Chapter 16:
Intrinsic and acquired cancer immunotherapy resistance

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Abstract

Cancer immunotherapies, such as immune checkpoint inhibitors (ICIs), have revolutionized the treatment of various cancers and have shown a great efficacy in inducing anti-tumor immunity. Cancer immunotherapy in the form of adoptive cell transfer (ACT) have also been developed to eradicate tumor cells in a specific and effective manner, and it includes the administration of autologous tumor-infiltrating T cells (TILs), T cell receptor (TCR)-modified T cells or genetically engineered chimeric antigen-receptor (CAR)-specific T cells (CARTs) in cancer patients. Additionally, cancer vaccines and recombinant cytokines can be used as monotherapy or adjuvant therapy. Despite the success of immunotherapies in treating various solid tumors and hematologic malignancies, a significant number of patients do not benefit from these therapies and exhibit limited or no response. Some cancer patients do not respond to immunotherapies as a result of primary or intrinsic tumor resistance, while others respond to immunotherapies but develop resistance over time, referred to as adaptive or acquired tumor resistance. Tumor intrinsic and extrinsic-mediated mechanisms, including genetic and epigenetic alterations, tumor mutational loads, overexpression of co-inhibitory immune checkpoints and elevated levels of suppressive immune cells and cytokines, can lead to a compromised anti-tumor immunity favoring tumorigenesis and cancer progression. This chapter outlines mechanisms of intrinsic tumor resistance and the emergence of acquired tumor resistance to cancer immunotherapies. Moreover, this chapter describes combined cancer immunotherapies which may offer a great therapeutic potential to overcome tumor resistance against therapy and improve clinical outcomes in cancer patients.
Key Words:

Cancer; immunotherapy; immune checkpoint inhibitor; adoptive T cell therapy; tumor microenvironment; intrinsic resistance; acquired resistance; epigenetics; therapeutic strategies
1. Introduction

In light of cancer immunoediting, tumor cells can continuously evolve to alter their immunogenicity and establish an immunosuppressive milieu within the tumor microenvironment (TME) comprising of cellular and soluble network which promote immune escape and tumorigenesis [1, 2]. Tumor cells can escape the recognition of effector immune cells by diminishing the expression of neoantigens and antigen presentation molecules [3-6], release suppressive cytokines, growth factors, and matrix metalloproteinases to induce immunosuppression and favor tumorigenesis [7-10]. Moreover, tumor cells can express high levels of co-inhibitory immune checkpoint (IC) ligands and can also upregulate the expression of IC receptors on tumor-infiltrating immune cells; increase the induction, expansion and activation of immunosuppressive cells, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs) and tumor-associated dendritic cells (TADCs); and increase the induction and differentiation of cancer-associated fibroblasts (CAFs) within the TME [11, 12]. Collectively, cellular communication between immune cells and non-immune cells within the TME can contribute significantly to cancer progression and suppress anti-tumor immune responses [13-15].

Cancer immunotherapy in the form of immune checkpoint inhibitors (ICIs) has made a breakthrough in cancer treatment of various types; however, a high number of cancer patients show limited response rates [16-23]. In addition to ICIs, the use of adoptive cell transfer (ACT), including the transfer of autologous tumor-infiltrating T cells (TILs) or genetically engineered chimeric antigen-receptor (CAR)-specific T cells (CARTs) have been developed to eradicate tumor cells in specific and efficient manners [24-28]. The efficacy of adoptive T cell therapy has been demonstrated in multiple cancer settings including hematologic cancers [29], melanoma [30],
Despite the success of cancer immunotherapies, the effectiveness of these therapies in cancer patients is limited due to intrinsic and extrinsic-mediated factors including genetic and epigenetic alterations, tumor mutational loads, and the overexpression of co-inhibitory immune checkpoints and surplus population of immunosuppressive cells in the TME [23, 36]. Tumor immunogenicity, dictated by the molecular and cellular composition of the TME, is one of the key aspects which dictates the response to therapy [3, 4, 37]. Altogether genetic/epigenetic and phenotypical changes acquired in the TME can collectively induce resistance against therapy [14, 38]. Consequently, therapeutic strategies to alleviate tumor resistance (intrinsic or acquired) against cancer immunotherapies are crucial to maximize the efficacy of cancer treatment and revert tumor resistance to ICIs [38, 39] or ACT [40, 41].

2. Tumor microenvironment

The TME is a composite milieu of multiple cell types embedded in an extracellular matrix, which enables cancer cells to interact with other cell types and favors their own progression and survival [42]. These dynamic interactions are indispensable for the heterogeneity, clonal development, drug resistance and metastasis of malignant cells [42]. The extracellular matrix (ECM) is molded with glycoproteins, polysaccharides and cell adhesion proteins including laminin, fibronectin and collagen that actively support the survival of both cancer cells and stromal cells [43]. Stromal cells in the TME comprise of immune cells (T cells/natural killer (NK) cells/ macrophages), stem cells, adipocytes and fibroblasts (Figure 1) [44]. Additionally, endothelial cells and pericytes present in the TME favor the formation of blood vessels and lymphatic vesicles (Figure 1) [45]. Tumor cells favor the trafficking of stromal cells into the TME by secreting various cytokines and chemokines.
(Figure 1) [46]. Notably, non-malignant cells are the major contributors of metastasis throughout the phases of cancer development/progression [46].

The TME represents a complex network with inherent modulations acquired during the progression of disease or upon various therapeutic interventions. Thus, understanding the biochemical characteristics of malignant cells/non-malignant cells within the TME is crucial to uncover the molecular mechanisms behind such modulations. However, the contribution of the TME to tumorigenesis encompass multiple genetic/epigenetic/metabolic alterations, which can also determine the therapeutic outcomes in cancer patients [47]. Importantly, numerous therapeutic modalities fail to show clinical efficacy in targeting tumor cells due to their dynamic characteristics within the TME. Therefore, better understanding of the TME can potentially reveal targets for novel therapeutics or improve current therapeutic modalities to achieve favorable clinical outcomes. In this section, we will focus on the complexity and heterogeneity of the TME and discuss the key factors which hamper anti-tumor immune responses.

### 2.1 Immune cells

Tumor-infiltrating immune cells (TIICs) play a dual role in promoting and hampering the onset and progression of cancer, and their definitive roles in cancer development/progression rely on the modulations within the TME. TIICs primarily comprise of T lymphocytes, B lymphocytes, NK cells, dendritic cells (DCs), macrophages, neutrophils and MDSCs.

Tumor-infiltrating effector T cells are the key players to elicit adaptive anti-tumor immune responses; their abundance, functionality and distribution have crucial diagnostic/prognostic values [48]. For instance, cytotoxic CD8+ T cells and CD4+ T helper-1 (Th1) cells are the major producers of IFN-γ and IL-2, which are indispensable in creating a robust effector mechanism for the elimination of tumor cells, thereby resulting in favorable disease prognosis [49]. On the other
hand, Th subsets including Th2 and Th17 are mostly associated with tissue inflammation and favor tumor progression and metastasis [50, 51]. Furthermore, the effector mechanism of CD8$^+$ and CD4$^+$T cells can be hampered by tissue-resident Tregs, thereby favoring tumor progression [52]. Thus, higher ratio of Tregs:T effectors within the TME usually suppress anti-tumor immune responses [52]. Notably, the roles of tumor-infiltrating Tregs in certain cancer types including colorectal cancer and gastric cancer remain controversial [53-55].

In addition to T cells, B cell infiltration and localization usually occur in the tumor-invasive margin and draining lymph nodes, and have both beneficial and harmful roles in cancer [56]. In melanoma tumor models, it has been reported that B cells assist T cells in eliciting anti-tumor responses [57]. Furthermore, in multiple malignancies including cervical, gastric and ovarian tumors, B cell infiltration is associated with favorable prognosis [58-60]. However, reports show that B cell infiltration in squamous carcinoma and prostate cancer mediated by CXCL13 can promote the onset and progression of tumor [61, 62].

Tumor-residing NK cells are innate cytotoxic cells, which are responsible for recognizing and eliminating malignant cells, and have higher potentiality to influence adaptive anti-tumor immune responses through the production of various cytokines and chemokines [63]. Within the TME, NK cells represent a highly heterogenous distinct populations and express unique surface molecules [64]. Furthermore, NK cells inhibit metastasis through the upregulation of IFN-γ production and subsequent modulation of fibronectin-1 expression on tumor cells [65]. On the other hand, it has been reported that NK cells could modulate T cells and favor angiogenesis and tumor progression [66].

Antigen presenting cells (APCs), such as DCs and macrophages, are innate heterogenous immune cell populations within the TME with specific immunological functions [67]. The function of DCs
in the TME includes antigen presentation, priming of cytotoxic CD8⁺ T cells, activation of humoral immune responses, secretion of cytotoxic molecules, immunotolerance and suppression [68]. Additionally, majority of macrophages residing in tumor stroma are TAMs, which play a major role in regulating the inflammatory responses within the TME [69]. The accumulation of TAMs is predominantly associated with poor prognosis [70]. Pro-inflammatory macrophages referred to as M1 subtype are primed by cytokines including IFN-γ and TNF-α favoring Th1 responses and eliminating tumor cells [71]. Meanwhile, M2 macrophages, polarized by IL-4 and IL-13 favor tumor promotion and create an immuno-subversive environment by interfering with T cell functionality [71]. In addition to these, MDSCs are considered as one of the major immunosuppressive cells, which abundantly exist in the TME [72]. MDSCs are heterogenous population including two major subtypes; polymorphonuclear (PMN-MDSC) or granulocytic (G-MDSCs) and monocytic (M-MDSCs), which have potent immunosuppressive characteristics and favor tumorigenesis and progression [73, 74]. Furthermore, MDSCs play a key role in the promotion of epithelial mesenchymal transition (EMT) and tumor invasiveness [75].

Lastly, neutrophils comprise a major leukocyte population and elicit primary defense mechanisms against infection [76]. Within the TME, similar to macrophages, neutrophils are also polarized into two differing populations; N1 (anti-tumorigenic) and N2 (pro-tumorigenic) tumor-associated neutrophils (TANs) [77]. It has been shown that cancer-associated fibroblasts (CAFs)-mediated secretion of TGF-β is responsible for the polarization of N2 phenotype and inhibition of N1 neutrophils [78]. Importantly, TAN phenotype can play an indispensable role in the development and progression of tumor [79].
2.2 Non-immune cells

The TME consists not only of immune cells, but also of extracellular matrix (ECM), endothelial cells, fibroblast and pericytes [80]. Similar to other components within the TME, ECM plays a major role in facilitating signaling, migration and metabolism of malignant cells and also has a major effect on the immunogenicity of many solid tumors [81]. It has been reported that ECM has multiple roles including the progression/proliferation of tumor and response to therapy [82]. Furthermore, ECM consists of collagens, proteoglycans, hyaluronic acid, elastin and laminins, which are non-redundant for tumor progression. However, a clear understanding of the characteristics of ECM is necessary for developing novel therapeutic modalities.

In solid tumors, tumor vascularization can occur as a result of endothelial dysfunction and the induction of tumor-associated endothelial cells (TECs) caused by hypoxic factors and the chronic release of growth factors [83]. Unlike normal endothelial cells, TECs have distinct molecular and phenotypic peculiarities, which are similar to tumor cells but are not immortal [83]. TECs play a major role in the cellular determination within the TME, control cell trafficking and nutrient supply, thereby shaping the TME [84]. Moreover, TECs support the survival, functionality and dissemination of malignant cells [85]. Within the non-neoplastic cells in tumor milieu, CAFs are considered as a predominant stromal element favoring tumor progression [86]. CAFs are responsible for TGF-β secretion, which can drive EMT by restructuring ECM, leading to tumor invasiveness and metastasis [87]. In addition to TGF-β, CAFs can also produce soluble IL-6, which favors not only EMT but also chemo-resistance to malignant cells [88]. However, depletion of CAFs favors IFN-γ production and reverts immunosuppression within the TME [89]. Additionally, vasculature development within the TME is instituted through the perivascular cells, commonly called as pericytes [90]. Reports show that pericytes could serve as stem cell reservoir favoring
tissue regeneration and angiogenesis [91-93]. Altogether both immune cells and non-immune cells have distinct and dynamic contributions within the TME.

3. Cancer Immunotherapies

3.1 Currently approved immune checkpoint inhibitors

ICIs aim to interfere with the inhibitory pathways, which negatively modulate T cell activation, in order to ensure sustained T cell effector functions [94]. In addition to activation signals via TCR and co-stimulatory molecules, T cell activation is also regulated by inhibitory molecules referred to as ICs and the balance between co-stimulatory and inhibitory signals determines the magnitude of T cell responses [94]. ICs are vital for maintaining self-tolerance and modulating the extent and duration of immune responses in tissues to prevent tissue damage [95]. T cells are vital in anti-tumor immune responses via direct killing of tumor cells through cytotoxic T cells (CTLs) [96]. ICs are expressed on highly activated T cells; however, prolonged antigen stimulation may lead to sustained upregulation and co-expression of multiple IC expression on T cells, leading to a state of impaired activity and loss of effector functions, disruption of key transcription factors, and failure in transition to quiescence and acquire antigen-independent memory T cell homeostatic responsiveness, referred to as T cell exhaustion [97]. Multiple ICs may be expressed on T cells, which exert their stimulatory or inhibitory effects on T cell activation. ICs and their ligands are abundantly expressed in the TME of various malignancies and constitute vital components of the tumor immune resistance mechanisms [98]. Several studies have reported associations between IC expression in the TME with disease progression and/or worsened disease outcomes. Therefore, ICIs result in durable responses, due to the generation of improved anti-tumor T cell responses, but notably only in a fraction of patients [99]. Nonetheless, ICIs are at the forefront of all promising cancer immunotherapy strategies.
Leach et al., were one of the pioneering groups to propose the use of ICIs as a novel anti-cancer strategy to treat various malignancies [100]. Eventually, monoclonal antibody targeting interactions between cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) on T cells and its ligands on tumor cells was developed (ipilimumab) as the first class of ICIs, which were granted US Food and Drug Administration (FDA) approval, following improved survival rates in patients with metastatic melanoma [19]. Following CTLA-4 blockade, interactions between programmed cell death-1 (PD-1) and its ligands PD-L1 and PD-L2 were extensively studied, which lead to FDA approvals for anti-PD-1 antibodies such as nivolumab and pembrolizumab, and anti-PD-L1 antibodies avelumab, atezolizumab and durvalumab to treat selective cancers [101]. At present, seven ICIs have been approved for cancer therapy; however, the criteria for their application in different disease settings have been clearly laid out [102]. For instance, pembrolizumab was initially approved for treating advanced melanoma [103] and non-small-cell lung cancer (NSCLC) [104, 105] but eventually approved to treat unresectable or metastatic, microsatellite-high (MSI-H) or DNA mismatch repair deficient (dMMR) solid tumors that progressed after prior treatment [106], and more recently as a front-line treatment for MSI-H/dMMR colorectal cancer (CRC) patients based on KEYNOTE-177 study [107]. Here, we focus on the current FDA-approved ICIs; anti-CTLA-4, anti-PD-1 and anti-PD-L1 therapies, and discuss the rationale behind their utilization for therapeutic benefits.

3.1.1 Anti-CTLA-4

CTLA-4 (CD152) belongs to B7/CD28 proteins and is constitutively expressed on Tregs; upregulated on activated T cells, and inhibits T cell functions by affecting signaling through the co-stimulatory receptor CD28 [108]. CTLA-4 interactions hinder antigenic T cell responses via blocking CD28-mediated signaling [109]. These effects were most evident in studies showing
development of spontaneous autoimmunity, elevated lymphoproliferation and potent anti-tumor immunity in CTLA-4-deficient FoxP3+ Treg models [110]. Therefore, anti-CTLA-4 could potentially prompt the priming and expansion of tumor-specific naive T cells and elevate effector functions of memory/effector tumor reactive T cells by ensuring TME devoid of immunosuppressive mechanisms. Ipilimumab and tremelimumab are considered as pioneering fully human T-cell immunomodulatory monoclonal antibodies targeting interactions between CTLA-4 and its ligands to promote anti-tumor immunity [111]. Anti-CTLA-4 therapy showed efficacy in a wide range of cancers including melanoma, NSCLC, prostate, breast and ovarian cancers; however, immune-related adverse events (irAEs) were also prevalent in a significant percentage of patients [112]. As a result, till present only ipilimumab is granted FDA approval as the sole anti-CTLA-4 therapy in clinical use to treat metastatic melanoma [113].

Immune-related toxicities are the primary factor, which have limited the clinical translation of anti-CTLA-4 therapies in oncology. Larkin et al., showed that a higher percentage of patients exhibited side effects following treatment with anti-CTLA-4 therapy (27.3%) compared with anti-PD-1 therapy (16.3%), while combining both therapies resulted in even higher percentage of patients exhibiting side effects (55%) [114]. Therefore, utilization of biomarkers for response and efficacy may be considered utmost important for initiation of anti-CTLA-4 therapy. For instance, melanoma patients with high prevalence of neoantigens showed better response to anti-CTLA-4 therapy [115], and thus the presence of neoantigens on mutated tumors favors anti-tumor immunogenicity, which in turn results in improved treatment efficacy.

3.1.2 Anti-PD-1/PD-L1

PD-1 signaling pathway is a vital regulator of T cell responses and is critical in maintaining peripheral tolerance [116]. PD-1 engagement interferes with TCR/CD28 signaling and therefore,
the IL-2-dependent positive feedback, leading to decreased cytokine (IL-2, IFN-γ, and TNF-α) release and cell proliferation and decreased expression of the transcription factors related to effector T cell functions including T-bet and Eomes [22]. PD-L1 (CD274) and PD-L2 (CD273) are identified as putative ligands for PD-1 expressed on antigen-presenting cells and also on various non-lymphoid cells, and are involved in mediating peripheral tolerance [117]. Tumor cells can also upregulate PD-L1 and its ligation to PD-1 on antigen-specific T cells can affect anti-tumor immunity. Moreover, it was reported that elevated levels of CD8⁺ T cells at the invasive margin are negatively regulated by PD-1/PD-L1 expression in melanoma patients [118]. Increased PD-1 expression on T cells has been associated with poor prognosis in several cancers including pancreatic, liver, breast and head and neck [119]. Blocking the PD-1 receptor has shown extraordinary clinical responses in various cancers [21, 120]. In addition, the prognostic significance of elevated PD-L1 expression has been shown in various solid tumors and was associated with poor overall survival in breast, renal, urothelial and gastric cancers, and disease-free survival or progression-free survival in melanoma, hepatocellular carcinoma and renal carcinoma [121, 122].

Molecules designed to block signaling through PD-1 receptor include nivolumab, pembrolizumab, cemiplimab and lambrolizumab [123]. Pembrolizumab, nivolimumab and cemiplimab have been approved by the FDA for treating selective malignancies [102]. Blocking the PD-1/PD-L1 axis implicates targeting the receptor or the ligand to barricade the signaling pathway. Therefore, anti-PD-L1 antibodies have also shown synergistic effects in several cancers as anti-PD-1 therapy. Atezolizumab, durvalumab, and avelumab are FDA-approved anti-PD-L1 drugs, which are currently approved for different cancers including NSCLC, urothelial cancer, renal cell carcinoma and merkel cell carcinoma; atezolizumab plus nab-paclitaxel are also approved for patients with
locally advanced or unresectable/metastatic TNBC [124]. Notably, PD-L1 expression is proposed as a predictive biomarker for therapy and testing for PD-L1 expression has been established as a prerequisite for initiation of anti-PD-L1 therapies. However, PD-L1 expression threshold, immune-related toxicities associated with all ICIs and resistance mechanisms remain the primary challenges associated with utilization of anti-PD-L1 therapies for all cancers.

### 3.2 Adoptive T cell therapy

ACT is a cell-based cancer immunotherapy, which induces an antigen-specific T cell response via autologous, genetically-engineered CARTs [26, 28] or TCR-modified T cells [24]. The development of engineered CARTs involves the modification of isolated T cells from cancer patients in a manner that enable them to recognize tumor-specific and/or tumor-associated antigens (TSAs/TAAs), without relying on antigen processing and presentation. Following their *ex vivo* expansion, these modified, activated CARTs are passively transferred back to the patients [28]. The advantage of using CAR T cell therapy is encapsulated in the ability of CARTs to eliminate tumor cells with a better cytotoxicity and specificity, and induce a long-lasting anti-tumor immunity given that CARTs can act as memory T cells [25, 28]. The effectivity of ACT is dictated by 1) the level of immunogenicity of the target antigen selected, 2) efficiency of CAR or TCR-modified T cell trafficking to tumor sites, and 3) the accumulation of the engineered T cells within the TME.

#### 3.2.1 Chimeric-antigen receptor (CAR) T cell therapy

Up to date, improved response rates using CAR T cell therapy has only shown success in hematologic cancers [29]. In a proof-of-concept clinical trial, the therapeutic potential for using CARTs targeting CD20, B cell surface antigen, was shown in patients with mantle cell lymphoma (MCL) and B cell non-Hodgkin lymphoma (NHL) [125]. Targeting CD19, another B cell surface
antigen, by CARTs resulted in durable, sustained anti-tumor immunity in acute lymphoblastic leukemia (ALL) [126, 127], chronic lymphocytic leukemia [25, 128], multiple myeloma [129], and diffuse large B cell lymphoma (DLBCL) patients [130, 131]. These promising findings led to the development of ‘CTL019’ by Novartis, the first CAR T cell therapy approved by FDA for young patients with refractory B cell ALL [132].

The success of CAR T cell therapy in hematologic cancers has prompted its application in solid tumors to examine its therapeutic efficacy. CARTs targeting epidermal growth factor receptor (EGFR) variant in glioblastoma (GBM) showed an enhanced anti-tumor immune response associated with tumor cell elimination in an antigen-specific manner [133-135]. These data suggest that CAR T cell therapy has a therapeutic potential for treating solid tumors [29].

3.2.2 T cell receptor (TCR)-modified T cell therapy

The development of TCR-modified T cells involves the identification of antigen-specific TCRs and their introduction into autologous T cells [24]. TCR-modified T cells could be utilized for treating multiple solid tumors [30-33, 136]. Early phase clinical trials showed a sustained antigen-specific immune response induced by TCR-modified T cells targeting the antigen New York esophageal squamous cell carcinoma (NY-ESO-1) in myeloma [136] and synovial sarcoma [31].

The use of TCR-modified T cells targeting HLA-A*2402-restricted MAGE-A4 antigen in 10 patients with esophageal cancer showed moderately durable, sustained anti-tumor immune responses in three patients [137]. Unlike CARTs, TCR-modified T cells can only recognize intracellular TSAs or TAAs as their function is dependent on the TCR-mediated signaling, which involves antigen processing and presentation [24].
### 3.3 Recombinant cytokines and cancer vaccines

The utilization of human recombinant cytokines as a monotherapy in cancer patients was approved many years ago [138]. Proleukin (recombinant IL-2) was approved to treat renal cancer and melanoma patients, while Sylatron (IFN-α2b conjugated to polyethylene glycol) was approved to treat patients with hairy cell leukemia, advanced follicular non-Hodgkin’s lymphoma and resected melanoma [139-143]. More recently, Zhang et al. reported that systemic administration of IFN-γ in patients with synovial sarcoma and myxoid/round cell liposarcoma (MRCL) can increase tumor antigen presentation and reduce exhausted phenotypes of tumor-infiltrating T cells [144]. Based on the important roles of cytokines in regulating anti-tumor immune responses and T cell activation/proliferation, systemic administration of human recombinant IL-2 or IFN-γ as adjuvant therapy has been utilized to be used in conjunction to ICIs aiming to expand the effectivity of cancer therapy and improve response rates in patients [145, 146].

A wide range of vaccines have been assessed in clinical trials including the application of pulsed DCs with MHC-binding peptides of TAAs or tumor peptide as adjuvant therapy [147, 148]. The efficacy of using peptide-pulsed or viral vector–infected DCs has been tested in patients with glioma, melanoma, prostate cancer and colorectal cancer [149, 150]. Indeed, Sipuleucel-T vaccine comprising APCs stimulated with the prostate antigen (PAP) fused to granulocyte macrophage colony-stimulating factor (GM-CSF) was approved by the FDA to treat patients with metastatic castrate-resistant prostate cancer (mCRPC) [151]. TAA peptide-derived vaccines can be an excellent therapy to be applied as an adjuvant therapy; they are cost-effective and tumor-specific [152]. Nonetheless, the efficiency of TAA-derived vaccines could be potentially compromised as a result of tumor evolution and the development of acquired resistance caused by the loss of target antigen.
4. Mechanisms of resistance against cancer immunotherapies

Durability of treatment response is one of the main factors for favorable clinical outcomes. Large subsets of cancer patients do not benefit from cancer immunotherapies due to primary resistance, or they initially respond to therapy then relapse over time and develop acquired resistance [38, 153]. Mechanisms underlying the development of primary and adaptive resistance to cancer immunotherapies could be driven by tumor cell-intrinsic or extrinsic factors [38] (Figure 2). There is a vital need to identify reliable predictive biomarkers for tumor resistance to immunotherapy. Moreover, further understanding of the molecular pathways, which facilitate resistance mechanisms should be helpful in designing and developing alternative or combinatorial therapeutic approaches to overcome such resistance and enhance clinical outcomes in cancer patients (Figure 2). In addition, the mainstream use of ICIs in treating cancers is largely thwarted by low response rates and irAEs reported in some cancer patients [154]. This is because ICIs intervene in various immune response pathways and are thus, predisposed in affecting various molecular pathways downstream of checkpoint signaling pathways. Importantly, irAEs also occur due to blockade of IC pathways against autoimmunity, which leads to local and systemic autoimmune responses [154].

Tumor cell-intrinsic mechanisms leading to primary or adaptive resistance to immunotherapy involve low mutational burden promoting tumor cells to evade T cell recognition, exclusion of T cells from the tumor core, diminished anti-tumor immune responses due to high mutational rates in the IFN-γ pathway [38, 155-158]. In contrast, tumor cell-extrinsic mechanisms are related to the molecular and cellular components of the TME, such as suppressive immune cells (e.g. Tregs, MDSCs, TAMs, TADCs, TANs), adenosine, chemokines, cytokines, growth factors, and inhibitory ICs, resulting in the suppression of tumor antigen-specific TCR signaling, induction of
T cell exhaustion or apoptosis, overexpression of inhibitory immune checkpoints and the maintenance of a suppressive microenvironment favoring tumor growth and progression [23, 38, 55].

4.1 Tumor cell-intrinsic mechanisms

4.1.1 Low tumor mutational loads and loss of tumor antigens

As a result of cancer immunoediting, tumor cells can acquire mechanisms by which they can escape immune cell recognition via genetic and epigenetic alterations leading to impaired production of neoantigens or loss of target antigens and subsequently inhibit the activation of anti-tumor immunity [8] (Figure 3A). Alternatively, tumor cells can lose sensitivity to immunotherapy and develop resistance due to the survival and expansion of non-immunogenic tumor cell clones [8]. The selection of target antigens to develop genetically modified T cells is a crucial requirement for an effective therapy. In support of this, it has been reported that tumor resistance against CD19-CAR T cell therapy diffuse large B cell lymphoma patient has occurred due to the loss of CD19 expression [159]. Furthermore, genetic mutations acquired by tumor cells can compromise antigen presentation, and T cell recognition and activation leading to resistance to therapy and insufficient response to ACT or ICIs [14, 160, 161]. Indeed, studies have shown a positive correlation between high tumor mutational burden and the response to ICIs [162-164].

4.1.2 Oncogenic signaling pathways

Tumor cell-intrinsic factors can be initially present in tumor cells causing primary resistance or can be acquired later as tumor cells evolve leading to the emergence of acquired tumor resistance to cancer immunotherapies. These factors include the loss of PTEN expression which activates PI3K signaling, increased activation of WNT/β-catenin signaling pathway, signal transduction via
mitogen-activated protein kinase (MAPK) pathway, impaired IFN-γ signaling pathways, and suppression of T cell responses [38].

Loss of PTEN and enhanced activation of PI3K signaling in several cancer types have been associated with tumor resistance against ICIs [5, 165-167]. Loss of PTEN gene expression has been inversely correlated with CD8+ T cell infiltration and expression of granzyme B and IFN-γ, and higher mutations of PTEN deletions have been observed in non-T cell infiltrated tumors [167]. Unlike wild-type mice, deficiency of PTEN in mouse tumor model can reduce the vulnerability to ACT [167]. Moreover, PTEN deletions and PI3K mutations in tumor cells can promote the upregulation of PD-1 ligands, PD-L1 and PD-L2 [168-173], which ultimately can suppress anti-tumor T cell functions (Figure 3B).

Constitutive signaling of WNT networks leading to the stabilization of β-catenin could result in the exclusion of T cell infiltration in tumor sites and therefore, reduces the sensitivity of tumor cells to ICIs or ACT [6] (Figure 3C). In support of this, it has been shown that human melanoma tumors with high expression of β-catenin signaling-related genes lacked the infiltration of T cells and CD103+ DCs in the TME [6]. Increased levels of β-catenin in mouse tumor model have been associated with reduced infiltration of CD103+ DCs in tumor tissues and tumor resistance to ICIs, compared to tumor-bearing mice lacking β-catenin expression [6].

Alternatively, activation of MAPK pathway in tumor cells promotes the secretion of various molecules, including IL-8 and VEGF, and leads to tumor angiogenesis and metastasis [174, 175] (Figure 3D).
4.1.3 Epigenetic modifications

Tumorigenesis and progression are closely related with the somatic epigenetic reprogramming [176]. These epigenetic alterations could influence potential modifications in gene transcription and favor mutational burden on host cells [177]. Moreover, TIICs possess an aberrant epigenetic profile, compared with normal immune cells [178]. For instance, tumor-infiltrating T cells showed a reprogrammed DNA methylation profile and favor tumor progression [178]. In addition to stromal cells, cancer cells per se have exhibited an altered epigenetic and considered as one of the hallmarks of cancer [179]. Accumulating evidences suggest that epigenetic modifications including DNA methylation and post-translational histone modifications are the predominant epigenetic alterations occurring within the TME [36]. Moreover, reports show that targeting epigenetic modifications not only favor the regulation of gene transcription but also revert anti-tumor immune responses and reinvigorate the exhaustion of T cells [180, 181] (Figure 3E).

Majority of studies on epigenetic modifications within the TME rely on DNA methylation, which could influence the onset and progression of tumors. DNA hypermethylation, either global or localized, of CpG islands (CpG-rich regions) in the promoter regions of key genes including tumor suppressor genes are evident in several cancers [182]. However, global hypomethylation of DNA creates a genomic instability and induce the transformation of cells. Global DNA hypomethylation is found in many cancers including prostate [183], leukemia [184], hepatic carcinoma [185] and cervical cancer [186]. In contrast, global DNA hypermethylation was reported in breast cancer [187]. It has been reported that cancer-associated hypermethylation happens in CpG islands, while hypomethylation happens in both CpG islands and repeated DNA sequences [188]. Moreover, the hypomethylation of these repeated DNA sequences could favor tumorigenesis by elevating karyotypic instability and gene expression [189].
Aberrations in the methylation profile of DNA is closely linked with post-translational histone modifications, which is crucial for determining the fate of tumor cells by reprogramming the chromatin architecture [190]. Major reprogramming of histones includes methylation, acetylation, ubiquitination and phosphorylation [190]. Various modifications coordinate together to regulate cellular and molecular events in the oncogenic transformation and progression of tumors [191]. For instance, histones are most frequently mutated proteins in malignancy and act as potent tumor drivers [192]. These mutations could influence invasion, dissemination, chemoresistance and abnormal proliferation of malignant cells [193]. Recent advances in high throughput technologies assisted genome wide characterization of chromatin modifications during the onset and progression of tumor [176]. It has been reported that tumor cells exhibited global loss of histone methylation and acetylation, with the loss of H4K20me3 and acetylated H4K16 [194]. These losses are closely related to hypomethylation of DNA-repetitive nucleotides, a characteristic feature of tumor cells [194]. DNA/histone epigenetic alterations can not only affect cancer cells to promote their growth and invasion, but can also affect T cells (Figure 4) [98]. The epigenetic modifications on IC promotor in T cells can impair effector T cell function, and increase Treg and MDSC levels in the TME [98].

4.2 Tumor cell-extrinsic mechanisms

One of the major challenges which adversely impacts anti-tumor immunity and limits the response to cancer immunotherapies in patients is tumor-induced immunosuppression [29, 195, 196]. An immunosuppressive environment within the TME is established via cellular and molecular networks, which chronically promote trafficking and accumulation of suppressive immune cells/molecules, and overexpression of inhibitory ICs [197, 198], and favor effector T cell and
CTL deactivation/apoptosis and tumor progression, therapy leading to resistance and sustained immunosuppression [14, 199].

4.2.1 Cancer-associated fibroblasts

Stromal cells within the TME of multiple solid tumors are predominantly CAFs [200]. Signals generated by tumor cells or the hypoxic condition within the TME can result in the conversion of normal resident fibroblasts into CAFs [200, 201]. CAFs can lead to cancer progression by inducing CD8+ T cell apoptosis, and supporting ECM degradation, tumor growth, invasion, and angiogenesis [13, 202, 203]. A study performed on lung carcinoma and pancreatic adenocarcinoma (PDAC) mouse models showed that specific knockdown of fibroblast activation protein (FAP) (a protein that is constitutively expressed by CAFs) positive cells resulted in the hypoxic necrosis of stromal and tumor cells [89]. In support of this, another study showed that CAFs expressing FAP can act as a compensatory resistance mechanism against anti-CTLA4 and anti-PD-L1 mAbs in PDAC mouse model [204]. Overall, these findings implicate the therapeutic potential of depleting CAFs in cancer to alleviate resistance mechanisms and maximize clinical benefits of cancer immunotherapies.

4.2.2Suppressive immune cells and cytokines

Immune cells within the TME can have both pro- and anti-tumor functions [205]; hence, they can determine the level of tumor immunogenicity and response to cancer immunotherapy [55, 206, 207]. Tumor cells and immune cells within the TME can cooperatively work together to induce the generation and activation of CAFs via the release of cytokines and growth factors (such as IL-6, IL-1β, VEGF and TGF-β), which in turn negatively modulate anti-tumor immunity and may lead to effector T cell apoptosis [23, 208, 209]. Furthermore, the suppressive milieu of the TME shaped by the types of infiltrated immune cells can promote the recruitment and favor the
accumulation of MDSCs in tumor sites [23]. MDSCs, in turn, inhibit effector T cell responses by various means, such as limiting the availability of tryptophan by the overexpression of IDO enzyme, production of reactive oxygen species, and release of suppressive molecules, for example TGF-β, which support the Treg function and survival [210-213]. Additionally, MDSCs can facilitate tumor resistance to therapy; reports have shown that intratumor depletion of MDSCs can restore the efficacy of anti-PD-1 therapy and BRAF inhibition [214, 215]. Moreover, gene signature derived from melanoma patients showing primary resistance to anti-PD-1 therapy comprised of genes involved in immunosuppression, EMT, angiogenesis and monocytes/MDSC chemotaxis [216], indicating that MDSCs could be also associated with the induction of primary resistance against ICIs.

Within the TME, Tregs are other immune cell subsets with suppressive properties can suppress effector T cell functions and may trigger effector T cell apoptosis leading to impaired anti-tumor immunity [217, 218]. Importantly, Tregs can contribute to acquired resistance and limit the effectiveness of ICIs against cancer [23]. Studies on mouse tumor models showed that targeting Tregs in combination with ICIs, such as anti-PD-1/PD-L1 mAbs, can improve response rates to cancer immunotherapy and prolong survival [219-221].

Tregs can express upregulated levels of TGF-β and IL-10 and inhibitory ICs, which in turn inhibit the activation of effector T cells and favor Treg survival and suppressive function [10, 55, 222]. Tregs also express high levels of ectoenzymes (CD73 and CD39) leading to the accumulation of high levels of extracellular adenosine which induce effector T cell apoptosis or cell cycle arrest and promote Treg function [223]. Tregs can also release high levels of perforin and granzyme B to mediate cytotoxic effects on effector T cells leading to reduced effector T cell:Treg ratio within the TME [23, 55]. Additionally, Tregs consume high levels of IL-2 leading to the deprivation of
effector T cells and apoptosis [224, 225]. It has been reported that high consumption of IL-2 by intratumoral Tregs can restrict the efficacy of adoptively transferred T cells, as well as effector T cells present in the TME (216). Conversely, the utilization of engineered CARTs, which are not capable of producing IL-2, in mouse melanoma models was shown to be effective in diminishing Treg infiltrate within the TME and thereby leading to prolonged, improved clinical outcomes and anti-tumor immune responses (217).

4.2.3 Upregulation of co-inhibitory immune checkpoints

There is a high likelihood for the emergence of acquired resistance post ACT therapy in cancer patients, as a result of increased expression of ICs on their cell surface leading to deactivation, exhaustion and/or apoptosis [24, 29, 226]. In support of this, it has been demonstrated that adoptively transferred TCR-modified T cells in mouse tumor models can express higher level of PD-1 upon their migration and accumulation in tumor sites [40]. Alternatively, direct contact between MDSCs/TAMs and tumor-infiltrating T lymphocytes, including effector T cells and adoptively transferred T cells, via the interaction between ICs and IC ligands could compromise the activation and proliferation of T cells [212, 227].

Apart from CTLA-4 and PD-1, blocking other ICs such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), T cell immunoglobulin and ITIM domain (TIGIT) and V-domain Ig suppressor of T cell activation (VISTA) among others, are being investigated in several preclinical studies and/or clinical trials [228]. These ICs are also overexpressed in the TME of various malignancies and have been shown to correlate with disease progression [98, 229, 230].

Studies have shown that blocking one IC or IC ligand can lead to the upregulation of alternative ICs and IC ligands, leading to acquired resistance against therapy. For instance, in vitro, breast
cancer cell lines co-cultured with activated PBMCs in the presence of anti-PD-1/PD-L1 mAbs showed elevated levels of TIM-3 and LAG-3 co-expression on CD4+ T cell subsets, including Tregs, suggesting the emergence of compensatory inhibitory mechanism of acquired resistance in breast cancer following PD-1/PD-L1 blockade [231]. In vivo, acquired resistance to anti-PD-1 mAb therapy in patients and mouse model with lung cancer have been associated with elevated levels of TIM-3 ligand, galectin-9, and CD4+ and CD8+ T cells expressing TIM-3, which potentially can impair the function of anti-tumor immune responses upon TIM-3/galaectin-9 interaction [232, 233]. In HNSCC mouse model, elevated expression of TIM-3 on intratumoral CD8+ T cells was detected following anti-PD-1 mAb treatment [234], while the overexpression of TIM-3 on Tregs and CD8+ T cells and Tregs in head and neck mouse tumor models was implicated in the emergence of acquired resistance to PD-L1 inhibition [221].

The overexpression of LAG-3 on CD8+ T cells has been detected in ovarian cancer mouse model following the blockade of CTLA-4 or PD-1 mAb, while the combined blockade of LAG-3 either with PD-1 or CTLA-4 showed beneficial effects evident by elevated levels of CD8+ T cells and reduced levels of Tregs within tumor sites [235]. Another study showed that increased expression of TIGIT could serve as a resistance mechanism to ICIs, evident by improved clinical outcomes post the inhibition of TIGIT in mouse tumors, which showed resistance to anti-PD-1 mAb [236]. Furthermore, increased expression of VISTA on M2 macrophages and TILs from prostate cancer patients treated with anti-CTLA-4 therapy, implicating that VISTA overexpression could be an acquired immunosuppression mechanism [237]. The interaction between VISTA and its ligand can suppress the function of effector T cells [238], and induce Treg differentiation [239], while the blockade of VISTA was effective in promoting effector T cell activation/accumulation within mouse tumors and reducing the number of MDSC and Tregs in the tumor [240]. Another study
showed that the co-blockade of PD-L1 and VISTA in mouse tumor models can enhance the anti-tumor responses and improve disease outcomes compared to their single inhibition [241].

5. Therapeutic approaches to overcome resistance

5.2 Predictive biomarkers for successful immune checkpoint inhibition

In addition to utilization of ICIs as therapeutic agents, the use of predictive biomarkers to identify patients for successful ICI-based therapies has also gained great interest in recent years [154]. Tumor-based predictive biomarkers for ICI primarily include immune cell infiltrates, protein expression, presence of neoantigens/tumor burden and gene/epigenetic signatures [154]. Chen et al. proposed classifying the TME into three major categories termed immune desert, immune excluded and immune inflamed, based on immune cell infiltration [242]. Tumors with high immune cell infiltration respond better to ICIs mainly due to the presence of additional targets on T cells, ensuring their sustained activation for potent anti-tumor immunity; therefore, some agents are also being designed to skew the TME towards an immune inflamed environment [243]. Similarly, elevated expression of protein targets of ICI such as IC ligands in the TME also potentially signifies improved response to IC inhibition. For instance, PD-L1 expression in the TME of NSCLC patients was approved by the FDA as a prerequisite for initiation of anti-PD-1 therapy [244]. However, studies have shown that PD-L1-negative tumors can also respond to blockade along PD-1/PD-L1 axis [245]. These findings suggest that investigating additional biomarkers may be required to ascertain response to IC-based therapies. Transcriptomic and epigenetic signatures could potentially aid as predictive biomarkers in such scenarios. Several groups have identified multiple dysregulated/mutated or silenced gene signatures across different cancers, which could potentially predict response to therapy; however, none have been recognized
as robust predictive markers and it is believed multiple biomarkers may need to be considered for accurate prediction of patient selection for effective response to IC inhibition [246].

5.3 ICIs and adoptive T cell therapy

The safety and efficacy of anti-VISTA mAb, anti-TIGIT mAb, anti-LAG-3 mAb and anti-TIM-3 mAb, in various cancer patients are under clinical trials [as reviewed in [23]]. Evidence from preclinical models showed promising clinical outcomes and durable immune responses upon the inhibition of multiple ICs, and could be used as a therapeutic approach to alleviate acquired resistance caused by the upregulation of ICs (Figure 5A and C). Furthermore, the utilization of ACT in combination with ICIs, such as anti-PD-1 and anti-CTLA-4 therapies [212, 227], or the application of CARTs with mutated forms of ICs using CRISPR/Cas9 technology [247, 248] could improve clinical efficacy than monotherapy. Other engineering approaches could be applied to alleviate the PD-L1-mediated T cell exhaustion by replacing the intracellular domains of PD-1 with that of CD28 to exchange the inhibitory signal with a co-stimulatory signal leading to the enhanced activation of CARTs and cytokine release [249].

5.4 Targeting immunosuppressive cells in combination with ICIs or adoptive T cell therapy

Combined targeting of immunosuppressive cells and their derived molecules with ICIs or ACT could offer promising clinical outcomes to enhance tumor sensitivity to therapy and prolong survival rates in cancer patients who did not benefit from ICIs or ACT alone and developed acquired resistance (Figure 5B).

Targeting chemokine receptors (for example CXCR2 and CCR2) to reduce the trafficking and infiltration of MDSCs and TAMs in the TME [250, 251] or blocking colony stimulating factor-1 (CSF-1) or its receptor (CSF-1R) [252, 253] to inhibit their survival and accumulation within the
TME could expand the therapeutic efficacy, if used in combination with immunotherapies. In vivo, the co-blockade of CSF-1R and PD-1/PD-L1 reduced the levels of MDSCs/TAMs in tumors, increased the number of activated effector T cells and led to superior clinical outcomes, unlike the blockade of PD-1/PD-L1 alone [254]. In another study, it was shown that the inhibition of CSF-1/CSF-1R signaling in mouse tumor models could re-program MDSCs and alter their suppressive function and enhance the clinical outcomes in combination with anti-CTLA-4 mAb [252]. Moreover, the combination of anti-PD-1 mAb and class IIa histone deacetylase (HDAC) inhibitor improved the survival and therapeutic benefits of anti-PD-1 mAb and caused transformation of TAMs to M1 macrophages [255].

The efficacy of ICIs along with drugs targeting MDSCs is under clinical investigations [256]. Alternatively, targeting Tregs via anti-CD25 mAb [257] or via anti-CCR4 mAb [258, 259] in combination with ICIs could boost the anti-tumor responses induced by cancer immunotherapies. Combined inhibition of ICs and the use of inhibitors against IDO, expressed by myeloid cells and tumor cells [41, 260], ARG1, expressed by MDSCs and TAMs [261], and the adenosine pathway (mediated by suppressive immune cells and tumor cells) [260] could also expand the sensitivity to ICIs by suppressing Treg differentiation and inducing effector T cell activation and proliferation [41, 260] (Figure 5B).

5.5 ICIs or adoptive cell transfer in combination with adjuvant therapies

The effectivity of TCR-modified T cell therapy is dependent on APC function; effective antigen processing and presentation to TCR-modified T cells is required for TCR-mediated signaling and anti-tumor immune response [24]. Cell-to-cell interactions between intratumoral TCR T cells and TAMs can impair tumor-specific T cell responses facilitating tumorigenesis [262]. Ultimately, chronic recruitment and accumulation of TAMs within the TME could induce the development of
acquired resistance. Such resistance could be resolved by the administration of human recombinant cytokines, such as GM-CSF, as an adjuvant therapy in combination with adoptive TCR-modified T cell therapy to alter the phenotype of TAMs and enhance T cell activation and function [263, 264]. Other adjuvant therapies which could be combined with ACT or ICIs are recombinant IL-2 and IFN-α2b (Sylatron) [265, 266] (Figure 5B); this could maximize the clinical outcomes in patients and sustain effective immune responses mediated by exogenous and endogenous T cells [265, 266]. Furthermore, cancer vaccines involving the administration of tumor-derived antigens and pulsed DCs could be utilized in combination with ACT to effectively suppress tumor growth/invasion in cancer patients [267].

5.6 Targeting CAFs and ECM components using CAR T cell therapy

CAFs is another promising therapeutic target for cancer, which could enhance the sensitivity of immune response to ICIs in patients with acquired resistance; for instance, TGF-β produced by CAFs has been linked with tumor resistance against anti-PD-1 therapy in metastatic urothelial cancer [268]. Indeed, the utilization of CARTs expressing FAP, have shown promising clinical benefits in a mouse tumor model [269]. In light of this, CARTs expressing FAP underwent clinical trial (NCT01722149) in cancer patients.

Furthermore, the application of modified T cells engrafted with an echistatin-containing CAR (T-eCAR) specific for αvβ3 integrin that is expressed on TECs, have shown promising outcomes associated with enhanced capacity of T cell trafficking into the TME and improved T cell function [270]. Similarly, the effectivity of CARTs specific for αvβ6 integrin that is expressed on breast, ovarian and pancreatic tumors has been shown in vivo [271]. The administration CARTs expressing ECM components could enhance the trafficking of CARTs to the TME and their activity [272, 273].
5.7 Epigenetic modifiers and ICIs

Epigenetic modifications have an indispensable role in the development and progression of cancer, as discussed in section 4.1.3. Pharmacological inhibitors, namely epigenetic modifiers, could revert these modifications and restore the host anti-tumor responses [36, 274]. Accumulating evidences suggest that the transcriptional regulation of multiple ICs and IC ligands including PD-1, CTLA-4, TIM-3, LAG-3, TIGIT and PD-L1 are controlled by DNA methylation and post-translational histone modifications on their promoter regions [36]. These data suggest the potential application of epigenetic modifiers together with ICIs or ACT as more effective immunotherapeutic modalities (Figure 5C).

DNA methyltransferase (DNMT), enzyme responsible for DNA methylation, could be blocked by DNMT inhibitors (DNMTi) including 5-azacitidine and 5-aza-2'-deoxycytidine and restore normal methylation profile on the promoter regions of key tumor suppression genes [275]. It has been reported that DNMTi could enhance the outcome of anti-CTLA-4 therapy by restoring the key hypermethylated genes in melanoma models [276]. On the other hand in myelodysplastic syndrome, demethylating agents lead to the transcriptional upregulation of PD-1, PD-L1 and CTLA-4, which could be beneficial for targeting aforementioned genes using appropriate checkpoint inhibitors [277]. Interestingly, multiple clinical trials have been initiated to measure the effectiveness of combinatorial therapeutic strategies using DNA demethylating agents and ICIs in various cancers [36].

In addition to DNA methyl modifiers, histone acetylation modifiers including HDAC inhibitors, also showed to enhance the efficacy of ICIs by upregulating corresponding immune checkpoints and tumor-associated antigens on malignant cells leading to their easiness in targeting and also upregulation in the production of cytokines/chemokine by TILs and decreases immunosuppressive
cell population within the TME [278]. Additionally, HDACi treatment prior to immunotherapy could enhance the efficacy of treatment [278]. For instance, HDACi treatment on melanoma preclinical model upregulated the expression of PD-L1 and PD-L2 and improved the PD-1 blockade therapy [279]. Moreover, in colon and breast tumor models, the efficacy of combinatorial inhibition of PD-L1 and CTLA-4 was enhanced by HDACi, entinostat [280]. Furthermore, combination of atezolizumab and entinostat has being tested in phase I/II clinical trials for TNBC patients (NCT02708680). Likewise, clinical trials of pembrolizumab and vorinostat are in phase I/II for advanced NSCLC (NCT02638090). In concordance with PD-1/PD-L1 interaction, CD80/86 and CTLA-4 interaction was also significantly influenced by HDACi [281]. It has been reported that HDACi could upregulate CD80/86 in the TME and significantly enhance the efficacy of anti-CTLA-4 targeting [282]. Combination of ipilimumab/entinostat for breast cancer (NCT02453620) and ipilimumab/panobiostat for advanced melanoma (NCT02032810) are under clinical trials.

6. Conclusions, challenges and future perspectives

Tumors are heterogenous and plastic and their molecular profiles can vary with cancer types and disease stages; therefore, it is imperative to stratify cancer patients and treat them accordingly using a personalized-medicine approach. Moreover, therapeutic strategies should be applied in logical and sequential manner to avoid any possible adverse immune-related and toxic events depending on the natural history of disease for cancer patients and the capacity of their anti-tumor immunity acquired within the TME.

The molecular and cellular components of the TME are the determining factors for tumor immunogenicity and can predict response to therapy and clinical outcomes in cancer patients. The presence of high tumor burdens and high levels of immune cell infiltrate within the TME make
tumors more vulnerable to cancer immunotherapy and regression. Nonetheless, tumor cells can gain the ability to escape immune cell recognition via intrinsic and extrinsic mechanisms and alter their transcriptional profile/molecular signature to promote their own survival, mediate immunosuppression and induce primary or acquired resistance to cancer therapies. Furthermore, the cross-talk between tumor and stromal cells within the TME can result in a positive feedback loop, which favors the recruitment and activation of suppressive immune cells, overexpression of inhibitory ICs and the suppression of effector immune cells, and promote the induction of CAFs, thereby leading to tumor growth, metastasis and angiogenesis. Importantly, these cellular interactions within the TME can lead to the emergence of tumor resistance to therapy.

Some cancer patients show no response to immunotherapies which is more likely due to primary resistance driven by genetic or epigenetic alterations or due to the exclusion of tumor-infiltrating T cells from tumor core, known as cold or desert tumor. Cancer patients who initially respond to immunotherapies have the potential to develop acquired resistance over time. To overcome tumor resistance, immunotherapies, epigenetic modifiers and small molecule inhibitors and/or monoclonal antibodies which specifically deplete immunosuppressive cells could be used in combination to promote the activation of anti-tumor immunity and eradicate tumor cells. Together, these combinatorial therapies could expand the efficacy of immunotherapies, and prolong anti-tumor immunity leading to favorable disease outcomes in cancer patients.
Figure 1. Cell populations within the TME. Construction of tumor microenvironment and various cell populations including tumor cells, epithelial cells and immune cells present within the TME are shown.
Figure 2. Determinants of tumor resistance to therapy and how to overcome them. In solid tumors, tumor cells are continuously evolving to establish an immunosuppressive environment, referred to as tumor microenvironment (TME), comprising of cellular and soluble components that favors tumor growth/progression and promote immune evasion. Over time, tumor cells within the TME become very heterogenous as they acquire genetic and epigenetic alterations with spatial and temporal diversity (1). Tumor cells with low growth rates (2) and low mutational burden (3) are usually incurable and resistant to cancer therapies (intrinsic or primary resistance). Surviving tumors cells following the administration of particular cancer therapy can acquire compensatory inhibitory mechanisms which allows them to escape immune cell recognition and induce immunosuppression. Under selective therapeutic pressure, these surviving tumor cells can
grow and expand leading to tumor recurrence or relapse (acquired resistance) (4). Cellular components of the TME can also impact the development of resistance and promote tumor growth and progression by suppressing the ability of effector T cells from eradicating tumor cells and enhancing the induction/function and recruitment of suppressive cells, such as TAMs, MDSCs and CAFs (5). Thus, therapeutic strategies to overcome intrinsic and acquired tumor resistance against cancer immunotherapies are crucial to maximize the efficacy of cancer treatment and revert tumor resistance to ICIs. Monitoring the immune response within the TME before and after the application of therapy could be helpful to identify biomarkers which could be related to resistance development (I). The utilization of cancer therapies with distinct mechanisms of action and multiple ICIs could be beneficial in enhancing the therapeutic response (II). Next Generation Sequencing (NGS) of DNA or RNA in cancer cell clones could be also helpful in revealing genetic factors which are important for cancer cell growth and survival (III).
Figure 3. Mechanisms of primary tumor resistance against cancer immunotherapy.

Tumor cells can escape immune cell recognition via genetic and epigenetic alterations leading to impaired expression of MHC I/immune cell recognition and impaired production of neoantigens or loss of target antigens and subsequently inhibit the activation of anti-tumor immunity (A). Loss of PTEN gene expression can reduce tumor vulnerability to immunotherapy by promoting the overexpression of PD-L1 and PD-L2, which ultimately lead to the suppression of T cell function (B). Constitutive activation of WNT signaling leading to the stabilization of β-catenin could result in the exclusion of T cell infiltration in tumor sites and therefore reduces the sensitivity of the tumor to ICIs or ACT (C). The activation of MAPK pathway in tumor cells leads to the secretion of various molecules, including IL-8 and VEGF, which exert immunosuppressive effects and promote tumor angiogenesis and metastasis (D). Epigenetic reprogramming in tumor cells can
promote tumor growth by enhancing the expression and production of growth factors and inhibiting the transcription of tumor suppressor genes (E).

Figure 4: Effects of DNA methylation and histone modifications on the promoter region of ICs/ligands within the TME. The effects of DNA/histone epigenetic alterations on T cells (A) and cancer cells (B) are illustrated. The epigenetic modifications of ICs on T cells can lead to less responsive T cells, increased Treg levels, increased MDSC trafficking and impaired effector cytokine release. The epigenetic modifications in tumor cells can facilitate metastasis and immune evasion, and establish an immunosuppressive microenvironment. Figure is adapted from Toor et al., Semin Cancer Biol [98].
Figure 5. Mechanisms of acquired resistance and potential therapeutic approaches.

Impaired generation of tumor-reactive T cells could be solved by the application of ICIs with ACT (CARTs) combined with cytokines, chemokines, TLR agonists or vaccines to restore and boost the anti-tumor immune response. The combined use of immunotherapy and radiation or chemotherapy could also enhance the clinical benefit and maximize tumor cytotoxicity (A). Inadequate activation of effector T cells (Teffs) could occur as a result of metabolic mediators,
increased levels of immunosuppressive cells/molecules and upregulation of alternate ICs. In this scenario, combining ICIs with cytokines, vaccines, depleting Tregs, MDSCs or TAMs, or targeting adenosine, ARG1, IDO or HDAC isoforms could restore APC function and the activation/proliferation of Teffs, and reduce immunosuppression (B). Impaired generation of Teff memory cells within the TME results in a loss of durability associated with T cell exhaustion and epigenetic changes. The use of multiple ICIs with ACT and epigenetic modifiers could augment the activation/proliferation and function of cytotoxic T cells and promote the generation of Teff memory T cells (C). Figure is adapted from Saleh & Elkord [23].
Chapter References


