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Immunotherapy in Colorectal Cancer: Where Are We Now?

Trevor R. Baybutt[1] · **Allison A. Aka**[1,2] · **Adam E. Snook**[1]

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Abstract
Purpose of Review This review examines the current state of colorectal cancer (CRC) immunotherapy across multiple treatment modalities and discusses some of the most promising approaches.
Recent Findings CRC immunotherapy involving viral vector and dendritic cell vaccines, checkpoint blockade, and adoptive cell therapy has been explored from preclinical to clinical studies. Despite successes in other malignancies, including melanoma, leukemia, lung, and renal cancers, immunotherapies have been FDA approved for only a small subset of CRCs. Recent studies leveraging greater understanding of cellular and molecular mechanisms underlying colorectal tumorigenesis and immunotherapeutic mechanism of action may be exploited in upcoming trials.
Summary While immune infiltration of CRC has been an established indicator of patient outcomes, immunotherapeutic strategies to date have not exploited its potential immunogenicity to benefit patients. New vaccine, checkpoint inhibitor, and CAR-T cell therapy paradigms promise to change that. With continued research, we could see a rapid increase in the number of FDA-approved immunotherapies for CRC in the coming years.

Keywords Colorectal cancer · Immunotherapy · Biomarker · Vaccine · Checkpoint inhibitor · CAR-T cell

Abbreviation

CAR	Chimeric antigen receptor
CEA	Carcinoembryonic antigen
CIK	Cytokine-induced killer cell
CIRC	Co-ordinate Immune Response Cluster
CTL	Cytotoxic T lymphocyte
CRC	Colorectal cancer
DC	Dendritic cell
GM-CSF	Granulocyte macrophage-colony stimulating factor
GUCY2C	Guanylyl cyclase C
HLA	Human leukocyte antigen
MDSC	Myeloid-derived suppressor cell
MMR	Mismatch-repair
MSI	Microsatellite instability
MSS	Microsatellite stable
NK cells	Natural killer cells
OS	Overall survival
PADRE	Pan DR Epitope
PD-1	Programmed death 1
PD-L1/2	Programmed death-ligand 1/2;
PFS	Progression-free survival
RFS	Recurrence-free survival
TCR	T cell receptor
TILs	Tumor infiltrating lymphocytes
Tregs	Regulatory T cells
TRICOM	TRIad of COstimulatory Molecules

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✉ Adam E. Snook
adam.snook@jefferson.edu

[1] Department of Pharmacology and Experimental Therapeutics, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA 19107, USA

[2] Department of Surgery, Sidney Kimmel Medical College at Thomas Jefferson University, 1020 Locust Street, JAH 368, Philadelphia, PA 19107, USA

Introduction

Immunotherapeutic approaches to cancer treatment have been clinically successful for multiple diseases including hematological, melanoma, and lung cancers; however, immunotherapy options for colorectal cancer (CRC) have yet to achieve similar outcomes. Given the prevalence of CRC, accounting for the second most cancer-related deaths in the USA [1], novel therapeutics are needed for patients whose tumors are unresponsive to current standard-of-care. However, many immunological approaches have been applied therapeutically without a complete mechanistic understanding of tumor-immune cell or molecular interactions. Given immunotherapeutic success in other malignancies, our growing understanding of the cellular and molecular events underlying immune control and evasion, and the ongoing development of new therapies for CRC, we believe that immunotherapy will undoubtedly contribute to the future CRC treatment armamentarium. Below, we explore the current state of CRC immunotherapy underlying this enthusiasm.

Genomics and Biomarkers

Although broadly applicable therapeutics is the ideal clinical goal, the remarkable specificity of immune responses and the emerging understanding of how immune responses and the heterogeneous tumor environment interact encourages more nuanced approaches to determine optimal patient management. Given the heterogeneous etiology of CRC, using genomics and biomarkers to identify appropriate therapies for individual patients is not only prudent, but paramount to clinical success. Indeed, studies published in 2012 demonstrated clinical responses in patients with melanoma, renal-cell cancer, and lung tumors treated with an antibody that blocks the checkpoint inhibitor PD-1 (programmed death 1) often overexpressed by cancer cells, while no responses were observed in patients with CRC [2]. More recently, using The Cancer Genome Project, 28 immune-related genes defining an immune signature were identified and together comprise the Co-ordinate Immune Response Cluster (CIRC) [3]. The gene cluster demonstrated a strong association with Th1 response and included genes for T cell activation, Th1 function, T cell chemoattractant chemokines, adhesion molecules, HLA Class II genes, checkpoint inhibitors, and genes associated with innate responses. Applying this gene set to patient data identified two groups of patients: those with high or low CIRC expression. Tumors with high CIRC expression were characterized by microsatellite instability (MSI) and polymerase mutations, as well as high Th1 immune cell infiltration and checkpoint inhibitor expression. The low CIRC expressing groups were microsatellite stable (MSS) with RAS mutations and lower immune cell infiltration. The checkpoint molecules PD-L1 and PD-L2 were also lower in this group, suggesting that antibody therapies against these molecules may be of little benefit to those patients. While genetic data support helper ($CD4^+$) T cell infiltration in MSI CRC, frameshift mutations in these patients positively correlate with cytotoxic ($CD8^+$) T cell responses. Examining 103 MSI^+ tumors, 19 target genes were identified within these tumors that correlate with frameshift mutations, compared with normal colonic tissue [4]. To demonstrate increased $CD8^+$ T cell activity to new antigens (neoantigens) produced by mutations in MSI^+ tumors, $CD8^+$ T cells from HLA-matched MSI^+ CRC patients and healthy controls were activated with T cell epitopes derived from frameshifted proteins. These activated T cells killed the MSI^+ colorectal cancer cell line HCT116, whereas the MSS^+ colorectal cancer cell line Colo205 was not killed by these frameshift-specific T cells. Taken together, these data suggest that MSI^+ CRC patients could be better candidates for checkpoint inhibitor antibody therapy than their MSS^+ counterparts, given their immune signature (CIRC), expression of PD-L1/2, and presentation of numerous neoantigens.

While MSI^+ status does appear to correlate well with potential immunotherapy responsiveness, this does not completely negate patients with MSS^+ disease from also being candidates. Recently, literature was published suggesting that PD-1 blocking therapy may be of value to patients with Mismatch-Repair (MMR) deficits. A subset of MSS^+ early-onset CRC patients was identified with a Proline (P) to Arginine (R) mutation in amino acid 286 of the DNA polymerase, epsilon, catalytic subunit (POLE) that leads to a hypermutated phenotype [5••]. The authors posit that mutational load, regardless of mechanism (MSI, POLE P286R, others), may help to define subsets of CRC patients who are good candidates for checkpoint blocking immunotherapy.

Biomarkers are not only being implemented in the diagnosis and prognosis of CRC and identifying patients who would make appropriate candidates for immunotherapy, they are also being used to determine the efficacy of immunotherapy. While immune infiltration of primary tumors is a good prognostic indicator [6, 7], determining immune infiltration of metastatic lesions is more difficult. Alternatively, peripheral blood cells could be used to provide a peripheral immunoscore prior to, and after, vaccination [8•]. Examination of refined immune cell subsets that reflect immune function such as central memory T cells, suppressor T cells (Tregs), myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, and others identified patients benefiting from breast cancer immunotherapy. While large, randomized trials are required to confirm its utility in both breast and other cancers, this peripheral immunoscore may have predictive value in identifying patients who would benefit from immunotherapy before treatment or who have successfully

responded to therapy before clinical endpoints have been reached.

Vaccines

Recent advances in CRC-specific vaccines have focused on the identification of novel target antigens or in finding appropriate adjuvants to accompany vaccines. One of the recurrent themes in vaccine-based CRC immunotherapy in the last few years has been the targeting of dendritic cells (DCs) using DC vaccines or cytokines to enhance DC recruitment and activity. Different research groups have taken different approaches, with many incorporating GM-CSF (granulocyte macrophage-colony stimulating factor). In a murine model, GM-CSF and interleukin-2 (IL-2) were effective adjuvants for an inactivated whole-cell vaccine, promoting survival in mice challenged with the colorectal adenocarcinoma cell line CT26 [9], suggesting that GM-CSF and IL-2 have potential clinical benefit. In fact, in a conceptually similar study, metastatic CRC patients were immunized with irradiated, allogenic human colon cancer cells and a GM-CSF-producing bystander cell line accompanied by a single, intravenous administration of low-dose cyclophosphamide to deplete Treg cells [10]. Nine patients were enrolled in the study and the median overall survival (OS) after the first vaccination was 51.2 months. Six patients with liver-restricted metastases who received chemotherapy following partial surgical resection remained disease free 36.2 to 53.5 months following the first vaccination. Three patients unable to have surgical intervention for liver metastases received multiple vaccination doses (no more than 4), but all experienced disease progression.

Two studies have been published examining the efficacy of the PANVAC vaccine in combination with GM-CSF. PANVAC consists of alternating doses of recombinant vaccinia (PANVAC-V) and fowlpox (PANVAC-F) vectors encoding carcinoembryonic antigen (CEA) and mucin 1 (MUC1) in addition to B7.1, ICAM, and LFA-3 termed the TRIad of COstimulatory Molecules (TRICOM). The first study compared the efficacy of (1) autologous DCs isolated, cultured in vitro with GM-CSF and IL-4, and infected with PANVAC before being given back to the patient versus (2) direct injection of the PANVAC vaccine followed by GM-CSF in patients who were disease free after complete metastasectomy with perioperative chemotherapy [11]. Overall, vaccinated patients had superior survival compared to unvaccinated patients, but there was no difference in recurrence-free survival (RFS) or CEA-specific immune responses between the DC + PANVAC and PANVAC + GM-CSF vaccines. In a separate study, the PANVAC vaccine was administered with GM-CSF and IFN-α. The rationale for this strategy is that in combination with GM-CSF, IFN-α enhances the expression of tumor antigens and is functionally superior to GM-CSF plus IL-4 [12]. This study recruited 33 patients who had metastatic cancer at enrollment. While CRC was not an inclusion criteria, 64% of patients had a diagnosis of CRC, with lung, breast, pancreas, appendix, esophagus, and bladder comprising the remainder. While the vaccine itself appeared to be ineffective, patients receiving IFN-α had a significantly improved OS compared with patients who did not receive IFN-α (6.40 vs. 3.94 months, $p = 0.02$). The authors used the T cell activation marker soluble CD27 to indicate that IFN-α recipients had enhanced T cell activation relative to those not receiving IFN-α and suggest further investigation of IFN-α and GM-CSF as adjuvants to vaccine immunotherapy.

An alternative method for stimulating DCs in vivo is to encode GM-CSF directly in the vaccine vector. The PexaVec (pexastimogene devacirpvec) vaccine is an oncolytic vaccinia virus vaccine encoding GM-CSF, while the thymidine kinase (TK) gene is deleted. The TK deletion limits viral replication only to cells with high TK activity (primarily cancer cells). Enrollment included 15 patients who had been heavily pre-treated with multiple lines of therapy, but remained refractory. Of the 15 patients enrolled, 10 had radiologically stable disease on day 29 [13]. Of note was the occurrence of skin pustules in seven of nine patients receiving a high dose of vaccine. The pustules appeared on the palms, soles, oral mucosa, and lips during days 3–7 of cycle 1 and resolved within five to 26 days. Further safety and efficacy studies have not yet been completed.

Overall, the lack of convincing efficacy data with DC-targeted vaccines encourages alternative strategies, including peptide vaccines, novel vectors, or novel antigens. Peptide vaccines present a viable approach, although they are restricted by patient HLA haplotypes and oncogene status. To overcome this restriction, personalized vaccines were designed that matched patient HLA haplotypes to specific peptides from a candidate peptide library for 60 patients with advanced CRC who had failed standard therapy [14]. Increased peptide-specific serum IgG titers and cytotoxic T lymphocyte (CTL) responses were observed in 49 and 63% of patients, respectively, with CTL responses after vaccination being significantly predictive of a favorable OS. In terms of prognosis, the authors found that patients with an IgG and CTL response survived better than patients without a response, whereas a single response was not significantly better than no response.

While vaccine vectors tend to be viral (such as poxviruses in PANVAC), there is increasing literature to support the use of bacterial vectors. In a rat model of chemically induced CRC, animals were immunized with the gram-negative gastroenteritis-causing microbe *Salmonella typhimurium*, encoding CEACAM6 (carcinoembryonic antigen-associated cell adhesion molecule), and the costimulatory molecule 4-1BB Ligand [15]. Eight weeks after tumor initiation, vaccination was begun by oral gavage four times per week for 2 weeks. At 18 weeks, animals were sacrificed and tissues

were harvested. The CEACAM6/4-1BBL combination vaccine resulted in increased intratumoral $CD8^+$ T cell and NK cell infiltration, decreased Treg infiltration, and fewer tumors than control treatments.

One novel tumor antigen that is showing promise is the mucosally restricted receptor guanylyl cyclase C (GUCY2C). This protein is expressed on the apical surface of normal intestinal epithelium; however, this polarity is lost in metastatic lesions of colorectal origin. This anatomical compartmentalization (luminal GUCY2C), along with immune compartmentalization restricting mucosal immune responses following systemic vaccination, make the likelihood of GUCY2C-induced intestinal autoimmunity low. The challenge of targeting a normal, self-protein is that GUCY2C-specific immune cells are partially self-tolerant. In that context, a replication-deficient human adenovirus (Ad5) encoding GUCY2C fused with the PAn DR Epitope (PADRE) overcame GUCY2C-specific tolerance by promoting PADRE-specific $CD4^+$ T cell help and GUCY2C-specific B and $CD8^+$ T cell responses [16–18]. Recently, 10 subjects were enrolled in a phase I study to determine the safety, tolerability, and immunogenicity of this vaccine (Ad5-GUCY2C-PADRE) [16]. GUCY2C-specific antibody and $CD8^+$ T cell responses were observed in 50% of patients while no adverse events were detected. GUCY2C vaccination has also been examined in an underexplored area of colorectal immunotherapy: combination radiotherapy and immunotherapy to promote synergistic tumor cell killing. In a therapeutic murine tumor model, a single, sub-lethal dose of radiation followed 7 days later by GUCY2C vaccination was superior to either treatment alone [19•]. While radiotherapy is not typically employed in colon cancer management, it is often used in rectal cancer, suggesting that neoadjuvant radiotherapy, followed by surgery and adjuvant Ad5-GUCY2C-PADRE, could prevent local and systemic recurrence and improve overall patient benefit.

Checkpoint Inhibitors

Multiple biomarker studies predicted positive responses to PD-1 blockade in patients with MSI^+ tumors ("Genomics and Biomarkers" section above) and subsequent clinical trials support that hypothesis. In a phase II study of the PD-1 antagonist pembrolizumab, patients with identified MMR deficiencies had a 90% disease control rate compared with 11% for MMR-proficient CRC [20••]. Disease control rate is the total percentage of patients having either a complete response, partial response, or stable disease. While PD-1 immunotherapy did not produce any complete responses, 40% of MMR-deficient CRC patients experienced a partial response, whereas the best outcome in MMR-proficient CRC patients was 12 weeks of stable disease, which occurred in only 2 of the 18 MMR-proficient CRC patients. In addition to single agent therapy, combination immunotherapy using both PD-1 and CTLA-4 antibody antagonists for MSI^+ disease is currently under investigation in the CheckMate142 (NCT02060188) trial. Preliminary data presented at the 2017 American Society for Clinical Oncologists (ASCO) Annual Meeting demonstrated an encouraging disease control rate of 78% at 12 weeks with the combination nivolumab + ipilimumab in MSI^+ patients [22]. Interestingly, MSI^+ status not only identifies which treatments are appropriate, it also identifies potentially harmful treatments. Standard-of-care chemotherapy including 5-FU may actually be harmful to CRC patients with MSI^+ tumors expressing high levels of PD-L1 with immune infiltrates based on RFS data generated through treatment interaction analyses [21]. Those results suggest that standard-of-care chemotherapy may be suppressing endogenous immune responses which are preventing MSI^+ CRC recurrence and that immune checkpoint blockade should be explored as an alternative first-line adjuvant therapy, rather than standard-of-care chemotherapy.

While results in MSI^+ CRC are very encouraging, checkpoint blockade has not been effective against MSS^+ metastatic CRC, which accounts for ~95% of CRC cases. The predominant hypothesis for this observation is that high mutational load in MSI^+ disease produces numerous neoepitopes promoting a robust T cell response which can be exploited by relieving the negative pressure of immune checkpoints with blocking antibodies. In contrast, MSS^+ CRCs may possess far fewer neoepitopes, necessitating enhancement of the tumor immunogenicity and induction of CRC-specific T cell responses in patients with MSS^+ disease. Indeed, PD-L1 blockade may be combined with pharmacological inhibition of the MAPK/ERK pathway, increasing HLA Class I expression and promoting intratumoral T cell infiltration in MSS^+ CRCs [23]. The initial study examining the safety and tolerability of this combination (cobimetinib + atezolizumab) was promising, with most adverse events attributed to the MEK inhibitor. A phase III study (NCT02788279) examining this combination is currently enrolling subjects. In the context of remarkable clinical efficacy against melanoma, lung cancer, and others, the hope that checkpoint inhibitor therapy could also be effective against CRC is understandable; however, it appears that additional combinations may be required to enhance the immunogenicity of most colorectal tumors (MSS^+). Studies of combination therapies in MSS^+ CRC are just beginning, but results with MAPK/ERK + PD-L1 blockade are very encouraging.

Cell-Based Therapies

While vaccines and checkpoint inhibitors are designed to induce an immune response within the patient (active

immunotherapy), cell-based therapies employ immune effectors generated in vitro or ex vivo and transferred to the patient (passive immunotherapy). The two predominant cell types employed in cell-based immunotherapy are NK cells and T cells, both of which have cytolytic activity and produce cancer-opposing cytokines. NK cells in the context of immunotherapy are typically referred to as cytokine-induced killer (CIK) cells because they are expanded and activated ex vivo with cytokines. While these two cell types have overlapping antitumor effects, they contrast each other in two important ways. NK cells rely on non-specific signaling mechanisms for their activation, including induction of stress response molecule upregulation or HLA downregulation in virally infected or cancer cells; however, T cells employ highly specific T cell receptors (TCRs) that recognize specific antigens on the surface of virally infected or cancer cells. Moreover, while NK cells are typically short-lived and provide no immunological memory, T cells can differentiate into memory T cells which provide life-long immunity. These differences result in advantages for each approach and consensus has not yet been reached on the ideal approach in CRC or other solid tumors. Below, we highlight encouraging examples of each approach.

CIK Cell Therapy

In a phase II clinical trial, CIK therapy significantly improved OS (19 vs. 8%) and progression-free survival (PFS; 36 vs. 16 months) in patients with metastatic CRC when combined with FOLFOX4, compared to FOLFOX4 alone [24]. In another study examining postoperative CIK combination therapy, disease-free survival was significantly increased in patients receiving immunotherapy, although OS was not different [25]. Another study used a slightly different approach, in which CIK were combined with dendritic cells (DC-CIK) in combination with chemotherapy [26]. Patients were randomized to receive either DC-CIK with chemotherapy or chemotherapy alone, consisting of six cycles of 5-FU, FOLFOX, or XELOX. The DCs were pulsed with a lysate of the human colon cancer cell line SW480 and IL-4 for 7 days before injection with CIK cells. Both PFS and OS were significantly increased in the combination therapy group relative to chemotherapy alone. Importantly, while adoptive immunotherapy using CIK is not a vaccination strategy, these cytokine-producing cytolytic CIK cells can increase endogenous immune responses after their transfer. Following radiofrequency ablation of liver metastases, CIK immunotherapy increased CEA-specific T cell responses as well as PFS compared to patients receiving ablative therapy alone [27]. Those results suggest that CIK therapy could be combined with active immunotherapy approaches, including vaccines, checkpoint inhibitors, and cytokines, to not only produce a short-term antitumor effect through CIK-mediated tumor ablation but also induce endogenous immune responses that provide long-term surveillance against recurrence.

T cell Therapy

In a compelling case report, a female patient with metastatic CRC in seven identified lung lesions was enrolled in a phase II clinical trial investigating the use of tumor infiltrating lymphocytes (TILs) expanded ex vivo to treat metastatic colorectal cancer [28••]. It should be noted that this is highly personalized therapy, with T cells collected from the patient, expanded ex vivo and transferred back to the patient. In this study, neoepitopes (new T cell epitopes produced by mutations in self-proteins) were identified in the patient's tumor and used to stimulate TILs to selectively expand neoepitope-specific T cells. In this patient, a high fraction of expanded TILs were $CD8^+$ T cells recognizing a mutant KRAS epitope and these were further expanded ($> 1 \times 10^{11}$ cells) and transferred back to the patient along with IL-2. At 9 months after therapy, six out of seven metastatic lesions had regressed or were regressing. The other lesion initially responded (40 days after therapy) before progressing and removal by surgical resection, at which point it was revealed that the lesion had lost expression of the HLA molecule required for presentation of the mutated KRAS epitope recognized by the transferred TILs, leading to immune evasion by the progressing lesion. Importantly, this case report demonstrates that adoptive T cell therapy for metastatic CRC is feasible and effective, confirming that metastatic colorectal cancer can be targeted and eliminated by T cells. However, this specific approach has limitations. First, identification of neoepitopes and isolation and expansion of neoepitope-specific TILs is expensive and laborious. Moreover, it may not be possible in many patients due to poor tumor accessibility for TIL extraction or failed TIL expansion. Moreover, loss of HLA expression will be a risk because it will render the tumor cells resistant to all T cell responses. Thus, an alternative approach to produce the same overall effect (transfer of large numbers of tumor antigen-specific effector $CD8^+$ T cells) without requirements for neoepitope identification and TIL expansion could produce a highly effective therapy with greater feasibility and scalability.

One such approach involves the genetic manipulation of patient T cells collected from peripheral blood to transform all T cells into tumor-specific effectors using chimeric antigen receptors (CARs) which directly recognize native surface tumor antigens, rather than peptide epitopes presented by HLA molecules (Fig. 1). Because CARs are HLA-independent, they can be used in all patients regardless of HLA haplotype and HLA loss is not a viable escape mechanism for tumor cells (unlike the KRAS-specific TILs described above). CAR-T cells have been remarkably successful in the treatment of various malignancies, especially hematological

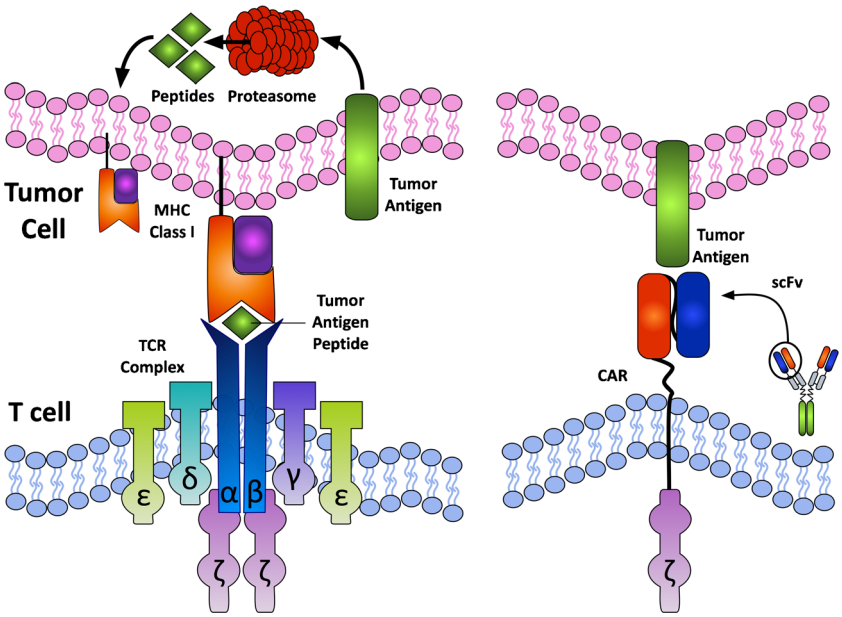

Fig. 1 Chimeric antigen receptors. Tumor antigens are processed into small peptides by the proteasome, loaded onto empty MHC (HLA) class I molecules and presented on the cell surface as peptide-MHC complexes. These peptide-MHC complexes are recognized by the T cell receptor (TCR) complex containing TCR αβ pairs and CD3 signaling molecules (γ, δ, ε and ζ) which induce T cell activation. In contrast, chimeric antigen receptors (CARs) contain only the antigen-recognition domain of a monoclonal antibody (scFv) fused to TCR signaling molecules. Therefore, CARs bind directly to surface tumor antigens, inducing T cell activation

malignancies expressing the B cell marker CD19 [29–32]. Meta-analysis of 14 early phase clinical trials including a total of > 100 patients with refractory B cell malignancies revealed an overall pooled response rate of 73% with CD19 CAR-T cells [33]. Moreover, 1-year progression-free survival across studies was > 75% with CD19 CAR-T cells, a remarkable outcome given the poor prognosis of patients with therapy-resistant leukemia.

While clinical studies of CAR-T cell approaches in metastatic colorectal cancer are just beginning, animal studies suggest that CAR-T cells targeting two colorectal cancer antigens may be promising therapies for clinical translation. CAR-T cells targeting the classic colorectal cancer antigen CEA induce tumor regression in mice, while also causing toxicity in the form of cytokine release syndrome [34]. Similarly, adoptive transfer of T cells expressing a conventional CEA-specific TCR resulted in transient serum CEA reductions indicative of an antitumor effect, as well as severe colitis in CRC patients, leading to study termination [35]. In that context, CEA CAR-T cell testing in humans has employed a more conservative design. In a phase I study, CEA CAR-T cells were targeted to hepatic CRC metastases by hepatic artery infusion (HAI) to limit extrahepatic toxicity while optimizing efficacy for treatment of liver metastases [36•]. While no patients experienced severe adverse events, five of six died of progressive disease. One patient remained alive with stable disease for 23 months and several patients showed temporary reductions in systemic CEA levels, indicating some antitumor efficacy. Further study is required to confirm the safety and efficacy of this approach for hepatic CRC metastases, but no safe approach for CEA CAR-T cell treatment of CRC metastases in other organs has been found.

In contrast to the well-established colorectal cancer antigen CEA, GUCY2C is an emerging immunotherapeutic target in CRC. While GUCY2C vaccine development has progressed to phase I clinical trials [16], GUCY2C-targeted CAR-T cells have been tested only in animal models. In a pulmonary metastases model using mouse GUCY2C-expressing CT26 cells, GUCY2C CAR-T cells significantly reduced tumor burden and prolonged survival [37•]. Importantly, GUCY2C-targeted autoimmunity was not observed in any organ or tissue. This murine study suggests that GUCY2C CAR-T cell therapy could be both safe and effective against CRC metastases in various organs. Phase I studies are now required to determine the safety and efficacy of this approach in patients with GUCY2C-expressing metastatic cancer, which includes not only colorectal cancer but also a subset of esophageal, gastric, and pancreatic cancers.

Conclusion

We propose that CRC immunotherapy should follow a bifurcated path of development and utilization dependent on disease staging: active immunotherapies for earlier stage patients and adoptive cell therapies for patients with late stage or bulky metastatic disease [38]. For many clinical trials examining active immunotherapy (primarily vaccines and checkpoint inhibitors), the patient population is often restricted to those with late stage disease who have undergone multiple lines of immunosuppressive chemotherapy regimens. It appears that an active immunotherapy approach is unlikely to be curative in these patients, as the endogenous response triggered by therapy can not eliminate bulky disease or overcome the

immunosuppressive tumor environment. As such, vaccines should be employed as a future immunosurveillance strategy for early stage (I–III) patients that may have dormant, undetectable micro-metastases that pose a recurrence risk following surgical removal of their primary tumor. Vaccines have favorable safety profiles as well as very manageable costs. As we discussed, there are numerous CRC-specific vaccines currently under development, but they need not be prohibitively expensive. A course of Sipuleucel-T, an autologous, DC vaccine for prostate cancer cost $93,000 [39]. However, direct injection of a vaccine vector with GM-CSF therapy was as effective as the ex vivo DC vaccine approach, at a fraction of the cost [11]. Given the lack of conclusive efficacy evidence with DC-based vaccine technology for CRC, a direct vector approach should be seriously considered in future vaccine trials.

The second arm of our bifurcated immunotherapeutic approach is using cell-based therapies, especially CAR-T cell therapies, for patients with late stage or bulky disease. CAR-T cells using the antigen specificity of antibody variable regions negate the necessity of considering individual patient HLA haplotype. While CAR-T cell therapy directed towards CRC is still very much in its infancy, therapeutic models in animal studies have demonstrated a significant level of efficacy. There certainly are barriers to treating solid tumors with CAR-T cells, but many of the immunosuppressive microenvironment challenges are common to multiple tumor types and as the field evolves more broadly, these insights can be extended to other tumor types, including CRC [40]. Nevertheless, the current limitations should not deter investigation, given the widely acclaimed results seen in hematological malignancies [41]. The therapeutic potency of these targeted tumor killers dwarfs that of conventional therapies, with considerably less off-target toxicity, and continued preclinical and clinical development of these promising therapies could transform colorectal cancer treatment in the coming decade.

Compliance with Ethical Standards

Conflict of Interest Trevor R. Baybutt was supported in part by a Percival E. and Ethel Brown Foerderer Foundation Fellowship from Thomas Jefferson University. Allison A. Aka declares that she has no conflict of interest. Adam E. Snook has received research support through grants from W.W. Smith Charitable Trust and Advaxis, Inc. during the conduct of the study. In addition, Dr. Snook has patents with royalties licensed to Targeted Diagnostics and Therapeutics.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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