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Authors
Hecht, J Randolph
Mitchell, Jasmine
Morelli, Maria Pia
et al.

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Next-Generation Approaches to Immuno-Oncology in GI Cancers

J. Randolph Hecht, MD[1]; Jasmine Mitchell, MD, MS[1]; Maria Pia Morelli, MD, PhD[2]; Gayathri Anandappa, MBBS, MPhil, MRCP[2]; and James C. Yang, MD[3]

Overview

Immunotherapy has only had a modest impact on the treatment of advanced GI malignancies. Microsatellite-stable colorectal cancer and pancreatic adenocarcinoma, the most common GI tumors, have not benefited from treatment with standard immune checkpoint inhibitors. With this huge unmet need, multiple approaches are being tried to overcome barriers to better anticancer outcomes. This article reviews a number of novel approaches to immunotherapy for these tumors. These include the use of novel checkpoint inhibitors such as a modified anti–cytotoxic T lymphocyte–associated antigen-4 antibody and antibodies to lymphocyte-activation gene 3, T cell immunoreceptor with immunoglobulin and ITIM domains, T-cell immunoglobulin-3, CD47, and combinations with signal transduction inhibitors. We will discuss other trials that aim to elicit an antitumor T-cell response using cancer vaccines and oncolytic viruses. Finally, we review attempts to replicate in GI cancers the frequent and durable responses seen in hematologic malignancies with immune cell therapies.

INTRODUCTION

Although immunotherapy with standard PD(L)-1 or cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) immune checkpoint inhibitors (ICIs) has revolutionized the treatment of melanoma[1] and non–small-cell lung cancer (NSCLC),[2] the benefits in GI cancers have been relatively limited. The most efficacy has been demonstrated in patients with GI tumors with deficient DNA mismatch repair (dMMR)[3] although these are only a small portion of patients with GI cancer particularly in pancreatic adenocarcinoma.[4] Furthermore, not all dMMR cancers respond to therapy with standard ICIs and some that do eventually progress. ICIs have become standard of care in metastatic upper GI malignancies,[5] hepatocellular carcinoma,[6] and bile duct cancers[7] although the benefits are not as great as those seen in more immunosensitive tumors. The most common GI cancers, proficient MMR colorectal cancer (CRC), and pancreatic adenocarcinoma remain resistant to these agents.[3] Therefore, new approaches to immunotherapy are needed. The most mature of these are novel checkpoint inhibitors, tumor vaccines, oncolytic viruses (OVs), and immune cell therapies. The data for their use in GI cancers will be reviewed.

NOVEL ICIs AND COMBINATIONS IN GI TUMORS

ICIs, notably anti–CTLA-4 and PD-1 and PD-L1 inhibitors, have improved treatment for solid tumors, including GI.[3,8-10] Combinations with chemotherapy[5,11] and the anti–human epidermal growth factor receptor 2 antibody trastuzumab are standard of care in cancers of the upper GI tract.[12] Unfortunately, outside of patients with dMMR, these benefits are rarely durable and are nonexistent in colorectal and pancreatic cancers. Therefore, researchers are looking at new versions of standard ICIs, new combinations with other agents to overcome immunosuppression, and finally inhibitors of novel checkpoints (Table 1).

Botensilimab, an Fc-enhanced next-generation anti–CTLA-4 antibody, in combination with balstilimab, a novel anti–PD-1 antibody, showed promising results in patients with microsatellite-stable (MSS), heavily pretreated metastatic CRC (NTC03860272).[16] Data presented by El-Khoueiry et al[17] recently showed that the objective response rate (ORR) was 24% (95% CI, 14 to 39), the disease control rate was 76% (95% CI, 60 to 84), and median duration of response was not reached. The 12-month overall survival (OS) rate was 63% (95% CI, 42 to 75). The patients who did benefit the most from treatment were those without liver metastases. About 12% of the patients had treatment-related adverse events resulting in treatment discontinuation. A phase II trial is currently enrolling (ClinicalTrials.gov identifier: NCT05608044).[18]

The presence of various immunosuppressive cells in GI tumors such as tumor-associated macrophages and regulatory T cells (Tregs) may limit the effectiveness of ICIs. In preclinical models, small-molecule tyrosine kinase inhibitors (TKIs) such as regorafenib can reduce this immunosuppression by inhibiting colony stimulating factor 1 receptor, vascular endothelial growth factor receptor, and other potentially immunosuppressive pathways.[19] The relatively small Japanese REGONIVO

Author affiliations and support information (if applicable) appear at the end of this article.

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PRACTICAL APPLICATIONS

- Further studies testing antibodies against LAG-3, TIGIT, T-cell immunoglobulin-3, and CD47 should be performed in GI malignancies as they have shown promising results in preclinical studies and phase I/II trials in other cancer types.
- Fc-enhanced cytotoxic T lymphocyte–associated antigen-4 inhibitor botensilimab in combination with an anti–PD-1 has shown remarkable activity in proficient MMR (microsatellite-stable) metastatic colorectal cancer in a phase I study, and results of further trials could have a major impact in standard-of-care treatments.
- Although cancer vaccines and oncolytic viruses have shown limited responses in early phase trials in GI cancers, there are still many unknowns in terms of which cancer types will respond and which combinations of chemotherapy/immunotherapy will improve efficacy.
- T-Cell receptor therapy may prove to be more advantageous than chimeric antigen receptor-T therapy in solid malignancies, but further research in this area is needed.
- Solving the problems of antigen selection and intrinsic tumor immune evasion will allow advances in genetic engineering of T-cell fitness to better promote durable antitumor responses.

trial combining regorafenib with the anti–PD-1 nivolumab showed encouraging results with the response rate (RR) of 44% in gastric cancer and 33% in MSS CRC. Many of these responses appeared to be durable.[13] A phase II North American trial, however, only showed a 7% RR with regorafenib and nivolumab, all in patients without liver metastases. In that small group, interestingly, the RR was 22%. Similar early data have been seen with newer small-molecule TKIs such as lenvatinib and cabozantinib that may inhibit additional immunosuppressive pathways.[20,21] Large-phase trials combining ICIs with lenvatinib (LEAP-017; ClinicalTrials.gov identifier: NCT04776148)[22] and zanzalintinib (XL092; STELLAR-303; ClinicalTrials.gov identifier: NCT05425940)[23] in metastatic CRC are currently underway.

There are multiple other immune checkpoints, and inhibitors of these alone and in combination with anti–PD(L)-1 inhibitors are the subject of active research in GI cancers. Lymphocyte-activation gene 3 (LAG-3) (CD223) is a cell surface molecule expressed on activated CD4 and CD8 T cells, Tregs, natural killer (NK) cells, B cells, and plasmacytoid dendritic cells (DCs).[24] In preclinical studies, the combination of LAG-3/PD-1 blockade resulted in synergistic activity, providing a strong rationale for a combinatorial strategy. In the randomized, phase II/III, RELATIVITY 047 study in patients with untreated or unresectable, advanced melanoma, the combination of relatlimab, a first-in-class, anti–LAG-3 antibody, with the PD-1 inhibitor nivolumab showed improvements in median progression-free survival (mPFS) compared with nivolumab alone (10.1 *v* 4.6 months [hazard ratio, 0.75; 95% CI, 0.62 to 0.92; $P = .006$]).[25] This combination is now approved for treatment by the Food and Drug Administration (FDA). A phase I trial with the anti–LAG-3 antibody favezelimab with pembrolizumab in metastatic CRC had an 11% RR. Combination trials with other LAG-3 antibodies are ongoing in GI cancers.[26]

T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), a member of the Ig superfamily and an immune inhibitory receptor, plays a key role in the suppression of T-cell proliferation and activation.[27] Among its functions, TIGIT inhibits NK cell–mediated tumor killing, suppresses CD8 T-cell priming and differentiation, and prevents CD8 T-cell–mediated killing.[28] Preclinical studies showed that TIGIT is coexpressed and associated with PD-1 expression and dual blockade of TIGIT and PD-1 in the restoration of T-cell[29] and NK cell immunity, providing a good rational for this combination.[30] The CITYSCAPE trial evaluated the efficacy and safety of tiragolumab in combination with atezolizumab as first-line treatment for NSCLC. The primary analysis from this randomized, double-blind, phase II trial showed clinically meaningful improvement in ORR and PFS compared with placebo plus atezolizumab in patients with chemotherapy-naive, PD-L1–positive, recurrent or metastatic NSCLC.[31,32] Unfortunately, these results were not confirmed by the SKYSCRAPER trial, which failed to confirm PFS and OS benefits in the tiragolumab arm.[33] There are ongoing trials of tiragolumab in combination with atezolizumab, chemotherapy, and targeted therapies in upper GI and CRCs (ClinicalTrials.gov identifier: NCT03281369, NCT04929223).[34,35] In the CITRINO study (ClinicalTrials.gov identifier: NCT03250832),[36] encelimab (TSR-033) was combined with dostarlimab and bevacizumab and chemotherapies in patients with CRC, but results are still pending. Vibostolimab (MK-7684), another anti-TIGIT antibody, was evaluated in combination with pembrolizumab in a phase I trial, showing a safe profile and a promising antitumor activity,[37] and is being looked at in MSI-high CRC (ClinicalTrials.gov identifier: NCT04895722).[38]

T-cell immunoglobulin-3 (TIM-3) is an immune checkpoint that promotes immune tolerance.[39] TIM-3 blockade results in decreased myeloid-derived suppressor cells (MDSCs) and increased proliferation and cytokine production by T cells.[40] Given its expression in a variety of T cells and its synergistic effects with other anti–PD-1 agents, several trials are ongoing to evaluate safety and activity of TIM-3 inhibitors in combination with anti–PD-1 antibody. In a

TABLE 1. Novel Immune Checkpoint Inhibitors and Combinations

Target	Mechanism of Action	Ongoing/Completed Trials in GI
Fc-enhanced anti–CTLA-4	Anti–CTLA-4 ab with enhanced FcγR-dependent functionality Promotes superior T-cell priming, memory responses, and depletion of intratumoral Tregs	Phase II of botensilimab with balstilimab in CRC (NCT05608044)
TKIs Regorafenib Zanzalintinib Lenvatinib	TKIs block potentially immunosuppressive pathways	REGONIVO Japanese trial combining regorafenib with nivolumab 11[13] Phase II trial regorafenib/nivolumab in North America (NCT04126733) Phase III zanzalintinib + atezolizumab in mCRC (NCT05425940) Phase III lenvatinib + pembrolizumab in mCRC (NCT04776148)
LAG-3	A cell surface molecule expressed on activated CD4/CD8 T cells, Tregs, NK cells, B cells, and DCs	Phase I trial with favezelimab with pembrolizumab in mCRC (NCT05064059)
TIGIT	Inhibits NK cell–mediated tumor killing Suppresses CD8 T-cell priming/differentiation Prevents CD8 T cell–mediated killing	Phase I trials in combination with ICI (NCT03281369, NCT04929223, NCT03250832, NCT04895722)
TIM-3	Blockade results in decreased MDSCs and increased proliferation and cytokine production by T cells	Phase Ib study of sabatolimab and spartalizumab 11[14]
CD47	Binds to SIRPα that inhibits macrophage phagocytosis	ELEVATE trial in combination with FOLFIRI and bevacizumab (NCT04827576)
ICOS (CD278)	Binds to an ICOS ligand expressed by B cells, macrophages, and DCs Costimulatory for T-cell proliferation and cytokine production Inhibition decreases intratumoral Tregs and increases T effector cells	Phase I/II trial in combination with atezolizumab in advanced malignancies (NCT03829501)
B7-H3 (CD276)	Inhibits CD4/CD8 T-cell activation, proliferation, and cytokine production	Phase I/II trial in advanced solid tumors with enoblituzumab 11[15]

Abbreviations: ab, antibody; CRC, colorectal cancer; CTLA-4, cytotoxic T lymphocyte–associated antigen-4; DCs, dendritic cells; FcγR, Fc gamma receptor; FOLFIRI, folinic acid, fluoruoracil, and irinotecan; ICI, immune checkpoint inhibitor; ICOS, inducible T cell costimulator; LAG-3, lymphocyte-activation gene 3; mCRC, metastatic colorectal cancer; MDSC, myeloid-derived suppressor cell; NK, natural killer; SIRPα, signal receptor protein-α; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3, T-cell immunoglobulin-3; TKI, tyrosine kinase inhibitor; Treg, regulatory T cell.

phase Ib study, sabatolimab (MBG453), a TIM-3 antibody, and spartalizumab, a PD-1 ICI, generated partial responses in two patients with CRC.[14]

CD47 is a don't-eat-me signal that is a truly novel checkpoint for macrophages and DCs. It binds to signal receptor protein-α that inhibits phagocytosis.[41] Studies with the anti-CD47 antibody magrolimab have shown promising activity in hematologic malignancies.[42] The randomized phase II ELEVATE trial is currently underway in second-line CRC in combination with FOLFIRI and bevacizumab (ClinicalTrials.gov identifier: NCT04827576).[43]

Other novel checkpoints such as V-domain immunoglobulin suppressor of T-cell activation (VISTA), inducible T cell costimulator (ICOS), and B7-H3 have not been closely examined in GI cancer. VISTA is an immunoregulatory molecule involved in maintaining T-cell and myeloid quiescence.[44] It is expressed on resting T cells, indicating its regulatory role in earlier stages, and is more abundant in MDSCs in the tumor microenvironment (TME). The nonoverlapping mechanisms of VISTA and PD-L1 make their combination an ideal treatment strategy to overcome immune suppression. ICOS (CD278) is a member of the CD28 coreceptor family, which includes costimulatory CD28 and coinhibitory receptor CTLA-4.[45] Yap et al[46] evaluated an ICOS agonist, vopratelimab, alone and in combination with nivolumab in patients with advanced solid tumors. The study showed a safe drug profile and efficacy only in a subset of patients, with potential biomarkers to be evaluated in prospective studies. B7-H3 (CD276) is a member of the B7 family, a family of transmembrane proteins that interact with CD28 receptors family and modulate wither stimulatory or inhibitory immune signals.[47] Several agents targeting B7-H3 are currently under investigation in clinical trials. The anti–B7-H3 monoclonal antibody, enoblituzumab (MGA271), was evaluated in combination with pembrolizumab in a phase I/II trial in advanced solid tumors, showing a safe profile and promising antitumor activity in checkpoint inhibitor–naïve patients.[15]

In summary, there are multiple approaches being examined to try to overcome resistance to standard CTLA-4 and PD(L)-1 inhibitors in GI cancers. These promise to improve outcomes in malignancies that have had little improvement over the past two decades. Further development will require more translational research and identification of robust biomarkers of activity.

CANCER VACCINES IN GI MALIGNANCIES

Adaptive immunity is mediated by cytotoxic CD8+ T cells, CD4+ helper T cells, and B cells. In cellular immunity, T cells can recognize and eliminate diseased cells.[48] Vaccines work by inducing an immune response to the antigen(s) encoded by the vaccine. Subsequently, immunologic memory and adaptive immunity elicited against the immunizing antigen can protect an individual against the pathogen from which the antigen was derived.[49] Cancer vaccines are designed with the intent to elicit an immunologic therapeutic response against tumor antigens. Tumor antigens can be divided into tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). TSAs are expressed only by cancer cells, not normal cells, whereas TAAs are overexpressed in tumor cells compared with normal cells.[50]

Despite numerous attempts over the past century, only two therapeutic vaccines have been approved to date, sipuleucel-T, a DC-based vaccine for the treatment of castrate-resistant prostate cancer,[51] and the Bacillus Calmette-Guerin (BCG) vaccine for early bladder cancer. Despite this track record, vaccines continue to be developed in GI malignancies because of the huge unmet need. There are multiple different vaccine approaches to stimulate anticancer immunity such as autologous or allogenic cancer cells, DCs, and vaccine vectors encoding tumor antigens.[52]

Early studies used whole cancer cells to induce an immune response. OncoVAX combined BCG with autologous cancer cells. In the phase III ECOG 5383 trial of patients with CRC treated with surgery with or without vaccine, there was no significant difference in overall or disease-free survival.[53] GVAX is an allogeneic whole-cell vaccine composed of two human pancreatic adenocarcinoma cell lines modified to express granulocyte-macrophage colony-stimulating factor (GM-CSF).[54] Promising early data with CRS-207, a listeria modified to express the common TAA mesothelin,[55] were not borne out in the larger ECLIPSE trial[56] or in combination with checkpoint inhibitors.[57]

DCs are antigen-presenting cells that can activate naïve T cells against various host insults. A MUC1 peptide–loaded DC vaccine was tested in a phase I/II trial in resected pancreatic cancer with some long-term survivors.[58] Many ongoing trials in CRC involve administrating DCs pulsed with autologous tumor lysates. Immune responses to tumor antigens found in CRC and pancreatic cancer can be generated after DC vaccination, but these have not resulted in improved clinical outcomes.[59]

Another class of vaccines use different vaccine vectors, such as peptides, DNA plasmids, viruses, or RNA, to encode specific tumor. Potential challenges include identifying tumor antigens that will be immunogenic in specific patients. In a phase II trial in advanced CRC, a mixture of five HLA-A*24:04–restricted peptides combined with oxaliplatin-based chemotherapy had no significant effect on clinical outcomes.[60] The RAS G12D/R peptide vaccine ELI-002 is currently being examined in patients with ctDNA-positive only pancreatic and other cancer (ClinicalTrials.gov identifier: NCT04853017).[61] Advances in sequencing and manufacturing of vaccine vectors have enabled the design of personalized and off-the-shelf vaccines that can target neoantigens (tumor antigens derived from mutations). As an example, the mRNA-based phase II trial, KEYNOTE-942 trial, showed encouraging activity and possible proof of concept with this approach, reducing recurrence or death by 44% in patients with stage II/IV melanoma.[62] These data support the concept that treating patients earlier in their course of disease may improve the efficacy of vaccine approaches. A phase I study of a prime boost strategy–personalized vaccine study using chimp adenovirus and self-replicating RNA resulted in robust antitumor immune response[63] and is being examined in the first-line colorectal maintenance setting in the phase II/III GRANITE trial (ClinicalTrials.gov identifier: NCT05141721).[64] New vaccine approaches even have the exciting potential to reduce cancer incidence in patients with high-risk premalignant conditions such as Lynch syndrome.[65]

Although several cancer vaccines have shown induction of vaccine-specific responses, these have not resulted in clinical benefits in GI or most other cancers. The quality and quantity of these immune responses, especially with respect to CD8+ and CD4+ T cells, remain incompletely characterized and are an important consideration in evaluating the effectiveness of cancer vaccines. The antigens targeted by vaccines have important implications in the quality of the immune response as one of the primary issues with overexpressed TAAs is that central and peripheral tolerance mechanisms limit the generation of autoreactive B and T cells that strongly recognize these sequences.[66] Vaccines need to overcome this immune tolerance to mount a response without causing autoimmune reactions. There is still much work to be performed to identify which class of tumor antigens delivered by which vaccine vectors results in an optimal immune response. Additional factors include how to combine vaccines with standard-of-care chemotherapy and other immunotherapy drugs and the treatment setting (ie, adjuvant, early *v* late metastatic disease). A successful vaccine approach aims to overcome tolerance, reverse

immunosuppression, cause tumor death, and generate long-lasting memory responses.

OVs IN GI MALIGNANCIES

The benefits of immune herapies seem to be greatest in immunologically hot TMEs.[67] These tumors have high mutational burdens, high levels of tumor-infiltrating lymphocytes (TILs), and increased PD-L1 expression. The lack of presentation and/or expression of TAAs; infiltration by suppressive neutrophils, regulatory T cells, macrophages, myeloid-derived suppressor cells, or NK cells; low density of TILs; and expression of immunosuppressive factors lead to an immunologically cold tumor.[67] OVs are an exciting class of anticancer immunotherapies that exploit viruses' innate ability to preferentially infect, self-amplify, and lyse tumor cells.[68] They hijack and reprogram the host's cellular machinery, expressing both therapeutic and virus transgenes.[69] OVs were initially designed to just kill tumor cells, but more recent data have shown that at least some of the anticancer effects are by infecting a tumor cell and induce apoptosis, triggering an inflammatory reaction.[70] This activates innate and adaptive immune responses by the release of TAAs, pathogen-associated molecular patterns, and danger-associated molecular patterns from lysed tumor cells to act like a cancer vaccine to achieve an abscopal effect.[69] Talimogene laherparepvec (T-VEC), a herpesvirus designed to produce GM-CSF in the tumor to enhance antigen release, presentation, and antitumor immune response, was the first OV approved for use in the United States and Europe.[67] In a phase III trial, intratumoral injection of T-VEC improved durable RR and other clinical outcomes in advanced, nonresectable melanoma, leading to full FDA approval in 2015.[71] Despite initial encouraging results together with pembrolizumab, in a phase III trial, the combination was not superior to pembrolizumab alone.[72,73]

Multiple classes of OVs have been developed. The nonenveloped double-stranded DNA (dsDNA) adenoviruses were some of the first.[69] ONYX-015 is a first-generation *E1B*-55kD gene-deleted replication-selective adenovirus that preferentially replicates in and kills malignant cells. In a phase I/II trial of endoscopic ultrasound injection of locally advanced or metastatic pancreatic cancer in combination with gemcitabine, two of 21 patients had partial regressions of the injected tumor and eight had stable disease.[74] Although not being developed further in GI cancers, a variant, H101, is approved in China for head and neck cancer.[75] TNFerade, an adenovirus encoding tumor necrosis factor alpha, was examined in combination with chemoradiation in locally advanced pancreatic cancer with encouraging phase I/II results, but a phase III trial was negative.[76,77] Other adenoviruses being examined in GI cancers include enadenotucirev (EnAd, ColoAd1) and telomelysin,[78] which are currently being studied in combination with pembrolizumab in a phase II trial for advanced gastroesophageal adenocarcinoma (ClinicalTrials.gov identifier: NCT03921021).[79]

Herpesviruses are characterized by an icosahedral capsid and a dsDNA genome. Oncolytic herpes simplex viruses (HSVs) have been extensively studied because of a large transgene capacity, lack of insertional mutagenesis, and ability to activate innate and adaptive immune responses against tumors.[69] A phase I study using T-VEC in combination with atezolizumab for triple-negative breast cancer and CRC with liver metastases, however, showed limited evidence of antitumor activity.[80] Other HSV derived agents are in development.[81]

Vaccinia virus has a large dsDNA genome[69] and replicates in the cytoplasm, thereby eliminating the risk of insertional mutagenesis. The best studied vaccinia OV is pexastimogene devacirepvec (Pexa-Vec, JX-594), an engineered thymidine kinase–mutant vaccinia virus armed to express GM-CSF and β-galactosidase as transgenes.[69,82,83] It was found to be trafficked to the tumor as evidenced by the viral genome found in tumor biopsies,[84] and there were early hints of anticancer activity.[85] Unfortunately, a randomized phase IIb trial in hepatocellular cancer was negative[86] and a phase I/II trial with durvalumab and tremelimumab in CRC showed modest benefit.[87]

Pelareorep (Reolysin) is an unmodified oncolytic reovirus, delivered intravenously, that can induce a T-cell inflamed phenotype in pancreatic ductal adenocarcinoma. In a phase Ib study in patients who had progressed after first-line treatment, pelareorep, pembrolizumab, and 5-fluorouracil, irinotecan, or gemcitabine[88] did not add significant toxicity and showed encouraging efficacy. Other phase II trials of pelareorep in pancreatic cancer in combination with carboplatin/paclitaxel have showed similar results of good tolerability but mixed responses in terms of RR, PFS, and OS.[89,90]

This is a nonexhaustive survey of OVs in GI cancers. There are many challenges to overcome in the development of effective OVs. The need for intratumoral injection of some OVs, proper spread and penetration of the therapeutic agent, tumor cell targeting, pre-existing immunity to the viruses, and hypoxia are all factors that can inhibit the effectiveness.[82] The site of injection may also affect efficacy. The liver is particularly immunosuppressive with multiple mechanisms including liver metastases siphoning activated CD8+ T cells from systemic circulation and within the liver, leading to acquired immunotherapy resistance.[91] The optimal degree of infectivity and oncolysis is also unknown. Other unknowns in the study of OVs are which patients and tumor types most benefit from this therapy and in which combinations of chemotherapy and immunotherapy. Potential combinations with cytokines, BiTEs, and even chimeric antigen receptor (CAR)-T cells may improve efficacy.[92,93]

IMMUNE CELL THERAPY FOR GI TUMORS

The ability of infused cultured tumor-reactive immune cells to induce the rejection of human cancers has been well demonstrated. Expanding the resident T cells in melanomas (TIL) and infusing them along with systemic interleukin-2 (after preparative lymphodepletion with chemotherapy) can result in an ORR of over 50%, with half of those responding patients apparently cured of metastatic disease.[94] Genetically modifying peripheral blood lymphocytes (PBLs) with a CAR targeting a B-cell antigen, CD19, can cause objective regressions of large B-cell lymphoma in 82% of patients with refractory disease, again with many of them achieving durable complete remissions after a single administration.[95] A third example is the introduction of a tumor-reactive T-cell receptor (TCR; cloned from a T cell specific for the NY-ESO-1 antigen) into the PBL of patients with synovial sarcoma or melanoma, which resulted in a 58% ORR.[96] The major goal at this time is to expand such results to the common epithelial cancers, and this has proven to be difficult. This review will clarify the differences between these three sources of tumor reactive T cells, review their results, and discuss future directions.

PBLs (or in some cases, NK cells) engineered with CAR-T cells have been quite effective in the treatment of several hematopoietic malignancies. The CAR consists of an antigen-binding domain coupled to the T-cell signaling machinery, often with an interposed costimulatory domain. The antigen-binding domain is typically an antibody single-chain variable fragment (scFv), the T-cell signaling moiety usually uses CD3-zeta, and the costimulator is often CD28 or CD134 although innumerable variations on this framework have been devised. The current obstacle to using CAR T cells against solid malignancies has been the identification of safe TAAs. First, these TAAs need to be outer cell membrane structures and then they must be invariant because of the complexities of creating Ag-binding domains and optimizing the CAR. Most have been normal differentiation antigens on disposable tissues. Targeting cell surface B-cell markers such as CD19 and CD22 to destroy both benign and malignant B cells is tolerable because patients can live without B cells. Unfortunately, the organs giving rise to GI cancers are typically not dispensable. Very limited efforts to target solid tumors with CAR-T cells have been pursued. Early efforts to target carcinoembryonic antigen (CEA) either were ineffective or generated normal bowel toxicity.[97] Targeting the GD2 ganglioside on neuroblastoma and some pediatric gliomas has shown some positive results in small studies, but it is not a target on common epithelial cancers.[98] One very interesting phase I trial targeted the tight junction protein Claudin18.2 with a classic CAR consisting of a scFv-binding domain, CD28 costimulation, and CD3 zeta signaling.[99] Cells were administered after cyclophosphamide and fludarabine preconditioning, but no interleukin-2 was administered. An ORR of 49% was reported in patients with predominantly gastric cancer despite administering a relatively low numbers of cells ($\leq 5 \times 10^8$ cells). All responses were partial, and many of short duration; yet, this represents one of the only CAR-T-cell trials relevant to GI cancers with significant objective responses. Another research initiative has been to apply gating strategies to CARs to allow immune attack on cancer, but block activity when the target is encountered on normal tissues.[100] Logic-gated CAR-T cells have shown activity and specificity in preclinical models, and trials are ongoing in GI cancers expressing CEA.[101-103] Alternatively, one can target two structures with imperfect specificity on cancer that do not coexpress on normal tissues to generate better specificity.[104] These promising ideas are poised to enter early clinical trials, and their effectiveness remains unknown. The idea of CAR-T cells for common solid tumors remains attractive because of the circumvention of the major histocompatibility complex (MHC) restriction of normal T cells, expanding the applicability to more patients. There is also a theoretical advantage to a novel synthetic receptor for cancer. As will be described below, tumors under siege by endogenous T cells rapidly develop diverse immune evasion and escape mechanisms. Using a novel non-native receptor to initiate a T-cell attack has the advantage of not encountering a priori escape mechanisms generated before the adoptive transfer. Yet, the main obstacle remains not having suitable and safe target antigens.

The alternative to using CAR-T cells is to use native T cells and TCRs. Their major disadvantage is that TCRs recognize small processed peptide epitopes presented on MHC molecules. Therefore, a TCR is only pertinent to tumors with both the antigen and the presenting MHC allele and a much larger array of receptors is needed to address a population of patientswith cancer. On the other hand, because the epitope is proteolytically processed and exported to the cell surface on the MHC molecule, the TAA can be any protein made in the cytoplasm, not just outer cell membrane proteins. Humans also have nearly 10^{11} premade T-cell specificities in their repertoire, so there is no manufacturing required. Again, the main problem is finding safe and effective TAA. Here, the critical role of tumor-specific mutations comes into play. It has become clear from laboratory work and checkpoint inhibitor therapies that these mutated proteins are the major driver of the immune response of humans to cancer. Their tumor-specific nature also makes them a safe T-cell target. Unfortunately, the array of tumor-specific mutations is highly specific to each patient and their tumor,[105] with a limited number of common, shared mutations. One method of identifying T-cell reactivities to mutated antigens (neoantigens) has been described and extended to clinical trials.[106] A cancer's mutations are defined by whole exomic sequencing, and those mutations are

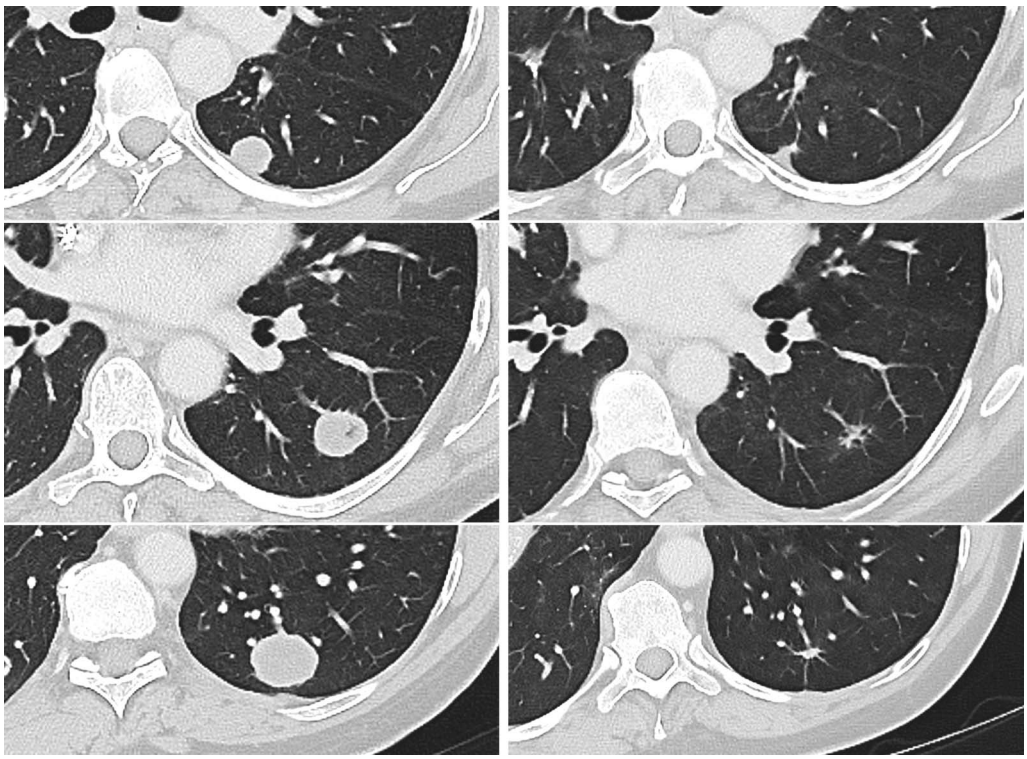

FIG 1. Responses to TIL reactive with neoantigens in colon cancer. Patient with colon cancer metastatic to lungs. Treated with lymphodepletion followed by adoptive transfer of TIL reactive with mutations in *DNMT3A* and *MUC6* and six doses of interleukin-2. The patient had near complete response and had received no other treatment. The left panel is baseline CT scan, and the right is 5-year follow-up showing all residual disease. CT, computed tomography; TIL, tumor-infiltrating lymphocyte.

expressed in autologous DCs by either minigene electroporation or loading synthetic peptides to create an avatar of that cancer's mutanome. This is then cocultured with TILs to identify which TILs are neoantigen-reactive. Patients are infused with subcultures selected for reactivity after undergoing preparative lymphodepletion with chemotherapy, and then systemic IL-2 is coadministered with the cells. The first patient to undergo this had cholangiocarcinoma and had a partial response of liver and lung metastases lasting nearly 3 years. She then relapsed but had persisting TIL from the infusion that expressed PD-1 and reresponded to a short course of pembrolizumab and remains free of disease, now 9 years after cell transfer.[107] Patients with breast cancer,[108] cervical cancer,[109] and colon cancer (Fig 1) have had durable complete responses to TIL reactive with neoantigens. Yet the RR is low despite the proven specificity of the infused TIL. Although this may in part be due to the exhausted phenotype of most TIL,[110] a host of tumor-related evasion mechanisms have been found as well. The simplest is the loss of the neoepitope or the restricting MHC allele. Although the latter was thought to occur from loss of both alleles of β-2 microglobulin (for MHC

TABLE 2. Advantages and Disadvantages of CAR-T Versus Native TCRs

Advantage/Disadvantage	CAR T Cells	T Cells/TCRs
Advantages	No MHC restriction	All proteins can be potential targets
	No previous immune resistance or evasion	Can easily target tumor-specific neoantigens
		Diverse repertoire naturally available
		Thymic tolerance prevents autoimmunity
Disadvantages	Targets essentially limited to shared (self) antigens	MHC restriction requires more TCRs
	Targets must be outer cell membrane structures	Prior selection for resistance occurs
	No thymic protection against autoimmunity	

Abbreviations: CAR, chimeric antigen receptor; MHC, major histocompatibility complex; TCRs, T-cell receptors.

class I), it has become apparent that loss of the single presenting MHC allele (seen as loss of heterozygosity at the MHC locus on chromosome 6) or downregulation[111] is more common and can even occur during the course of treatment.[112] Other evidence from checkpoint inhibitor therapy identified interferon-gamma signaling defects as a cause of tumor resistance to T cells.[113-115] Because the tumor and neoantigen-reactive T cells coexist for years, there is an opportunity for escape mechanisms to evolve; immune pressure leads to immune selection, which can lead to immune escape. Some mechanisms are reversable (such as T-cell inhibition by checkpoint receptors), some are irreversible (tumor MHC loss), some are T-cell–associated, and some are tumor-associated. One drawback of TIL therapy is that there is little control over the T cells that one recovers from a resected tumor. There can be problems with exhausted T cells, a low frequency of reactive cells, and inhibited TIL. One way to address issues with the quality of TIL is to clone the TCRs from neoantigen-reactive TIL and re-express them in fresh autologous PBL for transfer. This can also create an opportunity to target common recurring mutations with off-the-shelf reagents.

Mutations in *KRAS*, TP53, EGFR, BRAF, and PIK3CA, among others, are seen recurrently in many human cancers. Assembling libraries of TCRs specific for these mutations would allow the rapid generation of T cells for transfer by retroviral transduction. This would also allow one to select or genetically engineer optimized T-cell phenotypes to induce tumor rejection. Each mutation would require TCRs with specific MHC restrictions, greatly expanding the TCR libraries required. Yet, less than a 100 TCRs restricted by the most common HLA alleles would apply to the majority of human cancers. Most of the current efforts concentrate on *KRAS* (G12D, G12V mutations)[116,117] and TP53 (high-frequency hot spot mutations),[118] common in GI tumors. These mutations have been shown to be immunogenic, and in some cases, there is evidence that targeting them can be clinically effective.[112,119,120] Preclinical and clinical studies on genetic modifications to improve efficacy have looked at introducing cytokine secretion or orthogonal synthetic cytokine receptors into T cells,[113,121,122] and modifying function instead of just specificity represents the future of T-cell therapy. In summary, the adoptive transfer of tumor reactive T cells can cause curative regressions of some cancers. These T cells can be obtained from the natural repertoire of the patient via TIL or be genetically constructed by introducing either a CAR or a native TCR with tumor specificity. Each of these approaches has their advantages and disadvantages (Table 2). Solving the problems of antigen selection and intrinsic tumor immune evasion will allow advances in the genetic engineering of T-cell fitness to better promote the durable rejection of cancers.

CONCLUSION

Multiple immunotherapeutic approaches are actively being pursued in metastatic GI cancers. Although standard ICIs help some patients for a relatively short time, the immuno-oncology revolution in cancer care has bypassed most of these patients. Novel strategies including new checkpoint inhibitors, cancer vaccines, OVs, and immune cell therapies hold the promise of overcoming barriers to effective treatments. Progress has been slow, but the large number of ongoing studies may lead to improved outcomes.

AFFILIATIONS

[1]David Geffen School of Medicine at UCLA, Santa Monica, CA
[2]UT MD Anderson Cancer Center, Houston, TX
[3]National Cancer Institute, Bethesda, MD

CORRESPONDING AUTHOR

J. Randolph Hecht, MD, Division of Hematology-Oncology, David Geffen School of Medicine at UCLA, 2020 Santa Monica Blvd, #600, Santa Monica, CA 90404; email: jrhecht@mednet.ucla.edu.

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