IMMUNO-ONCOLOGY INSIGHTS

OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

REVIEW

Expanding the reach of immuno-oncology: considerations for optimizing treatment of solid malignancies in the future

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The treatment of cancer has been rapidly changing with the emergence of highly effective immunotherapies. The majority of this success stems from the development of monoclonal antibodies targeting negative regulatory immune checkpoint molecules. Despite the efficacy of these immune checkpoint inhibitors across a range of tumor types, unfortunately about 70% of patients [1] either do not respond to treatment or subsequently develop resistance to checkpoint inhibitor therapy. Here, we will review the current landscape of immune-modifying treatments, ranging from chemotherapy and radiation to cellular therapies, which have the potential to further increase the clinical impact of immunotherapy. We will also highlight some of the current challenges in the field. These include the need for further mechanistic studies to better understand the complex biology of the anti-tumor response and to identify better biomarkers to rationally inform the selection of novel immunotherapy combinations. Further insights in the function of the immune system will allow the maximal leveraging of the growing number of immunotherapeutic modalities available in the clinic.

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INTRODUCTION

In the past decade, immune therapy has revolutionized the treatment of cancer. Tremendous progress has been made in utilizing therapies which harness the immune system to fight cancer since the first observations in the late 19th century that Coleys cocktail of bacterial toxins could elicit regression of some tumors. Much of the recent success of immune therapy has been due to the introduction of antibodies blocking key regulatory molecules of the immune system, referred to as immune check point inhibitors (ICIs). Treatment with ICIs has resulted in durable tumor regression in multiple different solid tumors. Despite this success, sustained responses to treatment are not achieved by a significant number of patients treated with immune check point inhibitors [1]. This is particularly true of treatment with monotherapy targeting a single immune checkpoint molecule, such as programmed cell death-1 (PD-1/CD279), programmed cell death-ligand1 (PD-L1/CD274) or cytotoxic T-lymphocyte antigen-4 (CTLA-4/CD152).

This review will focus on highlighting the growing number of tools in the immunotherapeutic toolbox (Figure 1) and the current challenges presented in determining the ideal combination of these therapeutic modalities and approaches for each individual patient. Despite of all these emerging agents, the 'Holy Grail' of multimodal, personalized immunotherapy remains unrealized for most patients due to a lack of biomarkers to guide the integration of different immunotherapeutic agents.

IMMUNE CHECKPOINT INHIBITORS

Within the immune system, the process of immunosurveillance assessing for foreign pathogens or malignant cells is finely balanced against the development of autoimmunity. This balance is partially maintained by immune checkpoints [2], an array of receptors on the immune cell surface which, in turn, promote activation or suppression of the immune response. Seminal work in murine models in the 1990s established that preventing the ligation of the inhibitory receptors CTLA4 or PD-1 by their cognate ligands could result in the activation of T cells and tumor clearance [2,3]. More than a decade later, unprecedented durable responses were observed in roughly 20% of patients with advanced melanoma treated with an anti-CTLA-4 agent [4]. Increased numbers of responses were subsequently observed with anti-PD1 therapy for patients with advance melanoma [5,6]. Moreover, further increases in response rates and patient survival were observed when a PD1 blockade was combined with anti-CTLA-4 therapy, albeit at the cost of increased toxicity [7]. The success of ICIs targeting PD-1, PD-L1 and CTLA-4 has now been duplicated in multiple other tumor types. Interestingly, the enhanced efficacy of combined ICI therapy with anti-PD1 and anti-CTLA-4 observed in patients with melanoma has also been seen in patients with other solid tumors [8–10]. The hope was that this success of combined anti-PD1 and anti-CTLA-4 therapy could be replicated, and perhaps enhanced, with novel agents targeting other key immune inhibitory or costimulatory molecules. As recently reviewed extensively by Esfahani *et al.*, there are a multitude of other potential immune checkpoint targeting agents currently under investigation in various stages of clinical trial [11]. Many of these novel agents target other inhibitory checkpoint molecules, such as lymphocyte-activation gene 3 (LAG-3/CD223), V-domain immunoglobulin suppressor of T cell activation (VISTA/B7-H5) or T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). Others are agonistic antibodies which target co-stimulatory molecules on T cells, such as tumor necrosis factor receptor superfamily member 9 (TNFRSF9/ 4-1BB/CD137), inducible T-cell costimulator (ICOS/CD278) or CD28. To date, many of the results of these trials with novel ICI/costimulatory

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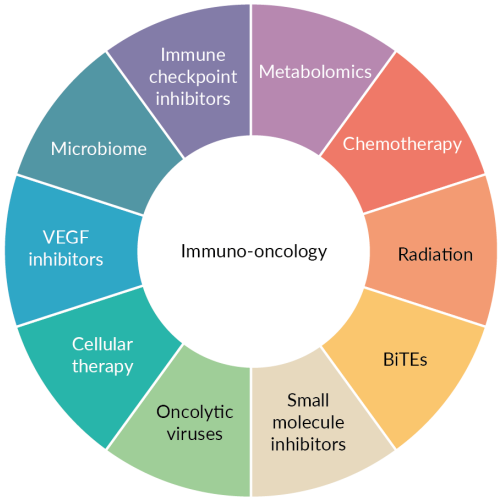
agonist combinations have been disappointing. Some agents, such as those early studies using an agonistic anti-CD28 antibody have displayed unacceptable toxicity [12]. On the other hand, many of the novel combinations of ICIs have not yet demonstrated significant clinical activity, although trials are ongoing [13]. A major limitation in the development of these novel ICIs/agonistic antibody combinations is lack of detailed mechanistic understanding of the underlying biology of many of these immune checkpoint molecules. The unique role of each immune checkpoint molecule in governing the anti-tumor immune response is unclear and unfortunately likely context-dependent. For example, recent studies have suggested that the inhibitory receptor V-domain immunoglobulin suppressor of T-cell activation (VISTA) is expressed on multiple tumor-infiltrating lymphocytes such as myeloid-derived suppressor cells (MDSCs) and T regulatory cells (Tregs). Interestingly, however, expression of VISTA is not sufficient for it to engage is cognate receptor and induce suppression of the anti-tumor immune response. For VISTA to be an active inhibitory receptor, it also requires an acidic environment [14]. Thus, measuring VISTA protein expression alone is not sufficient to predict the inhibitory activity of VISTA. Factors such as this have prevented the identification of robust biomarkers to predict the efficacy of many of these novel ICIs as well as hampering the rational selection of novel ICI combinations amongst the numerous possible combinations. As a result, no ICI combinations have yet demonstrated superior clinical efficacy than the original anti-PD1 and anti-CTLA-4 combination for most solid tumors. This failure to improve on the success of combination of anti-PD1 and anti-CTLA-4 also speaks to the complexity of immunoregulatory mechanisms in the tumor microenvironment (TME) and the need to target multiple different regulatory pathways in the TME beyond just immune checkpoint molecules to elicit an anti-tumor immune response in some patients.

FIGURE 1

The expanding range of treatments in immuno-oncology.



Immune therapy has expanded from the use of immune checkpoint inhibitors to include multiple other treatment modalities. These include the combination of immune therapy with chemotherapy and radiation, addition of VEGF inhibitors or small molecule inhibitors, cell therapy, bispecific antibodies, cytokines and tools to activate the innate immune system such as vaccines and oncolytic viruses. Immune-modifying therapies targeting manipulation of the microbiome and the metabolic composition of the tumor microenvironment are also being developed.

TUMOR MICROENVIRONMENT: BEYOND ICIS

The approach to immunotherapy needs to be undertaken in the context of the TME which encompasses the cellular milieux of tumor cells, stromal cells as well as a diverse array of immune cells such as T lymphocytes, dendritic cells, macrophages, polymorphonuclear cells and natural killer cells. The TME can show a wide degree of heterogeneity from patient to patient depending on tumor type, anatomical location and molecular characteristics of the tumor. Tumors can be conceptually divided into immunologically 'hot' or 'cold' microenvironments. 'Hot' tumors typically display evidence of robust infiltration of $CD8^+$ T cells and expression of IFN-γ or PD-L1 with high PD-L1 expression in NSCLC shown to be predictive of clinical response to ICIs [15]. Conversely, 'cold' microenvironments demonstrate limited or no infiltration of immune cells. 'Cold' microenvironments are associated with poor response to immune checkpoint inhibitors [16]. A myriad of

factors can contribute to this 'cold' immune phenotype. These can include the recruitment of immune suppressive cells, such as Tregs and MDSCs, or the expression of immune suppressing chemokines and cytokines. The tumoral stroma itself can create a barrier to the infiltration of immune cells [17]. Additionally, the TME is a harsh environment depleted of many nutrients required by T cells and other immune effector cells to function properly [18]. Accordingly, understanding additional approaches to transform immunologically 'cold' tumors to 'hot' tumors has been an ongoing area of investigation. Below, we will discuss additional therapies that help stimulate an anti-tumor immune response.

CHEMOTHERAPY

Chemotherapy was initially considered as a treatment modality which would potentially decrease or interfere with the use of immunotherapy due to its potential toxicity to myeloid cells and T-cell populations involved in immunotherapy responses. However, upon further investigation, it was discovered that chemotherapeutic agents given at specific doses and intervals could improve the response of immunotherapy. Chemotherapies, such as doxorubicin, mitoxantrone and cyclophosphamide, can induce immunogenic cell death via a number of cellular pathways. Immunogenic cell death leads to the activation of the innate immune system, and particularly antigen presenting cells such as dendritic cells (DCs), to support the activation of a tumor-specific adaptive immune response [19,20]. Pathways involved in immunogenic cell death include the activation of Toll-like receptors via post apoptotic release of nuclear chromatin binding protein HMGB1 [21,22]. Cytotoxic agents can also result in the release of ATP from lysosomal stores stimulating macrophage recruitment and maturation [23] and NK cell proliferation and IFNγ secretion [24]. Chemotherapy can also lead to tumor cell immunogenicity by inducing expression of MHC-I molecules and tumor specific antigens on the tumor cell surface [25]. Leveraging these effects, the use of chemoimmunotherapy combinations with standard chemotherapy regimens in combination with ICI has been studied in Phase 3 clinical trials and has been FDA approved for tumor tissue types including non-small-cell lung cancer, small cell lung cancer, triple negative breast cancer and head/neck cancer with evidence of clinical benefit [26–29].

In addition to standard-dose chemotherapy, continuous low-dose exposure to chemotherapy or 'metronomic' chemotherapy has been studied as a means to enhance the anti-tumor immune response. In clinical studies metronomic dosing of cyclophosphamide treatment of end-stage cancer patients (50 mg orally, b.i.d., 1 week on, and 1 week off, for 1 month or more) strongly curtailed immunosuppressive Treg cells, leading to a restoration of peripheral T-cell proliferation and innate immune cell killing activities [30]. Another study of metronomic cyclophosphamide in metastatic breast cancer showed a 40% reduction in T regulatory cells initially however these numbers recovered during the treatment course however the treatment induced a stable tumor specific T-cell response which correlated to improved clinically outcome [31]. Despite these promising results, metronomic chemotherapy has yet to show any synergistic activity with ICI or other immunotherapy modalities in prospective, randomized trials. Clearly, further research is warranted to be elaborate the ideal deliver of chemotherapeutic agents to optimize the activation of both the innate and the adaptive immune system and synergize with immunotherapy.

RADIATION

The addition of radiation is another potential tool which can be used to alter the TME potentially changing a noninflamed environment into a more immune sensitive environment. There is ongoing discussion regarding the pro-inflammatory versus the immune suppressive effects of radiation on

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anti-tumor immunity. The immune response to radiation is thought to depend on multiple factors including timing, dose, fractions, site radiated and also the tumor type. Low dose radiation at 2Gy has shown it can create an immunogenic environment via the innate immune system through macrophage stimulation [32]. In contrast high dose radiation has been thought to promote tumorigenic macrophages [33] and cause vascular damage limiting access of immune cells to the TME [34]. In a similar process to the effects of certain chemotherapeutic agents discussed above radiation treated cells undergo immunogenic cell death causing release of specific proteins which can activate Toll like receptors of the innate immune response [35]. DNA released from radiation damaged cells have also been shown to active the c-Gas-STING pathway causing increased type I interferon release by dendritic cells in the TME [36]. Radiation has also been shown to increase expression of major histocompatibility complex (MHC) I on tumor cells and increase T-cell activity [37]. In totality, these changes may be responsible for the 'abscopal effect' that has been described with radiation therapy; the abscopal effect is the observation of regression of non-irradiated metastatic lesions following the treatment with radiation of another site of disease, presumably due to the activation of the immune system. Unfortunately, to date, combinations of ICIs and radiation to induce the ascopal effect and enhance tumor immunity have proven difficult to demonstrate in clinical trials [38].

ACTIVATION OF THE INNATE IMMUNE SYSTEM: DANGER SIGNALS, ONCOLYTIC VIRUSES & VACCINES

Failure to active the innate immune system, and particularly DCs, can allow tumors to circumvent the immune response and undoubtedly contributes to the 'cold' tumor phenotype. Both chemotherapy and radiation can initiate cellular pathways that promote DC activation and allow for the bridging of the innate to the adaptive immune response, as activated DCs increase antigen presentation and provide costimulatory signals and cytokines to promote T-cell activation [39]. The pattern recognition receptor (PRR) family play a pivotal role in DC activation. These PRRs recognize bacterial or viral molecules called pathogen associated molecular patterns (PAMPs) or endogenous molecules called damage associated molecular patterns (DAMPs). Rather than trying to induce the release of DAMPs with chemotherapy or radiation, another approach is to directly provide DAMPs or PAMPs into the TME. This approach has been quite successful with non-metastatic tumors. The treatment of non-invasive bladder tumors involves the use of the attenuated bacillus Calmette–Guerin (BCG) and some superficial basal cell carcinomas of the skin can be treated with the synthetic TLR agonist Imiquimod. For metastatic disease, intertumoral injection of DAMPs or PAMPs have been found to synergize with ICI therapy and enhance the tumor clearance both of the injected lesion as well as at distant sites of disease. This systemic effect of local injection has been observed both in mouse models [40,41] and early clinical trials [42]. Additionally, synthetic DAMPS activating the cGAS-STING pathway which can be administered systemically but still result in potent tumor regression in preclinical models have been described [43]. These agents hold the promise to allow for the activation of DCs and the innate immune response in patients for which intra-tumoral injections are not safe or feasible. Unfortunately, these new STING agonists, as well as the other DAMPs and PAMPs that have been tested, have yet to demonstrate efficacy in large, randomized trials.

Oncolytic viruses have also emerged as an immunotherapeutic modality whose mechanism of action relies heavily upon activation of DCs and the innate immune system. Early in their development, oncolytic viruses (OV) were envisioned as engineered therapeutic that would selectively infect and lyse tumor

cells. Further research, however, has indicated that the dominant mechanism of action of these viruses is to induce an anti-tumor immune response [44]. To date, only one OV has been licensed for clinical use in the USA, Europe and Australia. Talimogene laherparepvec (T-VEC) is a modified herpes simplex virus type I that results in the expression of the human granulocyte-macrophage colony stimulating factor (GM-CSF) in infected cells. The combination of the expression of GM-CSF to attract immature DCs and the natural PAMPs in the T-VEC virus and DAMPS released by virally-lysed cells, co-ordinate to boost DC activation in the TME and promote anti-tumor immunity. Indeed, T-VEC treatment was demonstrated to induce durable clinical response in patients with advance melanoma when used as monotherapy [45]. Combination trials of T-VEC, as well as other OVs, with ICIs are ongoing with some encouraging early results being reported, albeit in small number of patients [46,47].

Finally, tumor-specific vaccines are another potential tool to provide both tumor-specific antigen as well as molecular signals to activate innate immune cells. Historically, tumor vaccines do not have a tremendous track record of success for the treatment of advanced cancer, even when used in combination with ICIs. For example, the seminal trial that established the potential of ipilimumab to induce durable responses in patients with melanoma also included treatment with a therapeutic vaccine targeting the gp100 melanoma peptide which did not display any added therapeutic benefit [48]. Since then, however, it has been discovered that tumors contain multiple mutated proteins that can give rise to novel 'neo-antigens' that can be recognized by the immune system. Vaccine strategies utilizing neo-antigen targets have shown promise in early clinical trials [49,50]. Moreover, novel vaccine platforms utilizing mRNA technology have improved the antigen expression and immunogenicity of the vaccine antigen [51]. There is now reason for growing enthusiasm that personalized, mRNA vaccines targeting tumor antigens or neo-antigens will be a key component of immune therapy combinations in the future.

CYTOKINE THERAPY

Early in the immunotherapy era stimulation of an immune response with provision of cytokine therapy was attempted in melanoma and renal cell carcinoma with high dose interleukin (IL)-2 or interferon alpha [52]. Unfortunately, these treatments had very high levels of toxicity with not very good efficacy. Subsequently, alternative cytokine therapies have been tested in early clinical trials with the aim of improving T cell and NK cell function, including Il-12, Il-15 and Il-21 [53]. Unfortunately, all of these treatments were also associated with high rates of toxicity. This has led to the development of modified cytokine agents, including bempegaldesleukin a polyethylene glycol–conjugated recombinant IL-2. These modified IL-2 agents have demonstrated anti-tumor activity but acceptable toxicity in animal models [54] and multiple clinical trials are ongoing. To date, however, no modified cytokine agents are approved for clinical use.

SMALL MOLECULE INHIBITORS: TARGETING VEGF & BEYOND

In parallel with the immune therapy revolution, there has also been an explosion of the number of tyrosine kinase inhibitors (TKIs) and other targeted therapies aimed at blocking key aspects of oncogenesis, from cell growth to angiogenesis [55]. For many malignancies harboring driver mutations, such as epidermal growth factor receptor (EGFR)-mutated non-small-cell lung cancer (NSCL), these targeted therapies are the standard first line therapy and have demonstrated impressive clinical activity [56]. There is now increasing interest in combining many of these targeted therapies with immune therapies, as there is emerging evidence that many of these targeted therapies may also aid in enhancing the anti-tumor immune response. This is

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particularly true of agents targeting the vascular endothelial growth factor (VEGF) receptor signaling pathway. The VEGF family of growth factors bind to VEGF receptor tyrosine kinase triggering their signal transduction pathways. VEGF stimulates formation of new blood vessels to help supply growing tumors. Signaling via VEGF also potentially suppresses immunity through effecting accumulation of immature dendritic cells, myeloid derived suppressor cells and inhibiting of T cell migration to tumors [57]. This pathway has been targeted clinically via the use of bevacizumab, a VEGF-A blocking antibody, in several solid malignancies and has shown varying degrees of clinical response [58,59]. Studies have shown that VEGF inhibition allows for dendritic cell maturation and treatment with bevacizumab have shown increased dendritic cell maturation [60]. Blockade of VEGF signaling with bevacizumab has also been used in combination of with ICI therapy. A combination of the anti–PD-L1 monoclonal antibody atezolizumab with bevacizumab and chemotherapy was assessed in first line treatment of metastatic nonsquamous cell lung cancers. This triplet regimen resulted in improved median overall survival compared with patients treated with bevacizumab and chemotherapy alone. Predictably, however, patients treated with the triple therapy also had higher rate of serious toxicity compared to the control group [61].

Echoing these data with bevazicumab, are the results of pre-clinical and clinical trials which combined ICIs with Lenvatinib. Lenvatinib is an oral small-molecule inhibitor of VEGFRs, as well as other receptors such as FGFRs, PDGFRα, KIT and RET proto-oncogene. Lenvatinib was previously used as monotherapy of several malignancies including hepatocellular carcinoma, renal cell carcinoma and thyroid cancer [62–64]. In murine models, Lenvatinib in combination with anti PD-1 blockade was shown to enhance anti-tumor immunity by reducing tumor associated macrophages and increasing the percentage of activated $CD8^+$ T cells secreting interferon IFN)-γ+ and granzyme B in the TME [65]. In trials with patients with various different cancers including, urothelial cancer, head and neck squamous cell carcinoma, melanoma, non-small cell lung cancer, renal cell carcinoma, colorectal cancer and endometrial cancer, combination Lenvatinib with anti-PD1 therapy displayed impressive rates of response ranging from 25–55% [63,66]. Again, these data clearly indicate the blockade of VEGF signaling is active in combination with ICI therapy. The challenge still remains as how best select patients for this combination, as the trials have yet to offer definite insight into this important question.

In addition to agents blocking the VEGF receptor signaling pathway, inhibitors targeting other signaling pathways have been suggested to have significant immuno-modulatory properties. For instance, combined BRAF and MEK inhibition as well as CDK4/6 inhibitors have been demonstrated to enhance the T cell response in pre-clinical models [67]. Evidence of these synergies are also emerging in the clinic as patients with BRAF-mutant melanoma treated with a triplicate regimen of a BRAF inhibitor, a MEK inhibitor and an anti-PD-L1 agent displayed increased progression free survival compared to patients treated with targeted therapy alone [68]. Similarly, in hormone receptor-positive breast cancer a triplicate regimen of anti-PD1, hormonal therapy and a CDK4/6 inhibitor displayed encouraging results in a Phase 1/2 trial [69]. Obviously, further trials are required, but these early data are notable as previously hormone receptor-positive breast cancer was considered an immunologically 'cold' tumor, refractory to ICI treatment. These data provide early clinical data that targeted therapies could hold the potential to expand the reach of immune therapy.

CELLULAR THERAPY

Adoptive cellular therapy (ACT) is a form of immunotherapy which uses *ex vivo* expanded T cells to generate an anti-tumor response. There are three major ACT modalities used

in treatment of solid malignancy: autologous tumor infiltrating lymphocytes (TIL), genetically engineered T cell receptors (TCRs) and chimeric antigen receptors (CAR) T cells. CAR T cells have demonstrated unprecedented results in the treatment of hematological malignancies but have yet to have the same level of success for solid tumor malignancy [70]. To date, ACT using TILs has displayed some of the best clinical responses in patients with certain solid tumors. Treatment protocols for ACT using TILs require the harvest of autologous $CD8^+$ and $CD4^+$ T from a tumor lesion via surgical resection. These TILs are then massively expanded *ex vivo* and reinfused back into the patients following preparative treatment with lymphodepleting chemotherapy. Engraftment and expansion of the infused TILs is then supported by interleukin-2 treatments. There has been promising clinical responses observed in patients treated with ACT using TILs, particularly for melanoma [71]. Importantly, significant clinical response have been observed in patients whose disease previously progressed on treatment with ICI therapy. For instance, a recent study assessed treatment with TIL product lifileucel in patients with unresectable melanoma after progression on either ICI or targeted therapy. The overall response rate was 36.4% and disease control rate of 80% [72]. Moreover, the reach of TIL therapy is now being studied beyond the treatment of melanoma. TIL therapy in combination with ICI treatment has shown promise in patients with NSCLC. Amongst 20 NSCLC patients treated with TILs and ICI after prior progression on anti PD-(L)1 inhibitors, 2 patients achieved durable complete response [73]. These data speak to the potential of TIL therapy to treat tumors that are refractory to ICI therapy alone.

Genetically engineered T-cell receptor therapy modifies naïve lymphocytes to recognize tumor antigen via the expression of T-cell receptor specific for a tumor antigen expressed in the context of the major histocompatibility complex (MHC). One of the major challenges of TCR therapy is identifying a tumor antigen which is specific to the tumor therefore avoiding activation of cells towards tissue other than the tumor. For example TCRs specific against the MART1 antigen [74] in melanoma and NY-ESO-1 and MAGE A3/6 antigens [75] have been used in clinical trials due to the high levels of expression of these antigens on certain tumor types. A drawback of TCR based therapies is the dependence of antigen presentation on MHC. CAR T cell lymphocytes are genetically modified to have specificity for tumor antigens however the engineered construct can recognize surface antigen that is not restricted based on MHC presentation. Several different tumor antigens have been targeted in CAR T cells for solid malignancy including IL-13 receptor α 2 (IL13Rα2) in a patient with multifocal glioblastoma multiforme [76]. Other targets have included mesothelin [77] and [78] HER2 both of which showed limited response to date. The next generation of engineered T cell products, T cells redirected for antigen-unrestricted cytokine- initiated killing' (TRUCKs) aim to combine CAR T-cells with inducible release of a transgenic protein, typically a cytokine at the time of activation to stimulate a wider immune response. These next generation cell therapies offer the potential for 'build in' combination immune therapy with ACT in addition to other therapeutics being expressed by the modified T cells. These approaches may help overcome some of the current issues of antigen-targeting currently impeding the development of gene-engineered T cells and CAR T cells and allow the engineered T cells to deliver agents to the TME that will support the reinvigoration of an endogenous, polyclonal anti-tumor T cell response.

The next generation of ACT may enroll the aid of gene editing technologies such as cluster regulatory interspaced short palindromic repeat/CRISPR-associated protein [9]. The addition of gene editing to CAR T cells has the ability to help enhance potency and safety of treatment via for example knocking out of inhibitory molecules such as PD-1 and TGF-beta which has shown increased tumor

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elimination in patient derived xenograft solid tumor models [79,80].

T-CELL ENGAGERS

T-cell engager are molecules that induce anti-tumor immunity by inducing the targeting and activation of polyclonal T lymphocytes to tumor-expressed antigens. Bispecifc T-cell engagers (BiTEs) are recombinant proteins made of scFv regions from two different antibodies. One scFV targets a specific tumor antigen and the other targets and activates T cells independent of antigen specificity, typically via engaging the CD3 complex. These molecules are able to reorient T cells that do not express a T-cell receptor specific for tumor antigens and thereby allow for an amplification of the anti-tumor response by recruiting 'bystander' T cells. As recently reviewed elsewhere [77], there currently are a range of target antigens expressed by tumors that are being tested as targets for BiTE therapy. These include HER2, EGFRvIII, mesothelin, GD2, CEA, PSMA, EpCAM and AFP. Similar to the challenge faced in the development of CAR T cell therapy for solid tumors, expression of the target antigen by tumor must be weighed against expression in healthy tissue. High expression of the target antigen in normal tissue can result in significant toxicity in what is terms an 'on target off tumor' effect. Despite this caveat, a modified T cell engager has recently demonstrated significant activity in the treatment of uveal melanoma.

Tebentafusp is classed as an immune-mobilizing monoclonal T-cell receptor against cancer (ImmTAC). The molecule differs from a classic BiTE as the tumor targeting is achieved via the use of a soluble, affinity-enhanced HLA-A*02:01–restricted T-cell receptor that is specific for a peptide from the glycoprotein 100 (gp100) protein. This soluble TCR is fused to an anti-CD3 single-chain variable fragment that induces activation of the recruited T cells. In a recent trial, first-line treatment tebentafusp was found to increase overall survival versus ICI monotherapy for patients with metastatic uveal melanoma [81]. These data establish that T cell engager therapies have the potential to be clinical benefit for solid tumors. Moreover, they reinforce that tumors thought of as immunologically 'cold', such as uveal melanoma, due to poor response to ICI therapy still can be amenable to treatment with a different modality of immunotherapy.

MICROBIOME

There has been growing interest in modulating the microbiome, the billions of bacterial that colonize the human skin, respiratory and digestive tracts, to enhance the outcomes of immunotherapy treatments. This interest stems from the multiple pre-clinical studies that have demonstrated the profound impact of the composition of the intestinal microbial flora has on the efficacy of immune therapy treatments, particularly ICIs [82,83]. Encouragingly, these results have been mirrored in cohorts of cancer patients, with different species of microbiota being found to be enriched in responders versus non-responders to ICI therapy. For example, patients with metastatic melanoma who were responders to anti–PD-1 therapy were shown to have enrichment of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* in pre-treatment stool samples [84]. Additionally, it has been found in cancer patients treated with ICI there exists a correlation between the microbiome and the toxicities experienced. A protective effect of a Bacteroidetes-rich phylotype against CTLA-4 blockade–induced colitis was observed in patients with melanoma [85]. Further studies have also correlated the microbiome with adverse events experienced by patients treated with combination PD-1 and CTLA-4 blockade [86]. Collectively, these data verify that the microbiome is an attractive target for modulation to enhance the efficacy and potentially lessen the toxicity of immune therapy.

One challenge has been to understand the best ways to modulate the microbiome to

improve immune therapy. It appears that a favorable microbiota contains a vast diversity of microbial species. Accordingly, promoting a diverse microbiome seems to be a key principle to guide potential therapeutic interventions. Avoiding concurrent therapies, such as antibiotics, that lessen microbial diversity has been suggested to improve outcomes with ICIs in retrospective studies. Wilson *et al.* reviewed 766 studies assessing the effects of antibiotic use in immune checkpoint blockade looking at the outcome of 2889 patients and showed an increased overall survival in patients that were not exposed to antibiotics during treatment [87]. Aside from preventing damage to microbial diversity, other treatment approaches have demonstrated promise to promote a varied intestinal ecosystem. A diet enriched for dietary fiber has recently been found to promote the diversity of the colonic microbiome and correlate with superior outcomes to ICI therapy [88]. Fecal microbiota transplant has also been utilized to attempt to repopulate the microbiome with flora supportive of response to immune therapy. To date, in early phase trials this approach has been able to rescue the response to anti-PD1 therapy in some patients whose disease initially progressed on treatment [89]. Collectively, all of these studies indicate that the microbiome has the potential not only to be an important biomarker for treatment selection but also an important therapeutic target for future immunotherapy regimens.

METABOLISM

Within the TME there are numerous suppressive factors that can blunt the anti-tumor immune response. In addition to the presence of many negatively regulatory cells such as Tregs and MDSC, there are multiple metabolic factors within the TME that can constrain T-cell activation and immunity. These include hypoxia, altered pH as well as the depletion of many key nutrients required for immune cell function [18]. In particular, the amino acids tryptophan and arginine are depleted in the TME via their catabolism by the enzymes indoleamine 2,3 dioxygenase (IDO) 1 and arginase 1 (Arg1) respectively, which are expressed by multiple cell types present in the TME [90]. Accordingly, agents that inhibit the enzymatic activity of IDO and ARG1 and prevent the depletion of tryptophan or arginine in the TME have the potential to help enhance anti-tumor immunity. Unfortunately, the first IDO inhibitor, epacadostat, to be trialed in combination with anti-PD1 agent in a randomized Phase 3 trial failed to demonstrate clinical benefit [91]. There remains, however, many questions as to the reason for this observed lack of benefit, ranging from the dosing regimen used to the trial design [92]. Further trials with novel, more potent IDO inhibitors that also inhibit the IDO2 enzymes [93] or ARG1 inhibitors are still required to fully evaluate this treatment strategy in the context of immune therapy.

Aside from enhancing amino acid levels in the TME, reducing hypoxia is another therapeutic approach that has shown promise in pre-clinical models. In mouse studies the commonly used diabetes drug metformin was shown to reprogram tumor metabolism, reducing oxygen consumption by the tumor cells and thereby increasing the oxygen available to immune cells in the TME. Treatment of mice bearing murine melanoma and colon cancers with metformin and anti-PD1 blockade demonstrated reduced hypoxia in the TME and increased efficacy of anti-PD1 therapy [94]. In patients, a retrospective cohort study that included patients diagnosed with metastatic malignant melanoma and treated immune checkpoint inhibitors plus metformin showed the overall response rate was higher in the combination group at 68% versus 54%, however this difference did not reach the threshold of statistical significance. The study did show a decrease in the mean number of new metastatic sites which appeared during in the combination group [95]. Again, prospective studies are indicated, but this shows proof in principle that medications with existing indications to treat metabolic diseases have the potential to be leveraged to

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alter the metabolic composition of TME and enhance the efficacy of immunotherapy.

CONCLUSION

Immunotherapy has caused a paradigm shift in the treatment of solid malignancy over the past decade providing the potential for durable long-term control of a broad range of malignancies. Unfortunately, however, treatment failure is still common. To overcome these failures, multiple different immunotherapeutic modalities have been developed. There exists solid evidence that combining these different modalities can over-come resistance and improve outcomes for patients. The challenge, however, is the emerging complexity; with each additional treatment modality the number of potential combinatorial treatments increases exponentially. This increasing complexity stands in stark contrast to the binary 'hot' and 'cold' tumor paradigm that has dominated the literature. To help resolve this conflict, studies integrating multiple different large-scale 'omics' approaches are needed. Integrated analysis of gene expression signatures, immune cell infiltrates and metabolic milieux within TME in conjunction with other patient factors, such as the microbiome, will hopefully aid in identifying novel biomarkers and global patient phenotypes to inform novel combination treatment regimens. These types of analysis, however, will likely have to leverage machine-learning approaches due the size and the complexity of the datasets. Artificial intelligence and machine learning technologies are being developed to aid in interpreting imaging studies, assessment of TME, prediction of immunotherapy side effects and treatment response [96]. To this end, a recent study utilized a transcriptomic-based analytics platform to characterize the TME of multiple different tumor types. This analysis allowed them to refine the 'hot' and 'cold' tumor model and define four different subtypes of TME, each conserved across multiple tumor types and each displaying a different responsiveness to immune therapy [97]. Importantly, these analyses also predicted the immune therapy approaches which would be potentially most efficacious for each of the novel TME subset defined. It will be of great interest to see if these predications can be validated in prospective trials. If these predicted treatment regimens prove to be effective, this would enable the use of TME characteristics, rather than just tumor histological type, to guide the choice of immunotherapeutic regimen. Encouragingly, studies like these suggest that the existing arsenal of different immune therapy modalities may already be large enough to greatly increase the clinical benefit for many patients; the challenge for the next decade of immune therapy is to match agents in the current treatment arsenal to the correct patients for maximal effect.

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