression.	Anti PD-L1 promotes tumor regression and disease stabilization. However, no objective responses in CRC.	Anti PD-L1 induced objective response and prolonged stabilization of disease in lung cancer melanoma, and renal-cell cancer patients.
Stewart R, 2015	Preclinical	PD-L1	Mouse	Monotherapy and combination with oxaliplatin.	Anti-mouse PD-L1 significantly improved survival of mice implanted with CT26 colorectal cancer cells.	Inhibition of PD-L1 leads to potent antitumor activity when used as monotherapy or in combination in preclinical models.
Dovedi SJ, 2014	Preclinical	PD-1 and PD-L1	Mouse	Radiotherapy combined with anti PD-L1.	Radiotherapy delivered in combination with αPD-1 or αPD-L1 mAbs improved local tumor control, long-term survival, and protection against tumor rechallenge.	Combination of radiotherapy and blockade of PD-1/PD-L1 signaling may improve CRC treatment outcome.
Mitsui J, 2010	Preclinical	GITR and CTLA-4	Mouse	Combination and monotherapy of each antibodies were tested in CRC model and Treg-mediated immune suppression was analyzed.	Anti-CTLA-4/anti-GITR mAb combination treatment exhibited far stronger antitumor effects than single antibody therapy.	Combined treatment with anti CITR and anti CTLA-4 can augment antitumor immune responses and should be explored in treatment in the clinic.
Pan PY, 2002	Preclinical	OX40	Mouse	Combination therapy with adenovirus-mediated gene delivery of interleukin 12 (IL-12) + anti-4-1BB + anti OX40 agonistic antibody.	Combination therapy of IL-12, anti-4-1BB, and anti-OX40 resulted in a significantly higher survival rate.	Combination therapy with the adenovirus encoding IL-12 (Adv. mIL-12) + anti-4-1BB + anti-OX40 antibodies may provide useful treatment modality for advanced CRC.
Adarini S, 2004	Preclinical	OX40 Ligand	Mouse	Intratumoral injection of a recombinant adenovirus vector expressing mouse OX40L (AdOX40L).	Anti-OX40L induced a significant suppression of tumor growth along with survival advantages in mice.	Modification of tumor cells with a recombinant OX40L adenovirus vector could provide benefit in cancer treatment.
Zhang Y, 2013	Preclinical	OX40	Mouse	Genetic depletion of OX40.	Tumors show reduced growth which is accompanied by increased T cell and NK cell infiltration, more vigorous Th1 cytokine response, and increased cytolytic T cell response.	Interaction between OX40 on T cells and OX40-ligand on B cells may be important in modulating anti-tumor immune response.

Note: CRC, colorectal cancer.

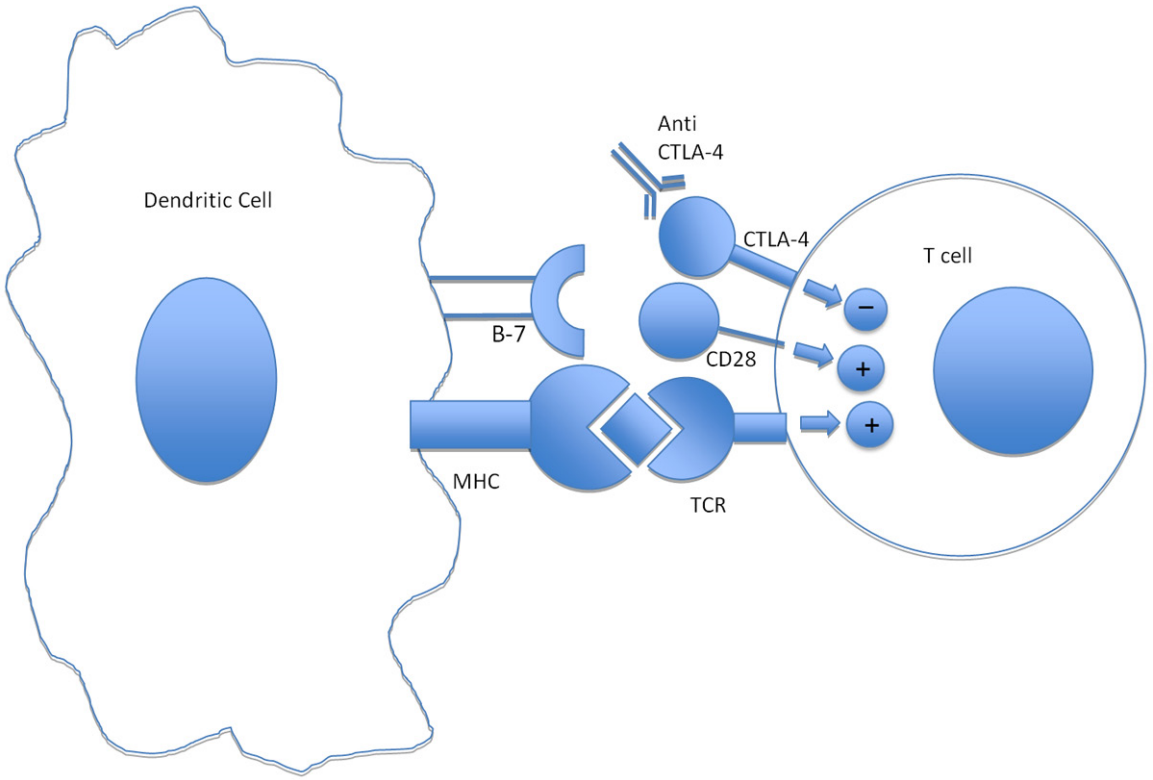

Figure 1. Mechanism of anti-CTLA-4. B7 and CTLA4 interaction leads to inhibition of T cell activation by dendritic cell. By blocking CTLA-4, antibody enhance T cell activation and expansion and potentiate the antitumor immunity. CTLA-4, cytotoxic T-lymphocyte-associated antigen-4; MHC, major histocompatibility complex; TCR, T cell receptor.

*Anti-PD-1 and anti PD-L1 antibody (**Figure 2**)*

Programmed death-1 (PD-1) is another negative regulator of T cells, with its ligands being PD-L1 and PD-L2 [40]. PD-1 acts to prevent the unrestrained activation of T cells that have been previously activated. Compared with anti-CTLA4, which potentiates the production of T cells of all specificities in lymph nodes, anti PD-1 or PD-L1 only act to overcome immunosuppression of antitumor T cells in the tumor bed. Antibodies against PD-1 and PD-L1 are under clinical investigations. PD-L1 is expressed by tumor cells or immune cells infiltrating into tumors. It prevents T cell hyperactivation. Tumor cells express PD-L1 to protect themselves against T cell killing by inhibiting tumor-specific IFN-gamma secreting cytotoxic T cells. In tumor bed, the binding of tumor PD-L1 to PD-1 on activated T cells exhausts T cells' ability to produce or use cytotoxic granules [41]. Therefore, antibodies against either PD-1 or PD-L1 can recover the T cell cytotoxic activity, leading to tumor regression [36, 42]. Nivolumab, an anti-PD-1 mAb was evaluated in a Phase I/II study in 296 patients with pretreated NSCLC, prostate cancer, renal cell carcinoma, colorectal cancer and melanoma. Nivolumab has not demonstrated objective responses in colorectal cancer and there was one drug-related death due to pneumonitis in patients with colorectal cancer [43].

Preliminary data suggested that a positive intratumoral PD-L1 expression is associated with objective response. Anti-mouse PD-L1 significantly improved survival of mice implanted with CT26 colorectal cancer cells [44]. In addition, combining anti PD-L1 and radiotherapy has shown signs of success [45].

Clinical responses were also seen in patient population with non-small-cell lung cancer, melanoma, colorectal cancer, renal-cell cancer, ovarian cancer, or pancreatic cancer after anti PD-L1 treatment. However, there's no objective response in patients with colorectal or pancreatic cancer [46]. Different anti-PD-L1 agents are currently being investigated in cancer patients. Data indicates that these monoclonal

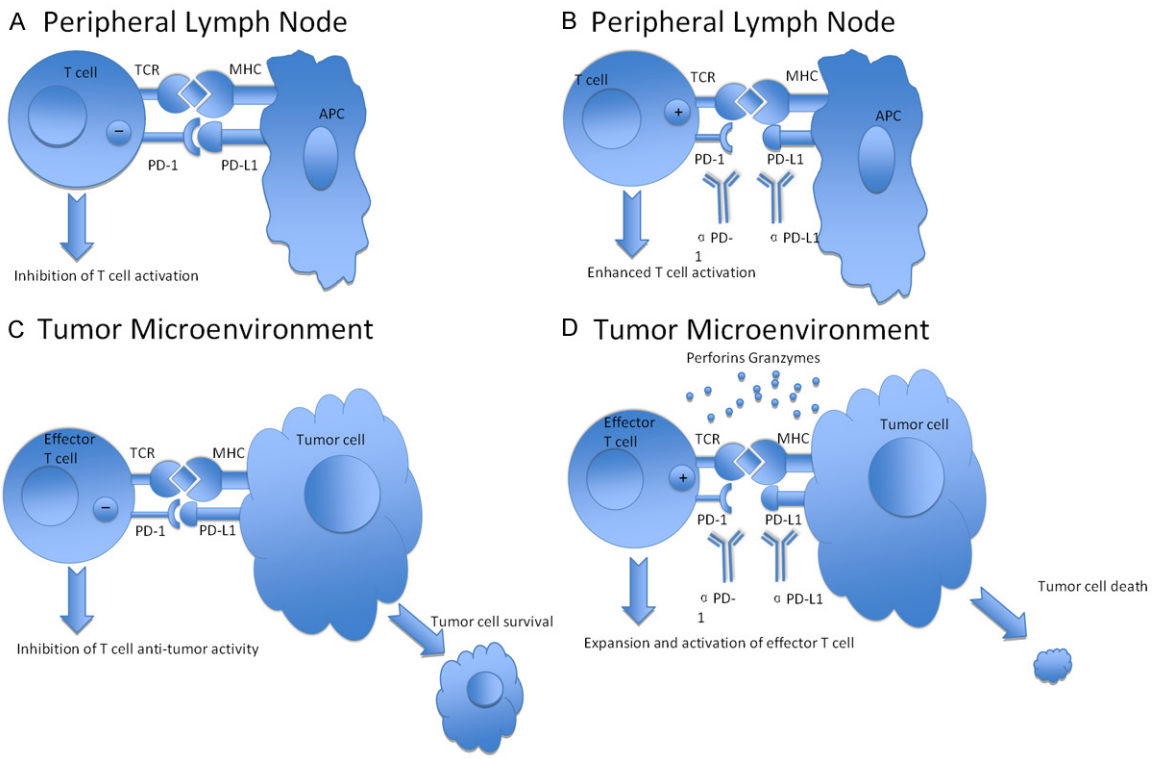

Figure 2. Mechanisms of anti-PD-1 and anti-PD-L1. PD-1 and PD-L1 interaction leads to inhibition of T cell activation by APC (A) or suppression of the antitumor immunity of of T cell (c). By blocking PD-1 and PD-L1 interaction between T cell and APC (B), antibodies enhance T cell activation and expansion and potentiate the antitumor immunity (D). PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T cell receptor.

antibodies are safe in multiple tumor types [47].

Growing evidence has shown that chemotherapy, local radiation and simultaneous blockade of immune checkpoints can synergize to promote antitumor immunity, resulting in improved clinical outcome in animals and patient [48-51].

Antibodies against tumor necrosis factor receptor family

Target of monoclonal antibody therapy for clinical development has been identified in ed to some members of the tumor necrosis factor receptor (TNFR) family.

Recent clinical trials on 4-1BB (CD137), OX40, glucocorticoid-induced TNFR-related gene (GITR), herpes virus entry mediator (HVEM), and CD27 seemed promising [52]. Agonist antibodies to these co-stimulatory molecules target both T and B cells, modulating T-cell activation and enhancing immune responses.

GITR (CD357)

Glucocorticoid-induced tumor necrosis factor (TNF) receptor related gene (GITR), expressed at low levels on resting T cells (including Tregs), DC, monocytes, and NK cells, is up regulated after stimulation. GITR ligand (GITRL) is highly expressed on activated APCs and endothelial cells [53]. Both GITR and its ligand are involved in immunomodulation. It has been shown that through the effect on both the effector T cell and regulatory T cells, GITR is able to induce immune response and promote tumor rejection [54]. Monoantibody against GITR provides immunostimulatory signals. Combination of GITR antibody with anti-CTLA4 and T cell transfer therapy improved immunity against fibrosarcoma and colorectal cancer in mice [55].

OX40 (CD134)

OX40 (CD134) is a tumor necrosis factor (TNF) receptor expressed primarily on activated T cells, neutrophils, dendritic cells, and Tregs [56]. Ligation of OX40 and its ligand transmits

a potent costimulatory signal. OX40 promotes T-cell activation, survival, proliferation, and cytokine production [57] and has been shown to promote both inhibition of colon cancer in mice [58] and eradication of hepatic metastases [59]. Also, adenovirus vector-mediated in vivo gene transfer of OX40 ligand to tumor cells enhances antitumor immunity in colon cancer bearing mice [60]. However, there is no report of clinical trial on GITR and OX40.

Cancer vaccines

Cancer vaccines are active therapeutic approaches based on immunizing cancer patients using whole tumor cells, dendritic cell, peptide, DNA, and viral vector-based vaccines. However, only very few suppressions of solid cancers progression have been reported by using this approach. The difficulty in identifying antigen target for vaccine and in designing vaccines that are capable of inducing anti-tumor response presents a great challenge for the vaccine development. Even as tumor specific T cells response could be induced by immunization, tumor progression can still occur due to the inhibitory endogenous factors or the low frequency of these tumor reactive T cells [61]. This has tempered the enthusiasm in developing cancer vaccine for CRC. Therapeutic vaccines in cancer have two objectives: one is transitioning from no immunity to immunity by generating new CD4+ or CD8+ T effector cells by "priming" a new immune response whereas the other is the modulation or reprogramming of memory cells, i.e., transition from one type of immunity to another (e.g., regulatory to cytotoxic) [62].

Autologous tumor cell vaccines

A strategy in anti-cancer therapies has been the use of cancer cells to develop vaccines. Cancer cells provide surface antigens that are targets for a desired immune response. Autologous tumor cell vaccines are tumor cells collected from patients, processed with adjuvant bacillus CalmetteGuérin (BCG) or infected with virus, and are re-administered to the patient.

Autologous tumor-based vaccination has led to some successes in the treatment of CRC. The National Surgical Adjuvant Breast and Bowel Project (NSABP) C-01 phase III trial demonstrated that tumor vaccine and BCG therapy improved overall survival in patients with resected Dukes' stage B or C colon adenocarcinoma [63]. After a 10-year follow-up, the authors concluded that there was no difference in disease-free survival but a statistically significant increase in overall survival for patients who received BCG. Tumor cell vaccines utilizing tumor cell lysates and adjuvant bacterial cell wall products did not show clinical activity [64].

An autologous tumor cell based vaccine, OncoVAX, was developed with irradiated tumor cells and adjuvant BCG.A multicenter, randomized controlled phase III clinical trial in Stages II and III colon cancer patients showed that OncoVAX improved not only recurrence-free interval, overall survival, and recurrence-free survival in Stage II colon cancer patients, but also provided health economics benefits [65] and reduction in recurrence [66]. This strategy is still undergoing evaluation.

Another tumor cell based vaccine involves irradiated tumor cells infected with Newcastle disease virus to produce an adjuvant effect. It has significantly augmented survival rate in patients with resected colorectal cancer, compared to vaccines using BCG [67].

A randomized phase III study of patients with resectable CRC liver metastases demonstrated no benefit in prevention of recurrence but showed increase in overall and metastases-free survival [68]. However, cancer cell-based vaccines has not produced significant benefits because they also contain many undesirable nonspecific antigens and intracellular contents that are not the intended targets of this vaccine strategy, and thus, the antitumor immune responses are poor [69]. In addition, in cancer cell vaccines, the expression of tumor-specific antigens is lower compared with vector-based and DNA vaccines, leading to an insufficient immune response. Utilizing the cell surface antigens instead of whole cell to develop vaccines may improve the detection of cancer cells by the immune system [69].

Peptide vaccines

A peptide vaccine employs the 8-11 amino acid epitope of an antigen recognized by effector T cells to induce tumor specific responses. Peptide vaccines have poor immunogenicity due to the HLA-restriction and antigenic escape

in cancer [70]. Peptide vaccines for CRC patients are generally well-tolerated; however, a high frequency of reactions were observed at the injection site due to the use of adjuvants such as incomplete Freund's adjuvant, IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), and BCG. Another kind of peptide vaccine is tumor associated antigens which are self-antigen expressed on CRC cells such as CEA, mucin [71-73], epidermal growth factor receptor [74], squamous cell carcinoma antigen recognized by T cells 3 (SART3) [75], β-human chorionic gonadotropin (β-hCG) [76], survivin-2B [77], p53 [78] or mutated KRAS [79]. Vaccination of these peptide vaccine elicit humoral and cellular immune response in some patients but is related with poor clinical activity.

Early data has shown that vaccination of peptide of β-hCG conjugated to diphtheria toxoid in patients with metastatic colorectal cancer could induce immune responses. Anti-hCG antibody induction was observed and was associated with longer overall survival [80]. Vaccinations with tumor associated antigen such as survivin, a member of the inhibitor of apoptosis protein (IAP) family, produced slight reduction of the tumor volume in one patient with advanced colorectal cancer, which was considered a minor responder [77]. Moreover, patients immunized with human-EGF linked to either tetanic toxoid or Neisseria Meningitidis recombinant protein demonstrated high antibody titers against self EGF [81]. In addition, synthetic mucin peptide admixed with BCG elicited delayed hypersensitivity [82].

Unfortunately, there was a discrepancy between clinical and immunological responses in some clinical trials. In SART3 peptide vaccine therapy, IgE-type anti-peptide antibodies were detected after vaccination; however, immunological responses were not detected in the patients. Significant levels of increased cellular immune responses to both HLA-A24+ colon cancer cells and the vaccinated peptide were observed after administration of SART3 peptide vaccines [75]. In a following clinical trial, SART2899, CyB91, ART1170, and ART413 were positive for immediate-type hypersensitivity in all patients tested and but only one partial response was observed in patients with regression of live metastasis. Another patient remained stable for 6 months and eight patients showed progressive disease [83].

To improve immunogenicity, long peptides -based vaccines was used. In a phase I/II trial, p53-specific T-cell responses were induced in 9 out of 10 colorectal cancer patients. However, there is no report on clinical results [78]. Recently, there was a novel strategy for delivery of β-hCG, which is an otherwise poorly immunogenic self-antigen, to antigen presenting cells (APC) by targeting the mannose receptors on APC. The β-hCG was delivered together with costimulatory signals such as GM-CSF and Toll-like receptor TLR3 and TLR7/8 agonists. This strategy led to enhanced antibody and T cell responses to β-hCG. However, only modest clinical improvement was seen in this trial [76]. Data indicates that expression of HLA-A2402-restricted epitopes, RNF43 (ring finger protein 43) and TOMM34 (34-kDa translocase of the outer mitochondrial membrane), is up regulated in CRC specimens [84]. Synthesized Peptide vaccines derived from RNF43 and TOMM34 were co-administered with chemotherapy in patients with metastatic colorectal cancer. In a phase I clinical trial, clinical responses was seen in CT scan. The cytotoxic T lymphocyte (CTL) responses against RNF43 and TOMM34 in peripheral lymphocytes were induced in these patients. Moreover, positive CTL responses against both antigens is correlated with longer survival [85].

Another peptide vaccine in phase I trials was testing a novel HLA-A24-restricted peptides made from RNF43, TOMM34, K homology domain-containing protein overexpressed in cancer (KOC1), and vascular endothelial growth factor receptors 1 and 2 (VEGFR1 and VEGFR2). The vaccine treatment using multiple peptides induced dose-dependent peptide-specific cytotoxic T lymphocytes responses and improved survival time [86]. Importantly, peptide vaccines have led to limited success in clinical trials [87]. However, down-regulation of certain antigens and MHC class I molecules, impaired dendritic cell function, and immunosuppressive tumor bed may limit the success of this method [88].

Dendritic cell vaccines

Dendritic cells (DCs) play a pivotal role in initiating and regulating the immune response and adjuvants act primarily as DC activators [62]. Developing DC-based vaccines against CRC has been going on for decades. DCs are isolat-

ed from a cancer patient. Then DCs are either loaded with peptides derived from tumor antigens, or loaded with tumor cell lysates in vitro. DCs can be engineered to express co-stimulatory molecules and cytokines by transfection with recombinant genes such as CD40L [89] to increase the ability of DCs to induce T cell responses. After activation, the DC vaccine is then re-administered back into the patient [62, 90].

A phase I clinical study based on autologous human DCs pulsed with a CEA peptide was performed in 21 patients with metastatic cancers. While the vaccine was safe and well tolerated, only one patient experienced stable disease after vaccination [71].

The ability of DCs pulsed with CEA to induce CEA-specific T cell responses was evaluated in patients with hepatic metastases of colorectal cancer. Vaccination with DCs induced potent CEA-specific T cell responses in these patients [91]. Other phase I clinical trials also showed modest effect of this vaccine [92, 93].

Effectiveness of tumor-lysate loaded DC vaccines in advanced CRC patients was tested. Six HLA-A*0201-positive patients received vaccine together with tetanus toxoid antigen, hepatitis B, and influenza matrix peptides. Transient stabilization of CEA levels or even reduction of CEA levels was observed. Specific immune responses and stabilization/reduction of CEA levels in the serum were confirmed [93]. Another study evaluated the clinical and immunological effects of a dendritic cell based cancer vaccine (MelCancerVac) which is dendritic cells pulsed with an allogeneic tumor cell lysate. A favorable anticancer response was achieved in 24% of the patients, indicated by elevated levels of GM-CSF, TNF-alpha, IFN-gamma, and IL-2 in patients achieving stable disease [94]. Lately, there was a combination of DC vaccine with cytokine-induced killer cells, which yielded clinical benefit in survival of CRC patients [95]. The results demonstrate significantly higher levels of IFN-γ and IL-12 and an increased overall survival.

DNA vaccines

This vaccine strategy uses the DNA sequence of known tumor antigens, often in association with genes encoding immunostimulatory molecules such as cytokines. These vaccines are injected at distal sites and are taken up by APCs [96]. DNA vaccines thus induce expression of tumor antigens in APCs.

The plasmid DNA is delivered into DCs for antigen expression and presentation or is delivered to parenchymal cells for antigen expression. The antigen was consequently acquired by DCs and presented to naive T cells [97]. Gene gun and electroporation were developed to increase transfection efficiency [98].

Studies with DNA vaccines for CRC in phase I clinical trials are rare and little is known regarding the efficacy in humans. There was a dose-escalation clinical trial of a plasmid encoding CEA and hepatitis B surface antigen (HBsAg) in 17 patients with metastatic colorectal carcinoma. DNA vaccination demonstrated no CEA-specific antibody responses, but 4 of 17 patients developed lymphoproliferative responses to CEA after vaccination. Unfortunately, no objective tumor responses were shown in this study [99].

Viral-vector vaccines

Viral-vector vaccines based on human tumor associated antigens have been tested in patients with CRC, in combination with or following standard therapy.

A phase II clinical trial in patients with metastatic CRC examined the effect of chemotherapy plus vaccination with a canary pox virus encoding CEA and the T-cell costimulatory molecule CD80 (ALVAC-CEA/B7.1). The trial demonstrated that anti-CEA–specific T-cell responses could be successfully generated. Objective clinical responses were observed in 40% of the patients [100, 101].

Several viral vector vaccines have been explored for CRC treatment in clinical trials. Vaccinia virus or replication-defective avian poxviruses expressing CEA promoted CTL responses but with no clinical activity in CRC patients was seen [102]. Insertion of CEA and co-stimulatory signal B7.1 into a canaryn pox viral vectors [101, 103] generated anti-CEA-specific T-cell responses. A more encouraging trial used vaccinations including vaccinia and fowlpox expressing CEA and three costimulatory molecules such as B7-1, ICAM-1, and LFA-3. This strategy enhanced immune responses and

produced prolonged disease stabilization in 40% patients [104].

In some early-phase clinical trials, patients with advanced CRC were vaccinated with DCs pulsed with CEA peptides [71, 91, 105] 73-76 or CEA mRNA [106, 107]. The majority of patients demonstrated positive CEA-specific T-cell responses after vaccination, and stabilization of diseases was observed in several patients.

Glycoprotein tumor antigen MUC1 is overexpressed on the colon cancer cells. A preclinical study with a vaccine consisting of MUC1 peptides, pan helper peptide, unmethylated CpG oligodeoxynucleotide, and granulocyte macrophage-colony stimulating factor has successfully broken MUC1 self-tolerance, induced strong antitumor immunity and reduced tumor burden [73] despite another report of failure to induce immunity with MUC1 vaccine [108]. MUC1 vaccine can target MUC1 that become aberrantly expressed in IBD and the associated colon cancer. This vaccination helped treatment and prevention of both chronic colitis and colon cancer [109]. Recently, MUC1 demonstrated high immunogenicity in some patients without cancer but with a history of premalignant lesions (advanced colonic adenomas, precursors to colon cancer) [14]. Guanylyl cyclase C (GUCY2C) is a mucosal antigens expressed on the cells that line the normal intestine and on metastatic colorectal cancer cells. Vaccination of GUCY2C using an adenoviral vector induced specific immune responses as well as prophylactic and therapeutic immunity against metastatic colorectal cancer in mice by addition of the cytokines GM-CSF and IL-2. The vaccination is able to prevent and treat metastatic colorectal cancer in animal models [110] and is mediated by induction of antigen-targeted CD8+ T cells [17]. One studie indicated that the tumor tolerance is associated with antigen-specific CD4+ T cell tolerance [111].

Adoptive cell therapy

Adoptive cell therapy (ACT) using autologous tumor-infiltrating lymphocytes has emerged as the effective treatment for patients with CRC (**Table 2**). The advance in genetic engineering of human lymphocytes has made it possible to provide ACT immunotherapy to patients with a variety of tumor [61].

Tumor-infiltrating lymphocytes

One strategy of adoptive cell therapy is autologous tumor-infiltrating lymphocytes (TILs), in which lymphocytes are isolated from resected tumors, activated and expanded and are given back to patients together with IL-2. TILs therapy is effective in melanoma [112, 113] despite the difficulty in collecting enough TILs and the poor ability of migration of these cells to tumor. Using autologous T cells may provide the advantage of breaking immune tolerance that inhibits T cell activation *in vivo*. Currently TIL therapy is under investigation for the treatment of many tumors [114]. In a phase I clinical trial, TILs from tumor specimen was activated and were administered with IL-2 to 14 patients with resected metastatic CRC [115]. The results showed that TILs immunotherapy and chemotherapy have similar effect on disease-free survival.

In another study, T cells were collected and expanded from the tumor-draining sentinel lymph nodes in patients with advanced CRC. Four of 9 patients experienced a complete response and survival was improved [116].

Cytokine-induced killer cells

Another adoptive cell immunotherapy involved cytokine-induced killer (CIK) cells. Compared to lymphokine-activated killer (LAK) cells, CIK cells can be obtained more easily and has demonstrated a higher tumor-specific cytotoxic activity [117-120]. So far, only one clinical trial with CIK cells for colorectal cancer patients was conducted [121]. Seven out of ten patients had colorectal cancer, two patients with lymphoma, and one patient with renal carcinoma. The patients received one to five intravenous infusions of IL-2-transfected CIK cells and five infusions of untransfected CIK cells; the second treatment cycle followed three weeks after the first one. Transfected cells could be detected even after two weeks with increased serum levels of IFN-γ, GM-CSF, and TGF-β. Also an increase in the cytotoxicity of PBLs was observed following CIK cell therapy. CT scans showed that after treatment three patients had stable diseases, and one patient had a complete response.

Engineered lymphocytes

Another way of adoptive cell therapy involves genetically engineered T cells expressing T cell

Table 2. Clinical studies applying adoptive cell therapy for the treatment of colorectal cancer

Study	Adoptive Cell type	Patients number	Therapy	Results	Conclusions
Gardini A, 2004	TIL	14	TILs from tumor specimen was activated with IL-2 and infused back.	There was no difference between TILs immunotherapy and chemotherapy in patient actual and disease free survival after 1, 3, 5 years.	TCR zeta-chain and epsilon-chain expression significantly increased after IL-2. But still not able to induce more clinical benefit than chemotherapy.
Karlsson M, 2010	TIL	16	Sentinel nodes were collected and lymphocytes were clonally expanded in vitro in the presence of autologous tumor extract and infused to patients.	Sentinel-node-acquired CD4+ lymphocytes were expanded in vitro and safely administered without side effects. In four out of nine stage IV patients, complete tumor regression occurred. Median survival time in the stage IV patients was 2.6 years, as compared with 0.8 years in traditional therapy group.	Sentinel-node-based adoptive immunotherapy is applicable and safe and appears to promote therapeutic antitumor effects.
Ren X, 2006	CIK	11 colorectal cancer patient in 66 cancer patients.	Enriched PBMCs of the patients were cultured with the mouse antihuman CD3 monoclonal antibody, IL-1alpha, IFN-gamma and IL-2. LAKs were cultured only with IL-2.	2 CRC patients received more than 3 times CIK and achieved stable disease.	Auto-CIKs is a suitable immunotherapy for those solid-malignance patients and could act to prevent recurrence.
Schmidt-Wolf IG, 1999	CIK transfected with IL-2 gene.	7 CRC metastatic patients out of 10 cancer patients	Autologous CIK cells generated from patients with colorectal carcinoma or lymphoma were transfected with a plasmid containing the interleukin-2 (IL-2) gene. Ten patients received 1-5 intravenous infusions of IL-2-transfected CIK cells. Clinical outcome was based on comparison of CT scans before and after treatment.	Transfected cells could be detected for up to 2 weeks after infusion. There was a significant increase in serum levels of IFN-gamma, GM-CSFand TGF-beta. A partial increase in cytotoxic activity in peripheral blood lymphocytes (PBLs) was documented. One patient with lymphoma developed a complete response. Three patients remain stable when measured by CT.	CIK cells transfected with the IL-2 gene can be safely administered.
Parkhurst MR, 2011	T lymphocytes engineered to express TCR against CEA.	3	Autologous T lymphocytes genetically engineered to express a murine TCR against CEA were administered to patients with metastatic CRC.	All patients experienced significant decreases in serum CEA levels and one patient had an objective regression of cancer metastatic to the lung and liver. However, a severe transient inflammatory colitis was induced in all patients.	Objective regression of metastatic colorectal cancer was seen after adoptive T cell transfer. However, there are severe limitations of using CEA as a target for CRC immunotherapy.

Note: CRC, colorectal cancer.

receptor (TCR) targeting antigen expressed on tumor cells. However, these TCRs would be limited due to MHC restriction. Alternatively, a new method used the antibody-based chimeric antigen receptors (CARs), which are single chain variable fragments derived from tumor antigen-recognizing monoclonal antibodies. These CARs are engineered to be fused to intracellular T cell signaling domains. Thus the CARs can both recognize native antigens on the surface of tumors and stimulate T cells without the need of receptor binding with MHC. So this strategy can bypass the MHC restriction. However, in a case report, one patient with metastatic colon cancer was treated with CAR T cells but passed away due to respiratory distress possibly induced by cytokine release caused by infusion of T cells [74]. In another phase I trial, autologous peripheral blood lymphocytes (PBL) were genetically engineered to express a murine TCR against CEA and consequently were given to three patients with metastatic colorectal cancer. All patients demonstrated considerable decreases in serum CEA levels and one patient had an objective clinical regression. On the other side, severe transient inflammatory colitis was also observed in all three patients [122].

Conclusions

For CRC patients, surgery and adjuvant therapies such as chemotherapy, radiotherapy have limitations. Based on results obtained over the past few decades, immunotherapy targeting various aspect of immune system offers an alternative promising way of treatment. With the tremendous effort in developing new therapeutic strategies against tumor, more treatment options are becoming available. Identification of appropriate antigen targets will be necessary for immunotherapy to achieve greater success. Although some clinical trials of vaccines have shown clinical responses in metastatic CRC, effective vaccine are still absent.

With the use of ipilimumab and anti PD-1 monoantibody, the boosting of T cell function can be achieved by blocking the immune checkpoint. Moreover, other methods activating the costimulatory mechanism by targeting TNF receptor family could prove to be useful. Combination of all modalities such as surgery, chemotherapy, radiation and immunotherapy may provide a better way of treatment and improve patient prognosis. Engineering personalized cancer immunotherapies will offer the promise of specificity and safety despite the difficulties in developing effective immunotherapeutic for CRC and will increase the responsiveness to the immunotherapy. Trials to explore the usage of immunotherapy are under active investigation and will continue to contribute to our increasing knowledge of immunotherapeutic and benefit CRC patients.

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