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REVIEW

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# Resistance mechanisms to immune checkpoint inhibitors: updated insights

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## Abstract

The last decade has witnessed unprecedented succusses with the use of immune checkpoint inhibitors in treating cancer. Nevertheless, the proportion of patients who respond favorably to the treatment remained rather modest, partially due to treatment resistance. This has fueled a wave of research into potential mechanisms of resistance to immune checkpoint inhibitors which can be classified into primary resistance or acquired resistance after an initial response. In the current review, we summarize what is known so far about the mechanisms of resistance in terms of being tumor-intrinsic or tumor-extrinsic taking into account the multimodal crosstalk between the tumor, immune system compartment and other host-related factors.

**Keywords** Immune checkpoint inhibitors, Resistance, Tumor-intrinsic, Tumor-extrinsic

## Introduction

Cancer is a major global public health burden and a leading cause of death worldwide with a recorded 19.3 million new cases and 10 million deaths each year [1, 2]. In the clinical settings, conventional cancer treatment modalities including chemotherapy and radiotherapy have been associated with inherent limitations such as lack of tumor specificity, poor tumor tissue penetration, chances of recurrence and systemic toxicities. Therefore, these limitations interfered with achieving beneficial therapeutic outcomes for most patients and highlighted the need to improve the strategies and modalities of cancer treatment.

After significant and concerted efforts and investigation, the critical roles of the immune system in controlling tumor development and progression have been established. This led to the emergence of immunotherapy as a new and promising modality to treat cancer. The increased understanding of immunosurveillance and the complex crosstalk between tumor cells and immune system compartment along with the development of molecular biology have nourished the field of cancer immunotherapy. Over the past decade, the emergence of immunotherapy has changed the paradigm of cancer treatment from the direct killing of cancerous cells to empowering the anti-tumor immune responses, and therefore is considered one of the most appealing and flourishing cancer treatment modalities of recent years [3]. Nowadays, several immunotherapeutic approaches, including immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T-cell therapy and cancer vaccines have been designed and implemented in the clinical use to treat a wide-spectrum of malignancies.

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ICIs are monoclonal antibodies targeting inhibitory immune checkpoint molecules such as programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) to inhibit T-cell negative costimulation and reinvigorate the anti-tumor immune responses [4]. In the past decade, ICIs have been shown to achieve sustained and unsurpassed efficacy in treating multiple cancer types including non-oncogene-driven carcinomas and unresectable metastatic cancers [5–7]. The durability of responses translated into long-term survival benefits is considered a significant hallmark of cancer immunotherapy. In 2011, ipilimumab (anti-CTLA-4) was the first US Food and Drug Administration (FDA)-approved ICI to treat patients with metastatic melanoma. To date, twelve ICIs have received FDA approval: two CTLA-4 inhibitor (ipilimumab and tremelimumab), six PD-1 inhibitors (nivolumab, pembrolizumab, cemiplimab, dostarlimab, retifanlimab and toripalimab), three PD-L1 inhibitors (atezolizumab, durvalumab, and avelumab) and one lymphocyte-activation gene-3 (LAG-3) inhibitor (relatlimab) as first-line, second-line, third-line and later-line cancer monotherapies or combination therapies [8, 9]. Others are in the late-stage clinical trials such as T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitors [10].

Despite the durable responses and dramatic clinical breakthroughs reported in some of ICIs-treated cancer patients, the majority showed slight to inconsiderable benefits [11–17]. The objective response rate (ORR) can range from 40–70% in some malignancies such as melanoma, Hodgkin's lymphoma and microsatellite instability (MSI)-high tumors, while it can be as low as 10–25% in most other cancers [12, 18, 19]. In addition, patients with initial favorable responses can develop resistance and eventually present with progressed disease due to acquired secondary resistance [5, 20]. Several lines of evidence attributed the heterogeneity in the therapeutic outcome among treated patients to several factors including cancer type, tumor intrinsic features and tumor microenvironment (TME) including its immune and non-immune compartments [18, 21, 22]. In the current article, we briefly highlight the role of immune checkpoint inhibitors in the Cancer-Immunity (CI) cycle and elaborate on reviewing the mechanisms that are known so far to underlie resistance to ICIs including both tumor-intrinsic and tumor-extrinsic factors.

## Cancer-immunity cycle and ICIs

The anti-tumor immune response is a complex process composed of a series of steps that can be linked in the CI Cycle [23, 24]. The cycle emphasizes on the iterative nature of the antitumor immune response in which tumor cell killing by T cells and release of tumor-associated antigens induce subsequent repetition of antigen presentation and T cell activation in order to propagate and amplify the immune response [23]. To ensure efficient tumor eradication while avoiding autoimmunity, different stimulatory and inhibitory checkpoints are at play in each phase of the CI cycle along with other pro-inflammatory cytokines, chemokines and co-stimulatory factors that regulate T cell activation and migration. For instance, CTLA-4 expressed on T cells binds with higher affinity- compared to CD28- to B7 ligands on the surface of antigen-presenting cells (APCs). This competitive binding interferes with CD28-B7 interaction and inhibits the full activation of T cells [25]. Another example is the engagement between PD-1 and its ligand PD-L1 that suppresses the immune response through inhibiting the effector stage of T cell activation [11]. Such interactions can influence the success or failure of different aspects within the CI cycle.

The increased understanding of CI cycle was associated with exceptional potential to enhance the discovery rate of new therapies that target and reinforce the anti-tumor immunity at different steps during the course of response and has become the framework for the development of different cancer immunotherapeutic approaches. Immunotherapeutic agents mainly function by targeting different steps within the context of the CI cycle with an ultimate goal of reinvigorating and expanding the pre-existing anti-tumor immune responses. ICIs including anti-CTLA-4 were configured to ameliorate the negative feedback mechanisms and reignite self-sustaining CI cycle during T cell priming and activation, whereas others including PD-1, PD-L1, T cell immunoglobulin and mucin domain 3 (TIM-3), LAG-3 and V-domain Ig suppressor of T cell activation (VISTA) inhibitors function at later stages during tumor cell killing [4, 23]. It is assumed that a functioning CI cycle in which each single step is activated, and function properly is needed for a clinically favorable response to ICIs [26]. In this context, it is worth noting that both primary and acquired resistance to ICIs could be driven by several mechanisms during antigen presentation and T cell activation, T cell trafficking and tumor infiltration in addition to T cell killing [27]. In other words, abnormalities or disruptions at any point within the cycle could result in resistance to ICIs, leading to an arrest in the anti-tumor immune responses and progression of tumor growth. Recently, Hou et al. highlighted the potential role of CI cycle in predicting the response to ICIs and adopting a beneficial treatment strategy for each patient [28]. This article reported the development and validation of a novel CI cycle-based signature that takes into account the status of CI cycle and

tumor immunophenotype to predict the responsiveness to ICIs among colorectal cancer patients.

## Mechanisms of resistance to ICIs

Despite the unprecedented and durable breakthroughs reported with the use of ICIs in treating cancer patients, the response rate remains rather modest in which some patients respond favorably to the treatment, while most of them don't show any clinical benefits. Considerable ongoing efforts to address resistance to ICIs have demonstrated that immune resistance is driven by complex, dynamic and interconnected processes. Understanding the underlying mechanisms and developing strategies to reverse the resistance remain one of the biggest challenges in the field of cancer immunotherapy. Resistance to ICIs can be classified into primary resistance and acquired resistance based on the mechanistic details, onset through the CI cycle (time of occurrence) and tumor immunophenotype. Lack of initial response to the treatment due to the baseline status is associated with primary resistance, whereas acquired resistance is seen in patients who experience initial promising responses that are ultimately followed by clinical and/or radiographic progression of the disease [29–31]. It is suggested that primary resistance occurs in tumors that lack adequate infiltration of immune cells (excluded or dessert tumors), whereas acquired resistance occurs in inflamed tumors [32]. Resistance can also be classified into intrinsic or extrinsic to tumor cells. Tumor intrinsic resistance occurs when cancerous cells alter processes that are related to cell signaling, gene expression in addition to immune recognition and effector function that ultimately result in therapy resistance, whereas extrinsic resistance happens outside tumor cells through multiple immunological and non-immunological interactions. Tumor intrinsic and tumor extrinsic resistance mechanisms are illustrated in Figs. 1 and 2, respectively. It is worth noting that the resistance to immunotherapeutic agents could be mediated by the cumulative effect of multiple mechanisms that are interdependent and seems to overlap. Herein, the mechanisms of resistance to ICIs are described in terms of being tumor-intrinsic or tumor-extrinsic.

### Tumor intrinsic resistance mechanisms

#### *Lack of neoantigens*

Several studies have documented that patients with high tumor mutational burden (TMB) and neoantigen load are more sensitive to ICIs, compared to patients with low TMB [33–37]. TMB refers to the total number of mutations found per coding area of cancer cell genome and has been utilized to determine tumor immunogenicity and sensitivity to ICIs. Tumor neoantigens induce

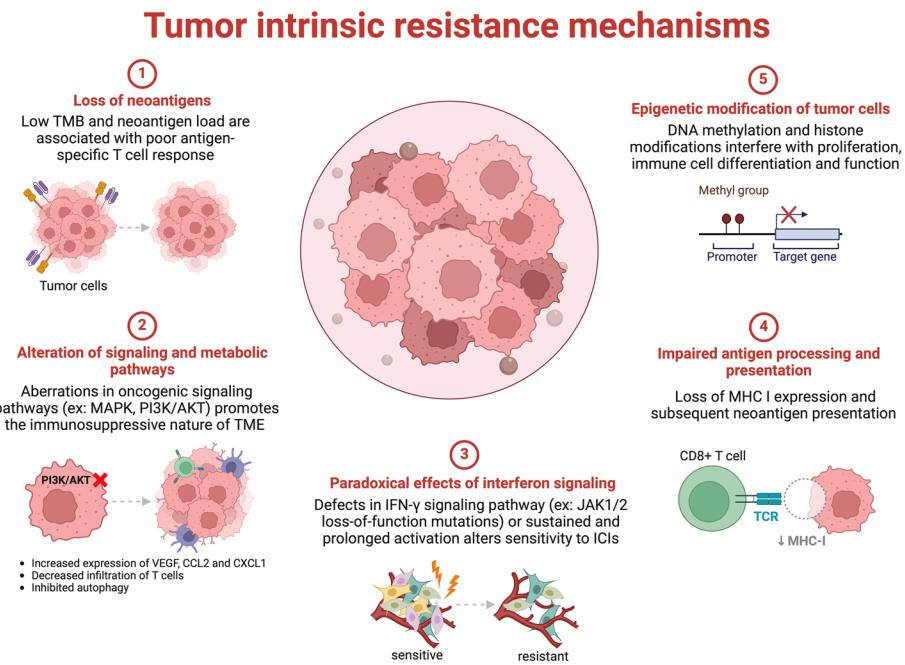


**Fig. 1** Schematic detailing putative tumor-intrinsic mechanisms to ICIs therapy. Generally, resistance to ICIs is driven through tumor-intrinsic and/or tumor-extrinsic mechanisms. Tumor-intrinsic mechanisms of resistance mainly include insufficient neoantigens, aberrations in oncogenic signaling and metabolic pathways, impaired interferon signaling, defective antigen processing and presentation machineries in addition to epigenetic alterations. TMB: tumor mutational burden; TME: tumor microenvironment; ICI: immune checkpoint inhibitors; TCR: T-cell receptor; MHC: major histocompatibility complex. This figure was created with BioRender.com

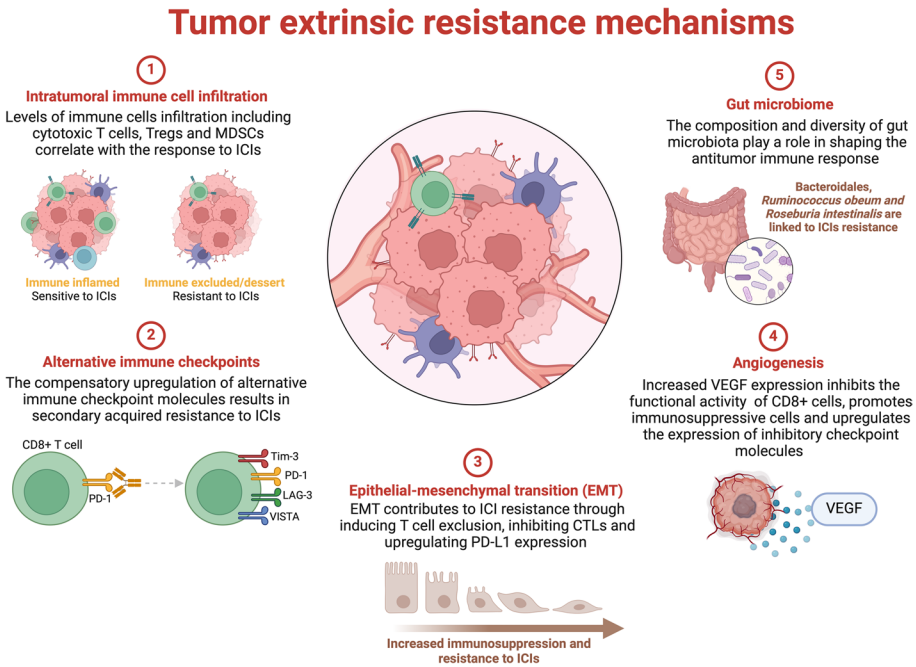


**Fig. 2** Tumor extrinsic resistance mechanisms to ICIs treatment. Resistance to ICIs could be derived through tumor extrinsic mechanisms that involve inadequate infiltration of T cells, high levels of intratumoral immunosuppressive cells such as MDSCs and Tregs, compensatory upregulation of alternative inhibitory immune checkpoint molecules including Tim-3, LAG-3 and VISTA, epithelial-mesenchymal transition, angiogenesis in addition to the composition of gut microbiota. Tregs: regulatory T cells; MDSCs: myeloid-derived suppressor cells; ICIs: immune checkpoint inhibitors; EMT: epithelial-mesenchymal transition; VEGF; vascular endothelial growth factor. This figure was created with BioRender.com

specific anti-tumor immune response mediated through antigen-specific activation of T cells. Ineffective T cell response resulting from low TMB accompanied with insufficient neoantigens has been shown to remarkably contribute to lack of sensitivity to ICIs [38]. Highly immunogenic tumors with high TMB and neoantigen load such as melanoma, head and neck squamous cell carcinoma (HNSCC) and non-small cell lung carcinoma (NSCLC) are known to be more responsive to ICIs, compared to poorly immunogenic tumors with low TMB such as prostate and pancreatic cancers [36, 39, 40]. In conflict, others claimed that TMB is not predictive for response to ICIs among all cancer patients [41].

Lack of neoantigens can underlie both primary and acquired resistance that result from acquisition of genetic changes. It has been suggested that the continuous interaction between cancerous cells and immune system prompts the selection of resistant tumor subclones that lack neoantigens expression [42, 43]. This, in turn, results in the development of poorly immunogenic tumors and compromises the efficacy of ICIs [44, 45]. In a study by Anagnostou et al., it was suggested that the acquired resistance to ICIs developed among NSCLC patients is due to mutations in genes encoding for tumor neoantigens predicted to have high affinity to T cell receptor (TCR) and MHC molecules [46]. Moreover, mutations interfering with the antigen presentation machinery of tumor cells including mutations in MHC molecules, transporter for antigen presentation (TAP) and/or beta-2 microglobulin (β2M) have been shown to be associated with compromised neoantigen presentation, lack of tumor antigen recognition by T cells and resistance to immunotherapy [47–49].

Moreover, genetic instability due to defective mismatch repair (MMR) system results in increased somatic mutations, tumor immunogenicity and neoantigen expression. It also promotes the accumulation of erroneous genetic products in microsatellites, leading to a status called microsatellite instability (MSI). Tumors with MSI-high were associated with higher TMB and neoantigen load, stronger local and systemic immune responses and higher sensitivity to immunotherapeutic agents, compared to microsatellite stable tumors (MSS) [50–52]. Furthermore, a study utilizing metastatic urothelial cancer demonstrated that alterations in DNA damage response genes namely FANCA, ERCC2, ATM, POLE and MSH6 accounted for high TMB and better response rate to ICIs [53]. Mutations in BRAC2 gene participating in DNA damage repair were also shown to be associated with high neoantigen load and better response to immunotherapy in melanoma and ovarian cancer patients [54, 55].

In order to enhance the therapeutic outcome of ICIs, the neoantigen load and corresponding tumor immunogenicity were targeted through combining ICIs with other modalities of cancer treatment. Several lines of evidence demonstrated the potential of chemotherapy and/or radiotherapy to induce tumor cell death, release neoantigens and improve T cell priming, activation and functional potential [56–59]. This, in turn, was shown to be associated with enhanced sensitivity to ICIs and improved therapeutic outcome across multiple types of cancer [60–62]. Nowadays, various combinations of ICIs and chemotherapy/chemoradiotherapy are approved by the FDA for the treatment of a broad spectrum of cancers including NSCLC and urothelial cancer and many others are still under clinical evaluation [63, 64]. In addition, several pre-clinical and clinical studies demonstrated that tumor vaccines are also capable to improve the efficacy of ICIs in terms of tumor regression and overall patient's survival [65–67]This was shown to be mediated through improving tumor immunogenicity and augmenting the long-term polyfunctional neoantigen-specific effector T cell responses [68].

#### *Alteration of signaling and metabolic pathways*

A growing body of literature demonstrated that aberrations in oncogenic signaling pathways can influence the sensitivity to immunotherapeutic agents through altering the immune system compartment throughout different stages of cancer development.

The mitogen-activated protein kinase (MAPK) pathway plays an essential role in several cellular processes including growth, proliferation, differentiation and apoptosis [69]. Thus, alterations in signaling cascades are implicated in cancer progression, invasion and therapeutic resistance across different types of tumors [70]. Increased MAPK signaling inhibits tumor infiltration of T cells through increasing the production of VEGF and other immunosuppressive cytokines, leading to resistance to ICIs [70, 71]. Moreover, mutations in components of MAPK pathway, namely EGFR, EML4-ALK and KRAS were associated with upregulated expression of PD-L1, which was accompanied with increased T cell apoptosis in NSCLC [72–74]. Consistently, some pre-clinical studies have documented that MAPK inhibition enhanced IFN-γ signaling and MHC-I expression and promoted tumor infiltration of $CD8^+$ cytotoxic T cells thereafter [75–77]. These findings highlight the involvement of MAPK pathway in immune escape and therapeutic resistance to immunotherapy.

The PI3K/AKT pathway is an intracellular signaling pathway involved in the regulation of cell cycle, growth and proliferation and has been shown to be overactivated in malignancies [78]. Loss of the tumor suppressor gene phosphate and tensin homolog (PTEN) enhanced PI3K pathway activation which, in turn, increased the expression of the immunoinhibitory cytokines VEGF, CCL2 and CXCL1, decreased tumor infiltration of T cells and inhibited autophagy [79, 80]. These alterations collectively have been shown to promote immune resistance and poor therapeutic outcome to ICIs [79, 81].

WNT/ β-catenin signaling is another regulatory pathway involved in essential cellular processes. Aberrations in this pathway were closely associated with cancer development, invasion, metastasis and resistance to immunotherapeutic agents [82]. In one study utilizing a melanoma model, it was demonstrated that the activation of WNT/ β-catenin signaling promoted the resistance to anti-PD-L1 and anti-CTLA-4 monoclonal antibodies through downregulating the expression of T cell specific genes and certain chemokines, resulting in poor intratumoral T cell infiltration [83]. In line with these findings, another study demonstrated that mutations in β-catenin were three-times enriched in non-T-cell-inflamed tumors in comparison to T-cell inflamed tumors [84]. Therefore, targeting WNT/ β-catenin signaling pathway may aid in restoring tumor infiltration and improving the efficacy of immunotherapy. A recent study of endometrial cancer revealed a new mechanism of immune evasion through dysregulating LATS1/2 that possess a key role in upregulating the expression of MCH I through the IFN-γ-STAT1-IRF1 signaling [85]. Therefore, dysregulating LAST1/2 was shown to be associated with the downregulation of MHC I expression and inhibition of intratumoral activated $CD8^+$ T cells providing that the dysregulation of this pathway could underlie primary and acquired resistance to ICIs [85].

In addition to the aforementioned signaling pathways, Jian et al. provided key insights into the involvement of RTK signaling pathway in shaping anti-tumor immunity and determining the therapeutic response to PD-1/PD-L1 inhibitors [86]. The research group demonstrated a correlation between high TYRO3 expression and resistance to PD-1/PD-L1 blockade in a mouse model of breast cancer and among patients receiving anti-PD-1/anti PD-L1 therapy. A high level of TYRO3 was shown to inhibit tumor cell ferroptosis that was triggered by PD-1/PD-L1 blockade and modulate the TME in favor of tumor growth through reducing M1/M2 macrophage ratio [86]. This, in turn, resulted in the resistance to PD-1/PD-L1 inhibitors that can be reversed by inhibiting TYRO3 [86].

Indoleamine 2,3-dioxygenase (IDO)−1 is a rate-limiting enzyme that catalyzes the conversion of the essential amino acid tryptophan to kynurenine metabolite. IDO-1 plays a vital role in suppressing effector T cells, inducing T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs), escaping immunosurveillance

and, therefore, promoting resistance to ICIs [87–90]. This comes in addition to adenosine, a derivative of ATP, that confer potent immunosuppressive role and promote resistance to immunotherapy [91, 92]. The hypoxic nature of the TME promotes the dephosphorylation of adenosine monophosphate by the ectoenzyme CD73 leading to the production of adenosine which- upon engagement with its receptor that is present on lymphocytes- inhibits the activation, proliferation and functional potential of effector T cells[93, 94]. Moreover, alterations in other genes involved in cancer metabolism namely LKB1, and MYC were associated with resistance to ICIs through their potential to hijack the immune system and promote the immunosuppressive milieu of the TME [95, 96]. Given their role in promoting resistance to ICIs, targeting these compounds showed remarkable potentials to alleviate the resistance and enhance the therapeutic potential of ICIs in multiple pre-clinical and clinical trials [27, 90, 97–99].

#### *Paradoxical effect of interferon signaling*

IFN-γ is a T-cell effector cytokine that plays a key role in anti-tumor immune responses by inhibiting cell growth, facilitating tumor cell apoptosis and upregulating MHC I and PD-L1 expression through JAK-STAT mediated signaling pathway [100]. Several lines of evidence have documented the paradoxical role of IFN-γ signaling pathway and demonstrated its positive and negative correlations with the response to ICIs. On the one hand, defects in IFN-γ signaling genes were shown to be associated with resistance to immunotherapeutic agents. For example, JAK1/2 loss-of-function mutations interfered with IFN-γ-induced PD-L1 expression and other interferon-stimulated genes resulting in primary PD-1 blockade resistance, regardless of the high TMB [101]. Acquired resistance to anti-PD-1 was also demonstrated in melanoma patients as a result of truncating mutations in JAK1/2 genes accompanied with loss of sensitivity to IFN-γ [48]. Another study demonstrated that defects in IFN-γ signaling pathways account for resistance to anti-CTLA-4 inhibitors in both melanoma-bearing mice and patients with metastatic melanoma [102]. Geo et al. demonstrated the genomic loss of key IFN-γ genes and amplification of essential IFN-γ pathway inhibitors, namely SOCS1 and PIAS4, in tumors from unresponsive patients [102]. On the other hand, some studies have shown that the sustained and prolonged IFN-γ signaling can elicit acquired resistance to ICIs through inducing STAT1-related epigenomic changes and enhancing the expression of IFN-stimulated genes along with multiple T cell inhibitory receptor ligands [103]. Another study using a pre-clinical lung cancer model reported that IFN-γ can result in secondary resistance to anti-PD-1 through promoting the nuclear translocation and phase separation of Yes-associated protein (YAP) which, in turn, leads to promoting the expression of multiple inhibitory genes and inhibiting the functional activity of cytotoxic T cells [104]. Beside IFN-γ, sustained stimulation of type I interferons was also shown to contribute to immunosuppression and resistance to antibody-based immunotherapy through increasing tumor infiltration of Tregs and myeloid cells [105]. Another study suggested that IFN-β in the TME can enhance the expression of CD38, which interferes with the cytotoxic T cell function via adenosine receptor signaling and inhibiting PD-1/PD-L1 blockade efficiency [106]. This comes in line with a recent study that linked the chronic stimulation of type I interferons to lipid peroxidation among $CD8^+$ T cells [107]. The later was shown to promote the metabolic and functional exhaustion of T cells resulting in resistance to ICIs [107].

#### *Impaired antigen processing and presentation*

Tumor neoantigens are recognized by the immune system after being processed and presented to T cells in the context of peptide-MHC complexes [108, 109]. Therefore, defects in the antigen processing and presentation machineries of tumor cells could interfere with establishing efficient anti-tumor immune responses and contribute to resistance to ICIs. In this context, downregulation of MHC I expression in addition to mutations in the β2M gene- that is essential for the surface expression and stabilization of MHC I molecules- were shown to negatively affect antigen presentation and compromise the efficacy of immunotherapy thereafter. Genetic and transcriptional loss of HLA I alleles were described as mechanisms of immune avoidance and resistance to immunotherapy in different cancer types [110–114]. Zaretsky et al. documented that homozygous β2M frame-shift deletion accompanied with decreased surface expression of MHC I in tumor tissues was associated with acquired resistance to PD-1 blockade in a metastatic melanoma patient [48]. In line with this study, others demonstrated that homozygous loss or heterozygous mutations combined with loss of heterozygosity in β2M gene in tumors could confer primary or acquired resistance to ICIs resulting from loss of MHC I expression [115, 116]. It is worth noting that the biallelic alterations of β2M loss were found only in non-responding patients, whereas monoallelic truncating mutations or loss of heterozygosity in β2M were shown to be associated with varying sensitivities to ICIs due to retaining a wide type allele of β2M [115]. Nevertheless, other researchers reported that β2M mutations were more likely to occur in MSI-high tumors and the β2M status was not correlated with the level of tumor infiltration of immune cells [117–119]. Furthermore, studies have demonstrated that MSI-high tumors

with inactivating mutations in β2M can still respond to ICIs, suggesting that neoantigen-rich tumors are capable to present their antigens independently from β2M protein [52, 119]. Moreover, other emerging factors such as Human Papilloma Virus E5, autophagy and IL-8 have been shown to alter the antigen presentation machinery and inhibit the efficacy of ICIs [120–122].

#### *Epigenetic modifications of tumor cells*

Several studies have documented that epigenetic alterations of tumor cells including methylation, histone modification and silencing could alter the expression of immune-related genes and, in turn, interfere with the antigen processing and presentation machineries, proliferation in addition to immune cell differentiation and function [123, 124]. For instance, in a study using an ovarian cancer model, epigenetic silencing consisting of enhancer of zeste homologue 2 (EZH2)-mediated histone H3 lysine 27 trimethylation (H3K27me3) and DNA methyltransferase 1 (DNMT1)-mediated DNA methylation inhibited the production of T helper 1-type CXCL9 and CXCL10 chemokines and subsequently decreased tumor infiltration of T cells impairing the therapeutic efficacy of PD-L1 inhibitors [125]. Other studies documented that methylation of MHC I transactivators and histone modification of MHC I APP gene promoters led to transcriptional silencing and downregulated expression of components of the MHC class I [126, 127]. This, in turn, was shown to interfere with the antigen presentation in cancer cells, promote immune evasion and confer resistance to immunotherapy. Additionally, recent studies illustrated that epigenetic modifications play crucial roles in regulating the expression of inhibitory immune checkpoints and their ligands within the TME [128–131]. For example, hypomethylation of the CpG islands in the promoter regions of PD-1, CTLA-4, and TIM-3 genes were linked to upregulated expression of the corresponding inhibitory immune checkpoint molecules [132]. Comparable findings were reported in colorectal cancer in which the increased histone modifications in the promoter region of PD-L1 gene was shown to underlie the upregulated expression of PD-L1 in tumor-infiltrating T cells [130]. Such alterations in the expression profile of these inhibitory molecules could confer the response to ICIs. In line with these findings, one study suggested that the efficiency of PD-1 blockade is, in part, mediated by the acetylation-dependent regulation of PD-L1 nuclear translocation [133]. Gao et al. reported that deacetylation of cytoplasmic PD-L1 by HDAC2 promotes its translocation to the nucleus in which the nuclear PD-L1 binds to the DNA and regulates the expression of multiple-immune-response-related genes [133]. Such observations provide valuable insights toward targeting nuclear PD-L1 translocation in order to enhance the efficacy of PD-1/PD-L1 blockade among cancer patients.

In support of these findings, combining epigenetic modulators including DNA methyltransferase inhibitors and histone deacetylase inhibitors has been shown to improve the therapeutic potential of ICIs [125, 134]. This was mediated through the capacity of combination treatment to inhibit the immunosuppressive milieu within the TME, modulate the expression of inhibitory immune checkpoint molecules and promote the functional activity of T cells. A very recent study demonstrated that histone macroH2A1 phosphorylation induced by lipoic acid-mediated p-AMPKα activation resulted in the nuclear compartmentalization of PD-L1 which, in turn, suppressed tumorigenesis and bypassed resistance to ICI treatment [135]. This was shown to be mediated by the potential of nuclear PD-L1 to upregulate the expression of MHC I and enhance tumor sensitivity to IFN-γ [135]. Currently, the translation of these promising results from pre-clinical to clinical settings is undergoing by a number of clinical studies [136–140].

### Tumor extrinsic resistance mechanisms

The TME is heterogeneous and composed of cancerous cells, immune cells, stromal cells, vasculature in addition to extracellular matrix. Accumulating evidence demonstrated that the complex multi-dimensional crosstalk between these multiple components plays a critical role in sculpting the immunosuppressive TME, modulating the anti-tumor immune responses and determining the sensitivity to cancer therapy. In addition to the aforementioned tumor intrinsic mechanisms, resistance to ICIs can occur external to tumor cells through immunological and non-immunological processes. In this section, we shed the light on the current understanding of the tumor extrinsic mechanisms that have been shown to underlie resistance to ICIs. Figure 2 summarizes the resistance-driven putative extrinsic mechanisms.

#### *Intratumoral immune cell infiltration*

It is known that the dynamic crosstalk between immune cells and tumor cells plays an essential role in shaping the TME and determining the tumor immunophenotype and sensitivity to immunotherapeutic agents. Tumor immune profiles are classified into immune-inflamed, immune excluded and immune-dessert tumors based on immune cell presence and localization within the TME [141]. Immune-inflamed tumors are characterized by the infiltration of T cells in the tumor parenchyma in close proximity to tumor cells and they are often associated with beneficial responses to ICIs. On the other hand, immune-excluded and immune-dessert tumors are identified by the presence of T cells in the tumor stroma but not in

the parenchyma or lack of T cell infiltration, respectively. Both phenotypes were linked to immune checkpoint blockade resistance [141, 142]. In a pre-clinical study using colon adenocarcinoma model, it was suggested that the level of intratumoral infiltration of $CD45^+$ immune cells correlate with the response to PD-L1 blockade in which favorable responses were observed in tumors that harbor higher proportion of $CD45^+$ cells [143].

$CD8^+$ cytotoxic effector T cells are known to play a critical role in mediating anti-tumor immune responses through inducing direct cytotoxicity to tumor cells. The clinical efficacy of ICIs has been positively correlated with T cell-derived biomarkers including the pre-existence of $CD8^+$ cytotoxic T cells at the invasive tumor margin and within the tumor, close proximity of PD-$1^+$ cells and PD-$L1^+$ cells in addition to the clonal T cell repertoire [46, 144]. Beside the tumor intrinsic factors affecting the level of intratumoral T cell infiltration (discussed in previous section), APCs were shown to play a role. It is evident that the stimulator of interferon genes (STING) signaling pathway significantly contributes to tumor surveillance and response to treatment through promoting the production of type I interferons which, in turn, facilitate dendritic cell maturation and presentation of tumor-specific antigens to T lymphocytes [145]. Moreover, the critical role of STING signaling pathway in promoting T lymphocyte trafficking and homing to the tumor site through upregulating the expression of T cell-attracting chemokines was demonstrated [145]. Loss of STING impaired interferon signaling and this has been associated with insufficient activation of dendritic cells, poor intratumoral cytotoxic T cell infiltration and lack of sensitivity to ICIs [146, 147]. In addition, abnormalities in several signaling pathways involved in the production of T cell-attracting chemokines- namely IFN-γ, WNT/β-catenin, PTEN, LKB1, and EGFR pathways- were associated with resistance to ICIs [148].

The Role of intratumoral immunosuppressive cells namely Tregs, MDSCs and tumor-associated macrophages (TAMs) in driving resistance to ICIs has been elucidated through tireless efforts of numerous researchers. Tregs are known to suppress the proliferation and activation of effector T cells and APCs through secreting immunosuppressive cytokines such as IL-10, IL-35 and transforming growth factor-β (TGF-β) in addition to the expression of inhibitory immune checkpoint molecules such as PD-1 and CTLA-4 [149, 150]. Some studies have demonstrated that depletion of Tregs following anti-CTLA-4 and anti-PD-1 treatment was accompanied with an increase in the advantageous effector T cell/Treg ratio within the TME [151, 152]. The effect of Tregs on the therapeutic potential of immunotherapeutic agents remains inconclusive, but some studies claimed their involvement in ICIs resistance. The incomplete depletion of Tregs following treatment with ICIs along with the upregulation of alternative immune checkpoint molecules on Tregs could result in the development of resistance [153–155]. Another study demonstrated that the selective inhibition of TGF-β produced by Tregs ameliorated the resistance to anti-PD-1 immunotherapy and promoted tumor regression suggesting that TGF-β may account for Tregs-mediated resistance [156, 157]. Others reported that the intratumoral apoptotic Treg subset was associated with immunosuppression and resistance to anti-PD-1 blockade through its potential to release and convert large amount of ATP to adenosine [93].

MDSCs are another group of immunosuppressive cells that contribute to immune evasion and tumor progression through suppressing the activation and proliferation of effector T cells and natural killer cells, inducing the differentiation of Tregs and inhibiting the antigen presentation potential of professional APCs [158, 159]. Several studies have established the negative association between the frequency of MDSCs within the TME and the therapeutic efficacy of ICIs among different tumor models [160–163]. Others demonstrated that resistance to ICIs can be reversed, and the response can be enhanced through inhibiting the trafficking of MDSCs to the TME suggesting that MDSCs could result in primary and acquired resistance to ICIs [164, 165].

TAMs play a critical role in shaping the anti-tumor immunity. Considering the complex plasticity of this population within the TME, TAMs can be identified as M1 or M2 macrophages in which M1 macrophages classically express pro-inflammatory cytokines and promote anti-tumor immunity whereas M2 macrophages function in favor of tumor progression through promoting angiogenesis, immunosuppression, hypoxia induction and metastasis [166]. Initially, M1 TAMs are among the primary cells to trigger inflammation in the early phase of cancer development then TAMs are educated by signals from the TME to undergo polarization towards the pro-tumorigenic M2 phenotype [166]. TAMs- through altering the expression of PD-L1 in tumor cells and secreting cytokines that promote tumor development- have been shown to unfavorably affect the response to ICIs [46, 167–169]. For example, a study of lung adenocarcinoma demonstrated that M2 TAMs inhibited $CD8^+$ T cell migration to the tumor site by forming long-lasting interactions that leads to immunotherapy resistance [170]. When depleted, intratumoral T cell infiltration was enhanced and beneficial effects of ICIs were restored [170]. In line with this study, others have shown that targeting M2 macrophages and re-directing their polarization toward the M1 phenotype can ameliorate the resistance and enhance the response to ICIs [171–173].

Moreover, Wang et al. suggested that a low ratio of adaptive immune response to pro-tumorigenic inflammatory gene signatures in myeloid phagocytic cells is linked to PD-L1 blockade resistance in urothelial cancer [174]. The potential of PD-1$^-$ TAMs to capture anti-PD-1 antibodies from the surface of PD-1$^+$ tumor-infiltrating T cells was also shown to mediate the resistance to PD-1 blockade [175]. In vivo imaging revealed that the administered anti-PD-1 initially binds to CD8$^+$ T cells and then got captured by PD-1$^-$ TAMs from T cell surfaces [175]. This was shown to be dependent on both the treatment Fc domain and Fcγ receptors that are expressed by host myeloid cells. Given that the composition of intratumoral immune cell repertoire plays a fundamental role in shaping the response to ICIs, converting the TME from being immunosuppressive to becoming more immunogenic was associated with enhanced tumor sensitivity to therapy. This can be achieved through combining ICIs with other modalities of cancer treatment such as chemotherapy, radiotherapy and immune stimulatory agents in which they can modulate the intratumoral immune cell compartment and render it more susceptible to therapy. Additionally, regulating the crosstalk between the intratumoral immune cells could play an essential role in shaping the response to ICIs. In support of this, Geels et al. suggested that, in mouse and human melanoma models, the accumulation of Tregs was mediated by activated CD8$^+$ T cells that produce IL-2 which, in turn, upregulate ICOS protein on intratumoral Tregs and promotes their accumulation [176]. They also demonstrated that disrupting the crosstalk between tumor-infiltrating CD8$^+$ T cells and Tregs, through inhibiting ICOS signaling, improved the overall therapeutic outcome of PD-1 blockade.

#### *Alternative immune checkpoints*

One of the main extrinsic resistance mechanisms that results in secondary acquired resistance to ICIs is the compensatory upregulation of alternative inhibitory immune checkpoint molecules namely TIM-3, LAG-3, B and T lymphocyte attenuator (BTLA), VISTA and TIGIT [9]. In the tumor context, these checkpoint pathways have been associated with T cell exhaustion and terminal dysfunction [177]. A study by Koyama et al. revealed that acquired resistance and tumor progression post promising response to anti-PD-1 treatment resulted from the upregulated expression of TIM-3 in a murine model of lung adenocarcinoma [178]. Sequential targeting of TIM-3 using blocking antibodies was associated with improved survival [178]. Furthermore, overexpression of CTLA-4 and LAG-3 on T cells was observed in resistant tumors [178]. Shayan et al. suggested that the compensatory upregulation of TIM-3 post treatment with PD-1 blockade is driven through PI3K/AKT pathway downstream of TCR signaling, but not cytokine-mediated pathways [179]. Another study of metastatic NSCLC illustrated that both primary and acquired resistance to anti-PD-1 therapy were associated with the accumulation of lymphoid cells and monocytic MDSCs that express TIM-3 and galectin-9, respectively, and resistance was shown to be reversed using TIM-3 blocking antibodies [161]. Moreover, Huang et al. suggested that targeting a single inhibitory molecule (PD-1, LAG-3 or CTLA-4) through genetic ablation or blocking antibodies resulted in the compensatory upregulation of alternate inhibitory checkpoint molecules and subsequent T cell-suppression whereas the dual blockade of PD-1/CTLA-4 or LAG-3/CTLA-4 or triple blockade of PD-1/LAG-3/CTLA-4 conferred durable anti-tumor immune responses accompanied with better tumor growth control [180]. In line with these findings, a cohort study of NSCLC patients demonstrated a link between acquired resistance to ICIs and upregulated expression of TIM-3 and/or LAG-3 inhibitory molecules [116]. VISTA is another inhibitory immune checkpoint molecule expressed on T cells and CD68$^+$ macrophages that was shown to be upregulated in a compensatory pattern following treatment with ipilimumab (anti-CTLA-4) in prostate cancer [181]. Another study of metastatic melanoma showed that acquired resistance to anti-PD-1 treatment among majority of patients was, in part, mediated by the upregulated expression of VISTA on intratumoral T lymphocytes [182]. Other studies illustrated that TIGIT immune checkpoint expressed on natural killer cells and Tregs could account for increased infiltration of intratumoral Tregs and resistance to ICIs [183–185]. Overall, the compensatory upregulation of alternative immune checkpoint molecules is a potential target for immunotherapy combinations and the beneficial outcomes observed as a result of targeting multiple inhibitory immune checkpoints were demonstrated by undeniable number of pre-clinical and clinical studies [186–190]. A recent trial revealed that combining nivolumab (anti-PD-1) and relatlimab (anti-LAG-3) was associated with durable safety profiles and clinical outcomes among heavily pretreated melanoma patients who previously progressed upon receiving anti-PD-1-containing regimens [189]. Interestingly, phase III CheckMate 067 trial that cobmined ipilimumab and nivolumab to treat advanced melanoma demonstrated unprecedented durable clinical benefits in comparison to monotherapy [190]. These promising observations, along with other clinical trials, have led to the FDA approval of multiple combinations to be utilized in the clinical settings including the combination of Opdivo (nivolumab) and Yervoy (ipilimumab) or Opdualag (combination of nivolumab and relatlimab)

for the treatment of unresectable or metastatic melanoma [191–194]. In line with this, bispecific antibodies that simultaneously target multiple inhibitory immune checkpoints such as Cadonilimab, that has been developed to bind PD-1 and CTLA4, were designed to reverse acquired T cell anergy-mediated resistance and restore anti-tumor immunity [195]. Despite the encouraging and durable anti-tumor activity observed in some patients, the results of the latest clinical trials that investigate the efficiency of bispecific antibodies against dual checkpoints revealed that the objective response rate ranges from 25%−40% associated with unneglectable incidence of treatment-related adverse events [196–199]. The low response rate along with other challenges such as mutational burden, tumor heterogeneity and insufficient level of T cell activation limit the implementation of this approach in the clinical settings [195, 200]. Additionally, it is worth noting that, up to date, there are no clinical trials comparing the efficiency of combining two monoclonal antibody-based ICIs versus bispecific antibodies against the same targets.

#### *Epithelial-mesenchymal transition*

Epithelial-mesenchymal transition (EMT) is a cellular process during which the phenotypic features of epithelial tumor cells change dynamically toward the mesenchymal phenotype and result in functional changes in cell migration, invasion and metastasis [201]. Several studies have identified EMT as a mechanism of resistance to immunotherapy due to the potential of mesenchymal-like tumors to evade the immune response through inducing T cell exclusion in the TME, promoting resistance to cytotoxicity of effector immune cells and upregulating the expression of inhibitory PD-L1 on tumor cells [202–204]. Using murine organotypic tumor spheroids, Sehgal et al. identified a subpopulation of immunotherapy persister cells that resisted anti-PD-1-mediated $CD8^+$ T-cell reinvigoration resulting in immune escape and resistance to PD-1 blockade [205]. This pre-existing subpopulation of cancer cells was shown to express Snail1 and Sca-1 that are associated with the hybrid epithelial-mesenchymal state and hematopoietic and tissue stem cells, respectively. Another study utilizing melanoma model demonstrated the involvement of SOX2 (transcription factor associated with EMT) in promoting resistance to $CD8^+$ T cell-mediated cytotoxicity and attenuating the sensitivity to immunotherapy. Moreover, it was suggested that mesenchymal tumors are associated with greater infiltration of immunosuppressive cells, more excluded $CD8^+$ T cells and elevated resistance to ICIs [205]. The exact mechanism by which EMT contribute to immunosuppression and therapy resistance remains unclear; however, recent studies collectively highlight the EMT as a potential target for ameliorating resistance to ICIs and improving the overall therapeutic outcome.

#### *Angiogenesis*

Angiogenesis plays an essential role in the process of tumor development and metastasis in solid tumors. Within the TME, several growth factors and receptors have been associated with angiogenesis including hypoxia-inducible factor (HIF), vascular endothelial growth factor (VEGF), VEGF receptor, basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor-α (TGF-α) and platelet-derived growth factor (PDGF) with VEGF family receiving more attention as a key driver for angiogenesis in tumor tissues [206]. Some studies suggested that VEGF promotes resistance to ICIs through promoting the immunosuppressive nature of the TME [206, 207]. It was shown that VEGF has the potential to enhance the expression of Fas ligand in endothelial cells which inhibit the functional activity of cytotoxic $CD8^+$ T cells [208]. This comes in addition to its ability to promote the inhibitory function of intratumoral immunosuppressive cells, namely MDSCs, Tregs and TAMs, and inhibit the maturation of dendritic cells [209, 210]. Moreover, role of VEGF in upregulating the expression of inhibitory immune checkpoint molecules, including PD-1, CTLA-4, LAG-3, TIM-3 and PD-L1, on tumor cells and intratumoral immune cells was documented [211–213]. Due to its immunosuppressive role in the tumor context, targeting VEGF enhanced the efficacy of ICIs among different cancer types [214–216].

#### *Gut microbiome*

A growing body of literature documents the relationship between gut microbiome and cancer development and progression, along with its potential to modulate the antitumor immune responses and influence the efficacy of different immunotherapeutic agents [217–219]. Other intratumoral, circulating and oral microbiota play undeniable role in modulating the TME and determining the response to ICIs in different cancer types, however, not sufficiently investigated [220]. The effect of gut microbiome on ICIs efficacy has been validated in several pre-clinical and clinical settings, in which the diversity and composition of the gut microbiota differs between responding and non-responding patients [221–225]. Gopalakrishnan et al. demonstrated that melanoma patients responding to anti-PD-1 immunotherapy have "favorable" gut microbiome with high diversity and relative abundance of *Faecalibacterium* and *Ruminococcaceae* accompanied with increased antigen presentation potential, improved function of effector T cells and augmented systemic and anti-tumor immune responses [224]. On the other hand, non-responding patients were

shown to have "unfavorable" gut microbiome with low diversity and high relative abundance of Bacteroidales along with limited intratumoral immune cell infiltration, compromised antigen presentation capacity and impaired overall immune responses [224]. Another study of metastatic melanoma examined the patient's gut microbiota composition before the initiation of anti-PD-1 treatment and concluded that bacterial species including *Enterococcus faecium, Collinsella aerofaciens,* and *Bifidobacterium longum* where markedly enriched in patients who responded favorably to the treatment [226]. Other species namely *Ruminococcus obeum* and *Roseburia* intestinalis were shown to be considerably abundant in the gut microbiome of non-responding patients [226]. The relative abundance of *Akkermansia muciniphila* and *Bifidobacterium breve* in the gut microbiome was correlated with beneficial responses to anti-PD-1 treatment in epithelial tumors and NSCLC, respectively [225, 227, 228]. Moreover, it was demonstrated that baseline gut microbiota enriched with Firmicutes including butyrate-producing bacterium L2–21, *Faecalibacterium prausnitz*ii L2–6, and *Gemmiger formicilis* ATCC 27749 were associated with beneficial responses to anti-CTLA-4 compared to initial microbiota enriched with Bacteroidetes [222]. A precedented study demonestrated that the efficacy of anti-CTLA-4 favored the outgrowth of distinct Bacteroides species namely *B. thetaiotaomicron* or *B. fragilis* that poses anti-cancer properties but not tolerogenic Bacteroides [229]. They also demonstrated that supplementation of germ-free or antibiotic-treated mice with these bacterial species enhanced the efficacy of anti-CTLA-4 treatment [229]. In addition to its role in the primary resistance, the composition of gut microbiome can also result in the acquired resistance. For example, the immune-related adverse events post treatment and antibiotic consumption could disrupt the gut microbiome and account for secondary resistance to ICIs [225, 230]. Immune cells were shown to play a critical role in altering the intestinal microbiota colonization and influence the anti-tumor immune response and ICIs efficacy. Goc et al. revealed that the crosstalk between Group 3 innate lymphoid cells and T cells through MHC II is essential to support microbiota colonization and subsequently promoting type 1 immunity in the TME [231]. This, in turn, was associated with shaping the anti-tumor immune response and sensitivity to ICIs in colorectal cancer. Overall, the impact of gut microbiota on the treatment efficacy has been demonstrated in different pre-clinical and clinical settings; however, more research is still needed to determine the exact mechanisms underlying its role in modulating the anti-tumor immunity and determining the sensitivity to immunotherapy. The increased understanding of the crosstalk between the gut microbiome and anti-tumor immunity highlighted the potential of modulating the gut microbiota in order to augment the anti-tumor immune response and alleviate resistance to ICIs. In line with this, the improved efficiency of ICIs following gut microbiome alteration, through the oral administration or fecal microbiota transplantation, was demonstrated in multiple pre-clinical studies [226, 229]. This, in turn, has paved the way for clinical studies that aim to evaluate the potential of improving the anti-tumor immune response and enhancing the therapeutic outcome of ICIs through fecal microbiota transplantation [232, 233]. In one study of refractory metastatic melanoma patients, fecal microbiota transplantation resulted in beneficial changes in the intratumoral immune cell infiltrates and gene expression profiles and this has been translated into favorable clinical responses in 3 out of 10 treated patients [232, 233]. Another study of refractory melanoma (phase I trial) demonstrated a promising overall response rate of 65% including 20% complete response following combined treatment with anti-PD-1 and fecal microbiota transplantation [234]. Other clinical trials that evaluate the potential of modulating the gut microbiome as a mean to enhance the therapeutic outcome of immunotherapy are summarized elsewhere by Kang et al. [235].

#### *Other host systemic factors*

Several lines of evidence have documented that various systemic host factors contribute to shaping the overall immune response and influence the sensitivity to cancer immunotherapies [141, 236, 237]. In addition to the previously discussed factors, some studies have demonstrated that diet and physical activity could influence the responsiveness to ICIs. For example, high-fiber diet and exercise were linked to beneficial responses to ICIs due to their ability to increase the diversity of gut microbiome and induce the enrichment of short chain fatty acids [238–240]. Additionally, it has been suggested that ketogenic diets have the potential to alter the intratumoral immune cell compartment, interfere with the immune-inhibitory process and, therefore, render the TME more conducive to beneficial responses with ICIs [241]. Although obesity has been implicated in tumor progression and immune cell dysfunction in several studies, others of melanoma, NSCLC and other solid tumors claimed that obesity could account for better therapeutic potential of ICIs and improved overall survival in both tumor-bearing mice and cancer patients [240, 242–244]. Interestingly, this effect was more prominent among men compared to women [240]. Furthermore, it is documented that sex hormones could influence both the systemic and anti-tumor immune responses [245]. In this context, some studies suggested that men have

higher susceptibility to malignancies but more favorable responses to ICIs [246–248]. This could be attributed to the lack of partially exhausted PD-1$^{high}$/CTLA-4$^{+}$ CD8$^{+}$ cells in women compared to men, lower levels of PD-L1 expression in addition to the potential of estrogen to promote the accumulation of immunosuppressive TAMs and indirectly inhibit T cell function [249–252].

## Conclusions and future perspectives

In the last decade, immunotherapeutic strategies have shown unprecedented and magnificent success in treating different malignancies becoming a major pillar of cancer treatment. Despite the promising and durable results, the clinical application of immunotherapeutic agents still poses significant challenges including the low response rate among treated patients, the dynamic and heterogenous nature of tumor tissues in addition to the complexity of the anti-tumor immune responses. Extensive efforts have been made to identify and understand the mechanisms that could underlie resistance to ICIs, in different pre-clinical and clinical settings, taking into account the multimodal interactions between host-related factors, tumor genetics in addition to immune and non-immune components of the TME and others are still under active investigations. Herein, the mechanisms of resistance are classified into two main categories namely tumor-intrinsic and tumor extrinsic mechanisms. These mechanisms could be primary or acquired post a beneficial response to the treatment and both patterns of resistance can occur at any point along the CI cycle. It is worth appreciating that either a single mechanism or multiple mechanisms combined could drive the resistance to ICIs and result in poor therapeutic outcome. Overall, the improved understanding of the resistance mechanisms facilitate identifying predictive biomarkers that aid in selecting patients who are more likely to respond favorably to the treatment. It also pave the way toward targeting these mechanisms utilizing adjuvant treatments such as chemotherapy, radiotherapy and targeted therapy in combination with ICIs. Such combination strategies demonstrated considerable success in overcoming resistance and enhancing the therapeutic potential of ICIs across multiple types of cancer in different pre-clinical and clinical models.

### Abbreviations

| | |
|---|---|
| ICIs | Immune checkpoint inhibitors |
| CAR | Chimeric antigen receptor |
| PD-1 | Programmed cell death protein 1 |
| PD-L1 | Programmed cell death ligand 1 |
| CTLA-4 | Cytotoxic T lymphocyte-associated antigen 4 |
| FDA | Food and Drug Administration |
| LAG-3 | Lymphocyte-activation gene 3 |
| TIGIT | T cell immunoreceptor with Ig and ITIM domains |
| ORR | Objective response rate |
| MSI | Microsatellite instability |
| TME | Tumor microenvironment |
| CI | Cancer-Immunity |
| APCs | Antigen-presenting cells |
| TCR | T cell receptor |
| TIM-3 | T cell immunoglobulin and mucin domain 3 |
| VISTA | V-domain Ig suppressor of T cell activation |
| TMB | Tumor mutational burden |
| HNSCC | Head and neck squamous cell carcinoma |
| NSCLC | Non-small cell lung carcinoma |
| TAP | Transporter for antigen presentation |
| β2M | Beta-2 microglobulin |
| MMR | Mismatch repair |
| MAPK | Mitogen-activated protein kinase |
| PTEN | Phosphate and tensin homolog |
| IDO | Indoleamine 2,3-dioxygenase |
| Tregs | Regulatory T cells |
| MDSCs | Myeloid-derived suppressor cells |
| YAP | Yes-associated protein |
| EZH2 | Enhancer of zeste homologue 2 |
| H3K27me3 | Histone H3 lysine 27 trimethylation |
| DNMT1 | DNA methyltransferase 1 |
| STING | Stimulator of interferon genes |
| TAMs | Tumor-associated macrophages |
| TGF-β | Transforming growth factor-β |
| BTLA | B and T lymphocyte attenuator |
| EMT | Epithelial-mesenchymal transition |
| HIF | Hypoxia-inducible factor |
| VEGF | Vascular endothelial growth factor |
| bFGF | basic fibroblast growth factor |
| TGF-α | Transforming growth factor-α |
| PDGF | Platelet-derived growth factor |

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### Authors' contributions

Conceptualization- BHA, BRA and EE; Investigation, original draft writing and visualization- BHA; review and editing- BHA, BRA and EE; Supervision- BRA and EE; All authors have read and agreed to the published version of the manuscript.

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### Data availability

No datasets were generated or analysed during the current study.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

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### Competing interests

The authors declare no competing interests.

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