Immunotherapeutic advances in gastrointestinal malignancies

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Cancer is an important global issue with increasing incidence and mortality, placing a substantial burden on the healthcare system. Colorectal cancer is the third most common cancer diagnosed among men and women in US. It is estimated that in 2018 there will be 319,160 new diagnosis and 160,820 deaths related to cancer of the digestive system including both genders in the United States alone. Considering limited success of chemotherapy, radiotherapy, and surgery in treatment of these cancer patients, new therapeutic avenues are under constant investigation. Therapy options have consistently moved away from typical cytotoxic chemotherapy where patients with a given type and stage of the disease were treated similarly, to an individualized approach where a tumor is defined by its specific tissue characteristics /epigenetic profile, protein expression and genetic mutations. This review takes a deeper look at the immune-biological aspects of cancers in the gastrointestinal tract (entire digestive tract extending from esophagus/stomach to rectum, including pancreatico-biliary apparatus) and discusses the different treatment modalities that are available or being developed to target the immune system for better disease outcome.

**INTRODUCTION**

A deeper understanding of the biology driving cancer has helped shape treatment approaches. Cancer therapy options have consistently moved away from typical cytotoxic chemotherapy where patients with a given cancer were treated equal, to an individualized approach where a tumor is defined by its genetic profile, pertaining to protein expression and gene mutations. The latest addition to the treatment arsenal is immunotherapy, where the patient’s own immune system is reprogrammed to recognize and target the tumor.

The relationship between immunology and cancer dates to the late 19th century. One of the first observation documented that an injection of heat-inactivated bacteria into sites of sarcoma sometimes lead to durable regression. Since then, an impressive amount of research has established that not only does the immune system provide initial identification and targeting, it also continues to protect against any residual or new cancer, engaging in a molecular game of “hide and seek” within the tumor microenvironment in a dynamic process now termed “cancer immunoediting.” This process essentially includes three phases: Elimination (initial response of immune system to tumor), Equilibration (immune-mediated tumor dormancy) and Escape (tumor evasion of immune response) phases (Fig. 1).

**ELIMINATION PHASE**

In the Elimination phase, the adaptive and innate branches of the immune system identify tumor-specific antigens as non-self and target the tumor cell for destruction. Important effector molecules of the former include T cells, important subtypes being CD8+ (cytotoxic), regulatory (Treg) and CD4+ (helper cells); Natural Killer (NK) cells; Antigen Presenting Cells (APCs), the macrophages and dendritic cells (DCs). Activation of T cells requires the presentation of tumor antigen by APCs, the most potent of which are DCs. Antigen presented by DCs on MHC Class I or Class II molecules are recognized by T cell receptors; CD8+ and CD4+, respectively. This results in secretion of anti-tumor cytokines namely Type I (IFN-α/β) and II (IFN-γ) interferons, interleukins (IL-12, IL-6) and chemokines (CCL2), which aids the destruction of the tumor cell. Type I interferons have been shown to be critical for the early activation of the antitumor response, by facilitating the cross-presentation of tumor antigens from CD8α+/CD103+ DCs to CD8+ T cells. Type I interferons are also thought to directly induce apoptotic and anti-proliferative responses in tumor cells, further supporting tumor suppression.

Unlike T cells, NK cells do not require antigen presentation by MHC proteins. Rather, NK cells are recruited to the tumor site by the latter’s expression profile of interleukins and chemokines. NK cells were shown to eradicate senescent tumor cells in a p53-dependent manner. Another key pathway to the innate immune response in the Elimination phase is the stimulator of IFN genes (STING) pathway of cytosolic DNA sensing. Phosphorylation of STING by TRAF2 (TNF receptor associated factor-2) binding kinase results in the binding, subsequent phosphorylation and release to the nucleus of IFN regulatory factor 3 (IRF3, a transcription factor), which then drives transcription of IFN-β. In the tumor microenvironment, IFN-β leads to the spontaneous generation of antitumor CD8+ responses and is critical for T cell priming. Intratumoral delivery of STING agonists is currently being explored. It should be noted that the STING pathway has also been demonstrated to be tumorigenic, and an optimal therapeutic level of activation is yet to be determined.

**EQUILIBRATION PHASE**

The molecular interactions that comprise the Equilibration phase have not yet been fully discerned, due to lack of appropriate animal model. However, evidence supporting the existence of this
markers (PD-L1, CTLA4), as well as (3) TREG inhibition (via PD-1/PD-L1 interaction) of CD8 variants utilize (1) decreased expression of antigenic cell surface markers, (2) increased expression of T-cell anergy-inducing cell surface from the tumor. Alteration of genetic pathways within tumor cells also generates new variants which can avoid detection. Escape—Tumor variants utilize (1) decreased expression of antigenic cell surface markers, (2) increased expression of T-cell anergy-inducing cell surface markers (PD-L1, CTLA4), as well as (3) TREG inhibition (via PD-1/PD-L1 interaction) of CD8+ T cells to overpower immune system. Steps (1), (2) and (3) ultimately result in growth, metastasis, angiogenesis and clinical presentation.

phase, as well as potential key interactions between the immune response and tumor do exist. In a mouse model of methylcholanthrene (MCA)-induced fibrosarcoma and p53 mutant tumors, it was shown that: (1) a Th1-like adaptive immune response facilitated tumor dormancy, (2) this dormancy may be a prolonged process, and (3) the balance between IL-12 (anti-tumorigenic) and IL-23 (pro-tumorigenic) in the tumor microenvironment is a determinant in whether the tumor dormancy is achieved.12,13 Another study in mice with islet adenomas demonstrated that transplantation of IFN-γ producing T-cell-antigen-specific Th1 cells inhibited angiogenesis and multi-stage carcinogenesis without tumor cell destruction.14 It was subsequently shown that this senescence was mediated by IFN-γ and TNF, by causing permanent growth arrest in the G1/G0 phase, activation of p16INK4a the inhibitor of cyclin-dependent kinases 4 and 6 and downstream hypophosphorylation of retinoblastoma (Rb) protein.15 These data strongly support an immune-mediated mechanism whereby tumors which survive the elimination phase are prevented from reaching full carcinogenic potential. Sadly, this is the proverbial "last stand" of the immune system, as the next phase of tumor development is escape.

ESCAPE PHASE

In this phase, the tumor becomes clinically apparent. Mechanisms employed by the tumor in this phase can be distilled into the following three categories:16 (1) reduced immune recognition and immune cell stimulation by downregulation of tumor antigens, antigen-expression machinery or co-stimulatory signals—all required for successful activation of APCs and thus T-cells, (2) upregulation of resistance against cytotoxic immunity or upregulation of pro-tumorigenic genes (e.g., STAT3 or Bcl2, respectively), and (3) creation of an immunosuppressive microenvironment.

The generation of an immunosuppressive microenvironment involves several tumors mediated cellular events. In addition to the production of cytokines like VEGF and metabolic factors like adenosine, PGE2, the tumor utilizes the recruitment of TREG cells or myeloid-derived suppressor cells (MDSCs), as well as the ligation of inhibitory receptors (e.g., CTLA-4, PD-1, Tim-3) on immune effector cells to generate adaptive immune resistance. TREG cells express the transcription factor forkhead box P3 (FoxP3) and are the subset of T cells that suppress the activation, proliferation and effector functions of a wide range of immune cells.17 There is evidence that due to the increased metabolic demands of the tumor microenvironment (the "Warburg effect"), tumor-infiltrating lymphocytes are directed towards the expansion of Tregs cells; glucose depletion ultimately inhibits adequate CD8+ and CD4+ T-cell control of tumor growth.18

IMMUNE INTERACTIONS IN THE GUT

The gastrointestinal system encounters the largest microbial activity in the body and thus requires multiple protective mechanisms to counter invasion by exogenous and endogenous/commensal microbes, as well as various infectious agents (e.g., viruses, parasites). Thankfully, mucosal immunity is a well-functioning, coordinated surveillance system, interwoven with the physiological and mechanical alterations that comprise gut homeostasis.19 Innately, intestinal epithelial cells (IECs); absorptive epithelial cells, goblet cells and Paneth cells provide physical and chemical barriers to infection, by the secretion of mucus and anti-inflammatory, via the secretion of cytokines to one another via direct contact, or by using cytokine signaling.22 The immunological diversity here includes CD4+ memory and effector (T helper 1 (Th1), Th17) T cells, and regulatory (Treg) T cells. Treg cells play a key role in suppressing inflammation, via the secretion of IL-10. Myeloid derivatives include dendritic cells and macrophages, and directly modulate immune cell responses via secretion of cytokines and chemokines.20,21 Additionally, the intestinal lamina propria contains a variety of myeloid and lymphoid cells, which can communicate to one another via direct contact, or by using cytokine signaling.22 The immunological diversity here includes CD4+ memory and effector (T helper 1 (Th1), Th17) T cells, and regulatory (Treg) T cells. Treg cells play a key role in suppressing inflammation, via the secretion of IL-10. Myeloid derivatives include dendritic cells and macrophages – subtypes of the latter have been shown to secrete IL-10 in response to commensal bacteria via the Toll-like receptor (TLR) signaling pathway, preventing inflammation.23 Conversely, immune cells can also interact with IECs. Th17 cells secrete IL-22, which upregulates secretion of AMP.24 IL-6 production from

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**Fig. 1** Elimination—(1) Apoptotic tumor cells release antigens which are collected by Dendritic cells, (2) Dendritic cells present antigen to CD4+ T cells in lymph node, which leads to the activation of cytotoxic CD8+ T cells and B cells, (3) B cells release antibodies; CD8+ cells release and Perforin/Granzyme, resulting in tumor destruction. Equilibrium—immune system keeps the tumor in a state of dormancy. Anti-tumor cytokines (IL-12, IFN-γ, TNF-α) and cytotoxic action is countered by pro-tumorigenic/energy-inducing molecules (IL-10, IL-23, PD-L1) from the tumor. Alteration of genetic pathways within tumor cells also generates new variants which can avoid detection. Escape—Tumor variants utilize (1) decreased expression of antigenic cell surface markers, (2) increased expression of T-cell anergy-inducing cell surface markers (PD-L1, CTLA4), as well as (3) TREG inhibition (via PD-1/PD-L1 interaction) of CD8+ T cells to overpower immune system. Steps (1), (2) and (3) ultimately result in growth, metastasis, angiogenesis and clinical presentation.
IEC lymphocytes facilitates proliferation of the intestinal epithelium, promoting healing after mucosal injury.\textsuperscript{25}

Mucosal immunity thus tightly regulates inflammation in the gut. This regulation is critical, as an unchecked and/or prolonged inflammatory state has been recognized as a potential driver for the development of colorectal cancer (CRC), the third most frequent cause of cancer related mortality amongst men and women in the US. The current understanding of tumor progression in CRC revolves around the initiation and maintenance of non-specific inflammation, which results in the production of pro-inflammatory cytokines (e.g., TNF-\(\alpha\), IL-1\(\beta\)). These cytokines can act directly on IECs, promoting proliferation, inhibition of apoptosis, invasion, angiogenesis, epithelial to mesenchymal (EMT) transition and metastasis.\textsuperscript{26} Additionally, anti-tumor (GM-CSF, IFN-\(\gamma\)) cytokines are depleted in CRC\textsuperscript{27} which not only accelerates tumor progression but also results in poorer outcomes for patients.

GUT MICROBIOME

In the past decade, as therapy options have expanded, we are learning to appreciate and possibly target this tumor microenvironment. \textsuperscript{28} Studies in mouse models of altered immune and in human patients who had not received antibiotics. Whether supplementing certain species of organisms through FMT can help increase/synergize response to immunotherapy is still under evaluation and is an important avenue for future research.

IMMUNE CHECKPOINT INHIBITORS

Immune “checkpoints” are inhibitory pathways which help differentiate self-antigens from foreign and suppress uncontrolled autoimmunity. Tumor cells evade these checkpoints by genetic and epigenetic alterations to influence neoantigen formation, presentation, and/or processing, as well as alterations in cellular signaling pathways that disrupt the action of cytotoxic T cells.\textsuperscript{41} Identification of the PD-1/PD-L1 and CTLA4 pathways have provided opportunity for manipulation of these checkpoints to block the immune evasion by cancer cells. This new class of drugs allows the host to mount a robust immune response to tumor cells, with scope for long lasting immunity by allowing cytotoxic T cells to recognize tumor antigens and subsequently generating memory T cells. Since the first approval of Ipilimumab for melanoma in 2011, this class of drugs has seen tremendous growth. Due to excellent tumor responses, limited side effect profile and efficacy in numerous solid organ tumors that are otherwise difficult to treat, these drugs have quickly proven themselves to be superior to many cytotoxic chemotherapy regimens.

COLORECTAL CANCER

Pembrolizumab was the first checkpoint inhibitor targeting the PD-1 pathway to demonstrate clinical activity in solid tumors, including CRC and gastric cancer with micro-satellite instability (MSI). Results from Keynote- 059 led the FDA to grant an accelerated approval to pembrolizumab as treatment for patients without other options with unpredictable or metastatic, MSI-H or mismatch repair deficient (dMMR) solid tumors. This was the first approval of a drug for a tissue agnostic indication and this has further paved the path for other clinical trials with umbrella or basket designs. The immune-related objective response rate and immune-related progression-free survival rate were 40% (4 of 10 patients) and 78% (7 of 9 patients), respectively, for dMMR CRC and 0% (0 of 18 patients) and 11% (2 of 18 patients) for pMMR CRC.\textsuperscript{42}

There are many theories as to why checkpoint blockers are not efficient in subjects with pMMR CRC. In CRC lesions that are largely infiltrated by effector memory T cell, immunological checkpoints might be intrinsically inactive. In this case, the exogenous administration of checkpoint blockers would be ineffective. Contrarily, CRC lesions with limited T-cell infiltration may not respond to checkpoint blockers because they cannot be properly
invaded, recognized or eliminated by the cellular immune system. This may re
fect the antigenic properties of malignant cells, their inability to adequately activate the immune system, or the activation of yet to be discovered immunological checkpoints that actively suppress immunosurveillance against CRC.10

On the basis of the promising efficacy from pembrolizumab for MSI-H colorectal cancer, it is now understood that somatic mutations have the potential to encode non-self-immunogenic antigens, making these otherwise non-responsive cancers, targetable by immune-mediated intervention. Checkmate 142 is an ongoing clinical trial (NCT02060188) investigating nivolumab in MSI-H metastatic or recurrent CRC, in combination with other checkpoint inhibitors with targets other than the PD-1/PD-L1 axis. The trial is designed to test the efficacy of nivolumab initially in dose escalation (completed) and then in combination with other drugs such as ipilimumab, cobimetinib and daratumumab (ongoing). Among pts treated with nivolumab 3 mg/kg Q2W (N = 74) OS rates were 83.4% (6 mo) and 73.8% (12 mo).43 Data from the above study is still maturing and the first full report on the nivolumab + ipilimumab cohort was presented at the ASCO GI Symposium in Jan 2018.44 Of 119 treated pts, 76% had ≥ 2 prior lines of therapy. Median follow-up was 13.4 months. The Objective Response Rate was 55% and Disease Control Rate was 80%. Combined treatment with nivolumab/ipilimumb also provided impressive benefits in progression-free survival, with rates at 1 year of 71 and 85%, respectively. Responses were observed regardless of tumor programme death-1 ligand 1 (PD-
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| Cancer type | Target | Drug | Year | Trial | Comparator arm | Benefit observed | Line of therapy | Toxicities of clinical interest | Ref |
|-------------|--------|------|------|-------|----------------|------------------|----------------|--------------------------------|-----|
| Colorectal  | PD-1   | Pembrolizumab | 2015 | MSS tumor | ORR 40% v 0% | Recurrent or metastatic | FFS 78% v 11% metastatic | Transaminase, transaminitis, colitis, diarrhea, fatigue (22%), pain, and arthritis (11%), acute kidney injury, adrenal insufficiency, hematuria, rash, and pruritus (1% each) | 42,43 |
|             |        |      |      |       |                |                  |                |                                 | 42,43 |
|             |        |      |      |       |                |                  |                |                                 | 42,43 |
|             |        |      |      |       |                |                  |                |                                 | 42,43 |
|             | PD-1   | Pembrolizumab | 2017 | CheckMate 142 | NA | NA | NA | Recurrent, locally advanced or metastatic | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             | PD-1   | Pembrolizumab | 2018 | CheckMate 142 | NA | NA | NA | Recurrent, locally advanced or metastatic | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             | PD-1   | Pembrolizumab | 2018 | CheckMate 040 | NA | NA | NA | Recurrent, locally advanced or metastatic | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             | PD-1   | Pembrolizumab | 2018 | CheckMate 040 | NA | NA | NA | Recurrent, locally advanced or metastatic | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             | PD-1   | Pembrolizumab | 2018 | Keynote 059 | NA | NA | NA | Recurrent, locally advanced or metastatic | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |
|             |        |      |      |       |                |                  |                |                                 | 74 |

Talimogene laherparepvec (T-VEC) for treating metastatic melanoma - has been approved by the FDA.33

Several trials have been performed to test the appositeness of OVs in CRC management. Biweekly intravenous administration of Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus (Vv), in CRC has been shown safe and well-tolerated.2 Pexa-Vec is currently being tested combined with durvalumab, an anti-PD-L1 agent, in phase I and with tremelimumab, anti-CTLA-4 antibody, in phase II in patients with refractory metastatic CRC. In another study, Vv’s combination with oxaliplatin or SN-38 has been shown to be increasing median survival in mice and synergetic cell killing and causing S phase arrest in cultured CRC cell lines.34 Enadenotucirev (formerly known as ColoAd1), a novel group B Ad11p/Ad3 chimeric adenovirus, in combination with PD-1 checkpoint inhibitor nivolumab is being tested as phase I dose-escalation study (NCT02636036). LOAd703, an immunostimulatory oncolytic adenovirus has been investigated as single agent in phase I/II trial of patients with CRC. Treatment of CRC stem cells with oncolytic herpes simplex virus in preclinical models has shown enhanced cytotoxic effect.55,56

Reovirus, a naturally occurring and ubiquitous double-stranded RNA OV - commonly infected in mammals, including humans and mice, asymptomatically - has intrinsic preference for replication in KRAS mutant cells causing apoptosis.57,58 Replication happens in the cytoplasm of infected cells and culminates in the formation of crystalline arrays of progeny virions within viral inclusions. Additionally, reovirus can be useful to trigger immune system to kill cancer cells. Reovirus serotype 3 - Dearing Strain (Reolysin) - has been studied in phase I in combination with FOLFIRI and bevacizumab, an anti-VEGF-A agent, in FOLFIRI naive patients with KRAS mutant metastatic CRC (NCT01274624). In a Phase 1b study, it was tested along with chemotherapy and pembrolizumab, anti-PD-1 antibody, in patients with advanced (unresectable or metastatic) histologically confirmed pancreatic adenocarcinoma (MAP).59 The combination therapy showed manageable safety profiles and antitumor activity in previously treated MAP patients.

**Vaccine Development**

Immunotherapy has opened avenues to develop treatment strategies with both prophylactic and therapeutic implications. Figure 2 depicts the various immunotherapeutic modalities currently available in practice. Vaccines work on the principle of artificial immunity where the immune system is primed by introduction of foreign antigen, to which an immune response is elicited, and thus immune memory is created.60 Vaccines against non-viral tumors have mainly targeted differentiation antigens, cancer testis antigens, and overexpressed neo-antigens.

A phase III trial was performed as early as 1999 to study OncoVax, a patient-specific vaccine composed of metabolically-active, sterile, irradiated, and non-tumorigenic autologous colon cancer cells. The study compared changes in overall and progression-free survival by the addition of the vaccine to surgery compared to surgery alone, in patients with stage II/III CRC. While OncoVax had a major impact on stage II disease with a significantly longer recurrence-free period and 61% risk reduction for recurrences, no significant changes were found in overall survival or stage III disease.61

There are numerous trials underway to identify and establish an effective vaccine in various gastrointestinal malignancies (Table 2). However, development of cancer vaccines has been challenging as the interaction of tumor and immune system is a dynamic, unremitting relationship. Evolution of a clone that has mutated or deleted the target antigen could become a resistance pathway of major clinical concern.52,63 Negative selection in the thymus against normal nonmutated antigens severely limits the ability to generate high avidity anti-cancer T cells. Such depletion can impair their antitumor activity and limit tumor elimination.
| Therapeutic Agent | Target | Clinical Setting | Phase | Comparator arm | Identifier |
|-------------------|--------|-----------------|-------|---------------|------------|
| Cancer vaccine    | Neoantigens Expressed by the Autologous Cancer | Metastatic gastrointestinal cancer | I/II | NA | NCT03480152 |
|                  | Long peptides and minimal epitopes from defined neoantigens or highly expressed mutations in tumor suppressor or driver genes | Melanoma Gastrointestinal Breast Ovarian Pancreatic | II | NA | NCT03300843 |
| Peptide loaded dendritic cell vaccine | Sterile, live but non-dividing tumor cells administered as vaccine | Stage II Colon CA | III | Surgery | NCT02448173 |
| Surgery and OncoVax | Globo H hexasaccharide 1 (Globo H) antigen conjugated to DT-CRM197, a non-toxic, mutated form of diphtheria toxin (DT) | Metastatic Gastric Metastatic Breast Metastatic Colorectal Metastatic Lung | I | NA | NCT02310464 |
| Adoptive T cell therapy | Peripheral Blood Lymphocytes Transduced with a Murine T-Cell Receptor Recognizing the G12V Variant of Mutated RAS in HLA-A*1101 Patients | KRAS G12V molecule on the surface of tumors. | I/II | NA | NCT03190941 |
|                  | Cytokine-induced killer cell immunotherapy along with radical surgery and adjuvant chemotherapy | Pancreatic Gastric Colon Rectal | II/III | NA | NCT03190941 |
|                  | Cryosurgery and natural killer (NK) cell immunotherapy | Advanced esophageal cancer | I/II | Cryosurgery | NCT02843581 |
|                  | Radiation Therapy and Peptide Specific CTL Therapy | Neoantigen peptide specific cytotoxic T lymphocytes (CTL) unresectable advanced esophageal cancer | II | NA | NCT03011255 |
| Combination Immunotherapy | Nivolumab + Ipilimumab | PD-1 + CTLA4 | Cholangiocarcinoma/ duodenal carcinoma Neuroendocrine tumors Rare Gynecological tumors | II | NA | NCT02923934 |
|                  | Oral cobimetinib with intravenous (IV) atezolizumab and bevacizumab | MEK + PD-L1 + VEGF | Metastatic colorectal cancer | Ib | NA | NCT02876224 |
Additionally, concerns about ballooning expenditures for new medical technologies certainly apply to these individually manufactured immunologically derived anticancer vaccines.

**ADOPTIVE T-CELL THERAPY**

Genetic engineering has allowed for ex vivo customization and expansion of autologous T-cells. In the CAR-T cell therapy, autologous T cells are extracted and transduced with a gene that encodes a chimeric antigen receptor (CAR) to direct the patient’s T cells against the tumor cells. These CAR-T cells are then expanded in a production facility and finally infused back to the patient. As a result, recognition of a specific cell surface antigen activates T-cell response independently of MHC recognition. This research has been most extensive in hematologic malignancies and the first CAR-T cell therapy, Tisagenlecleucel, was approved in Aug 2017, for relapsed refractory B-cell Acute Lymphoblastic Leukemia (B-ALL).64 There are numerous translational studies underway to identify a consistent antigen to serve as a target for the CAR-T cell therapy to be expanded to solid tumors as well. (Table 2)

Another form of adoptive T-cell therapy is ex-vivo expansion of tumor-infiltrating lymphocytes (TILs). TILs, as the name suggests, are immune cells such as Dendritic cells and Natural Killer cells isolated from the tumor tissue and thus recognize the tumor antigens. These are cultured ex-vivo with lymphokines such as interleukin-2 and then re-infused into the patient. The theory is that the immune cells are exhausted by the tumor microenvironment and the ex-vivo treatment allows them to be re-introduced at higher doses, by which they can overcome any tolerance by the tumor. While this form of therapy has been studied in various cancers such as melanoma, cholangiocarcinoma and cervical cancer, it has not yet received FDA approval in any cancer.65–67 A major limitation of this therapy is the unreliability of TIL extraction and expansion.

**MONOCLONAL ANTIBODIES**

The addition of biologic agents (i.e., bevacizumab [humanized monoclonal antibody to vascular endothelial growth factor], cetuximab [chimeric] and panitumumab [fully human; monoclonal antibodies to epidermal growth factor receptor]) have led to modest improvements in survival. For example, with bevacizu- antibodies to epidermal growth factor receptor]) have led to cetuximab [chimeric] and panitumumab [fully human; monoclonal antibody to vascular endothelial growth factor], the addition of biologic agents (i.e., bevacizumab [humanized MONOCLONAL ANTIBODIES

and expansion.

The major limitation of this therapy is the unreliability of TIL extraction and expansion.

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Newer mAbs such as LY3022855 are being designed, with novel targets such as Colony-stimulating factor 1 receptor (CSF1R), also known as macrophage colony-stimulating factor receptor (M-CSFR). CSF1R is a cell-surface receptor for its ligands, colony-stimulating factor 1 (CSF1) and IL-34.71 Increased CSF1 expression is implicated in tumor progression and metastasis, which is associated with poor prognosis in some cancers.72

**FUTURE DIRECTIONS**

It is evident that with the paradigm shift in oncology therapeutics, the field has consistently moved away from the cookie-cutter approach. Personalized medicine has taken the frontier where a tumor, its treatment and prognosis are defined by the genetic/epigenetic profile, protein expression and genetic mutations rather than a TNM stage. Tissue agnostic drug approvals are on the rise. It can be well perceived that there is an urgent need to identify additional biomarkers and cancer pathways, discern tumor heterogeneity at the molecular level, determine variability in cancer type and stage and have deeper insight into the underlying immunosuppressive mechanism of cancer to find the much-needed cure. Advances in preclinical knowledge and application of this at the bedside are quintessential for optimal outcomes.

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**AUTHOR CONTRIBUTIONS**

R.P. reviewed the mechanism of immune evasion. D.R. compiled the treatment modalities in various GI malignancies and emerging therapy options. D.R. synthesized the tables and R.P. created the illustration. T.A. discussed the viral therapy. R.M. conceptualized the review, discussed comprehensively with the authors to determine the contents and finally compiled the complete manuscript. Q.L. and S.G. reviewed the manuscript critically and made necessary additions and alterations to make the article comprehensive and up to date.

**ADDITIONAL INFORMATION**

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**Fig. 2** Overview of immunotherapeutic modalities
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