Mechanisms of resistance to immune checkpoint inhibitors in cancer

Tianshu Pan*

Department of Biological Chemistry University of Michigan School of Medicine Ann Arbor, MI USA

*Corresponding author: tshupan@umich.edu

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Abstract: Immune checkpoint inhibitors have shown promising clinical activity in various types of cancer and have improved the overall survival of patients in the past decades. However, the resistance to immune checkpoint inhibitors remains a challenging problem today and has been studied in different aspects. This review gave an overview of the current status of the resistance to immune checkpoint inhibitors in clinical treatments and the underlying mechanisms of drug resistance based on different stages of T cell immune cycle. Several possible solutions of the resistance to immune checkpoint inhibitors towards the future development of immune checkpoint inhibitors in cancer treatment were also discussed.

1. Introduction

As the second leading cause of death, cancer remains a main threat worldwide to human health. In 2018, there were about 18.1 million new cases and 9.6 million deaths in the world [1]. 6.9 million new cancer cases in patients older than 80 years were estimated by the time of 2050 [2]. The urgent demand of treating cancer led to the development of various strategies including immunotherapy. As one of the most promising immunotherapy options of cancer to date, Immune checkpoint inhibitors (ICIs) have achieved impressive therapeutic performances in clinical applications and prolonged the overall survival of patients with melanoma, non-small cell lung cancer (NSCLC) and other types of carcinomas [3]. However, commonly observed resistance to cancer patients receiving ICIs severely limited the treatment outcome of non-responders and led to greater occurrence of relapse. Only 33% of 655 patients with melanoma showed responses to pembrolizumab, an antibody to programmed cell death 1 receptor (PD-1). Moreover, 70–80% of the initially responding patients maintained their responses at 3 years [4]. Plenty of factors were indicated to be related to resistance generation, but many of them remain unclear. To achieve better understanding of this tumor tolerance, this review summarized the underlying mechanisms related to the resistance to ICIs and several possible ways to improve their clinical responses.

2. Main body

2.1 Immune Check Point Pathways

The immune system plays an essential role in controlling the development of cancer by recognition and T cell-mediated elimination of tumor cells. During the process of immunosurveillance, naïve T cells receive tumor antigens from antigen presenting cells (APCs) by combination of T cell receptor (TCR) and major histocompatibility complex (MHC) [5]. After that, further activation of T-cell necessitates various additional co-stimulatory signals including the B7: CD28 combination which contributes to cell growth, survival as well as differentiation to effector T cells. However, co-inhibitory signals also exist for protecting normal cells from autoimmune attack, inflammation, and tissue damage [6]. Various inhibitory immune checkpoint pathways, which serve as negative regulators of T cell proliferation, function and immune responses, provide a special escape mechanism for tumor cells to evade from the elimination by cytotoxic effector T cells. Among these immune checkpoints, the

cytotoxic T-lymphocyte–associated molecule 4 (CTLA-4) and PD-1 are two of the most essential pathways that have been broadly investigated in the past decades. CTLA-4 and CD28 are homolog receptors that share two same ligands, B7-1 (CD80) and B7-2 (CD86). The binding of these ligands to CTLA-4 leads to immunosuppressive reaction instead of T cell stimulation [7]. During the process of T cell activation, receiving positive signals from CD28, and TCR triggers enhanced translocation of CTLA-4 from intracellular vesicles to the cell surface, resulting in inhibitory signaling restraining IL-2 generation and cell survival [8]. PD-1 is also an immunosuppressive receptor found on the surface of various immune cells [6]. Binding of PD-1 to programmed death ligand 1 (PD-L1) leads to reduced production of interferon-γ and IL-2 and exhaustion of effector T cells [9]. The expressing of B7 and PD-L1 on the surface of tumor cells results in the suppressed anti-tumor immune response and T cell activation. And the immune checkpoint inhibitors on these targets of immune checkpoint pathway were discovered and proved to be a promising option to improve cancer treatment [10].

2.2 Clinical Response of ICIs and Resistance to ICIs

Immune checkpoint inhibitors, basically monoclonal antibodies, prohibit tumor escaping from T cell-mediated elimination by specific binding and blocking specific receptors

Table 1. ICIs in clinical trials.

Trial	Cancer	Agent(s)	Enrollment	OS	PFS	ORR	AEs
KEYNOTE-042 [17]	NSCLC	Pembrolizumab	128	NA	14.3	NA	19.50%
		Chemotherapy	134	NA	8.9	NA	68.80%
KEYNOTE-024 [19]	NSCLC	Pembrolizumab	154	6 (80.2%)	10.3	44.80%	26.60%
		Chemotherapy	151	6 (72.4%)	6	27.80%	53.30%
KEYNOTE-598 [18]	NSCLC	Pembrolizumab +Ipilimumab	284	21.4	8.2	18%	62.40%
		Pembrolizumab +Placebo	284	21.9	8.4	17%	50.20%
Lung-MAP S1400I [22]	NSCLC	Nivolumab/Ipilimumab	125	10	3.8	18%	39.50%
		Nivolumab	127	11	2.9	17%	33.30%
KEYNOTE-006 [15]	Melanoma	Pembrolizumab	279	12 (74.1%)	3.4	33.70%	13.30%
		Ipilimumab	278	12 (58.2%)	2.8	11.90%	19.90%
CheckMate 067 [16]	Melanoma	Nivolumab	316	NA	6.9	43.70%	16.30%
		Ipilimumab	315	NA	2.9	19%	27.30%
		Nivolumab + Ipilimumab	314	NA	11.5	57.60%	55%
KEYNOTE-061 [23]	Gastric Cancer	Pembrolizumab	257	NA	17.8	46.7%	NA
		Chemotherapy	257	NA	3.5	16.70%	NA
Motzer et al. [20]	RCC	0.3 mg/kg Nivolumab	60	18.2	NA	20%	11%

		2 mg/kg Nivolumab	54	25.5	NA	22%	NA
		10 mg/kg Nivolumab	54	24.7	NA	20%	NA
CheckMate 025 [21]	RCC	Nivolumab	406	25	NA	25%	19%

OS: overall survival, PFS: progression-free survival, AEs: adverse events, NA: not available, ORR: objective response rate, RCC: Renal cell carcinoma, NSCLS: non-small cell lung cancer.

in co-inhibitory signaling pathways to achieve net positive signal and thus reactivate T cells to kill tumors. Immunotherapies with FDA-approved immune checkpoint inhibitors include Pembrolizumab, Ipilimumab and Nivolumab have shown promising clinical results and therapeutic effects on treating metastatic melanoma, NSCLC, ovarian cancer and other types of carcinomas [11]. The clinical responses, resistance to drugs, efficacy and safety issues of these immune checkpoint inhibitors in various malignances have been examined by plenty of result-posted or ongoing experiments over decades, as shown in TABLE 1 about data in several clinical trials. Ipilimumab, as the first FDA-approved immune checkpoint inhibitor for melanoma [12], works as a CTLA-4 antagonist that breaks the self-tolerance of cellular immunity. Its utilization in melanoma prolonged the long-term survival rate of a remarkable proportion of patients, as shown in a pooled analysis on data for several studies include 1861 patients treated with ipilimumab 3 mg/kg or 10 mg/kg which indicated overall survival for 11.4 months and 20% patients demonstrated limited tumor progression for 5-10 years after the treatment [13]. And Ipilimumab treatment improved median overall survival rate to 23.5% at 2 years [14]. However, significant evidences of low response to Ipilimumab were also observed in several monotherapy trials, suggesting the resistance that prevented Ipilimumab from functioning in some cases. For example, KEYNOTE-006 phase III trial, the median progression-free survival for Ipilimumab-receiving group was only 2.8 months with an extremely low response rate of 11.9% [15]. A similar resistance to durable control of advanced melanoma could be indicated in Ipilimumab treatment alone group of a CheckMate 067 trail with merely 19% of 315 patients showed discernible responses [16]. Same problems exist on PD-1 inhibitors, Pembrolizumab and Nivolumab. Although Pembrolizumab delivery significantly increased the progression-free survival of 128 patients with NSCLC from 8.9 mouths of chemotherapy to 14.3 months in a KEYNOTE-042 phase III trial [17], the objective response rate of Pembrolizumab only reached 17% in 284 patients in another trial KEYNOTE-598 [18]. It is worth mentioning that, under similar trial conditions, the results from KEYNOTE-024 [19] revealed a much higher clinical response activity of Pembrolizumab (44.8%) than in KEYNOTE-598 (17%) [15]. However, the median duration of the KEYNOTE-024 trial was not available, making it difficult to evaluate the continued duration of tumor control of Pembrolizumab [19]. And the observed remarkable response rate (44.8%) in KEYNOTE-024 trail of Pembrolizumab may not be accurate considering the 27.8% response rate for chemotherapy that was also high in this trial [19]. The clinical outcome of Nivolumab surpassed Ipilimumab in CheckMate 067 trial comparing the PFS (6.9 versus 2.9), ORR (43.7% versus 19%) and AEs between these two ICIs, suggesting a possible higher efficacy of Nivolumab in melanoma treatment [16], despite the early cut-off time point of ORR data. In the renal cell carcinoma cases of a CheckMate 025 trial, the long-term survival of patients was risen to 25 mouths by Nivolumab with a dose-independent [20] low response rate of 25% [21]. The current ICIs especially PD-1 antibodies Nivolumab and Pembrolizumab showed their great potential for prolonging the life of patients with melanoma, NSCLC, renal cell carcinoma, gastric cancer and several other types of tumors with shared challenges of response deficiency in some patients due to complicated resistance mechanisms that remain to be further understood [22, 23].

2.3 Mechanisms of Resistance

Inhibited immune response and tumor resistance of ICIs have been studied in different aspects. Since the therapeutic effect of ICI depends on activation of cytotoxic T cells and their action on tumor, interference on T cell immune cycle (including tumor antigen releasing, recognition and presentation,

activation, specific targeting of T cells and tumor killing) may lead to suppressed anti-tumor response to ICIs.

2.3.1 Impaired antigen presentation and T cell activation

Normally APCs receive tumor antigens and present to CD8+ T cells to generate positive signals for tumor elimination. The impaired formation of sufficient neoantigens, which has strong association with tumor mutational burden (TMB) and non-synonymous mutations, was proved to be one of the reasons for restrained cancer immunity [24]. It was shown that high tumor mutational load is closely related to higher survival rate of patient receiving ICIs therapies [25]. And a meta-analysis study based on PD-1 or PD-L1 antibody treatment indicated a notable correlation between high TMB and high ORR to ICIs [26]. A clinical trial treating colorectal cancer patients with pembrolizumab showed obvious association between high response to PD-1 inhibition and mismatch-repair deficiency [27]. This deficiency may lead to increased non-synonymous single nucleotide mutations, enhanced production of neoantigens, and eventually promote the response to ICIs [27]. Dendritic cells (DC), the essential APCs for antigen presenting to activate T cells after accumulating in lymph node, require full maturation to play their roles in cancer immunity [28]. A study in 2008 found that the reactive oxygen species (ROS) oxidation of high-mobility group box-1 protein (HMGB1) stopped the DC maturation, which could be a potential mechanism of tumor interfering on immune response [29]. Moreover, high level of interleukin-10 (IL-10), indoleamine 2,3-dioxygenase (IDO), IL-35, overexpression of vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β) in the tumor microenvironment (TME) also block the maturation of DCs. They trigger the transformation into regulatory DCs, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) which further inhibit T cells and DCs by releasing more immunosuppressive factors [30-32]. For instance, arginase I (ARG1) in the TME produced by regulatory CD11bhighIalow DCs showed significant inhibition on T cell proliferation in lung tumor tissue [33]. During activation process in TME, T cells face the challenges on not only prominent co-inhibitory checkpoint signaling including CTLA-4 and PD-1 from tumor, but also the lack of co-stimulatory stimulations from APCs that may lead to insufficient production of IL-2, MHC and impaired T-cell priming.

2.3.2 Impaired T cell trafficking and infiltration

The proper trafficking of functional effector T cells to tumor tissue is essential for the efficacy of tumor killing and the clinical outcome of ICI treatments. It was reported that C-X-C motif chemokine receptor 3 (CXCR3) expressed on the cell surface of cytotoxic T cells plays an important role in assisting infiltration of T cells into tumor tissue [34]. However, reduced activity of interferon signaling pathway in TME results in decreased production of IFN-γ and thus the down-regulation of CXCR3 and its ligand, leading to low response to immunotherapy [35]. Additionally, an angiogenic factor basic fibroblast growth factor (bFGF) released by tumor cells significantly interfered T cell adhesion on endothelial cells of RCC by reducing the Intercellular adhesion molecule 1 (ICAM-1) [36]. This observation suggested a potential role of tumor angiogenesis in preventing T cell infiltration and tumor tolerance [36]. A study treated mice with CAR-T cells with antibodies targeting on receptor of angiogenic factor VEGF (VEGFR-2) dramatically restrained the size of melanoma, prolonged OS of mice, promoted T cell penetrating and maintained T cell accumulation in tumor tissue [37]. These results indicated a complicated VEGF-mediated regulation of tumor tolerance to T cells, including repression on T-cell adhesion and infiltration, triggering lymphocyte apoptosis, impairing DC maturation and inducing angiogenesis. After passing through endothelial cells into tumor tissue, lymphocytes are still under pressure in TME filled with inhibitory chemokines and cancer-associated fibroblasts (CAFs) [38]. Immunostaining on lung tumor slides revealed impeded T cell infiltration from matrix to the very central area of tumor, and decreased extracellular matrix was correlated with higher level of lymphocyte accumulation [39]. Consistent with this observation, the combination between chemotherapy and drug on fibroblast activation protein (FAP) significantly repressed collagen generation and tumor growth by inhibiting CAFs [40]. And the inhibitor on CAFs by binding

CXCL12 receptor induced remarkable T cell infiltration and suppressed pancreatic carcinoma together with anti-PD-L1 immunotherapy [41].

2.3.3 Impaired T cell function on tumor

Mutations in the pathways of various immune-associated factors could disable T cell recognition and killing of tumor cells. Alteration in antigen-presenting beta-2-microglobulin (B2M) led to decreased major histocompatibility complex (MHC) class I expression on the cell surface. The down-regulated MHC expression may inhibit both the antigen presentation and recognition of malignant tumors by cytotoxic T cells [42]. Coincidently, down-regulation of MHC could also be observed in malignant cells with increased levels of alterations in human leukocyte antigen (HLA) genes [43]. An exome sequencing analysis found that the expression level of HLA genes is proportional to that for T cell-related genes. This observation suggested a potential mechanism of obtained resistance due to accumulated tumor cells that survived T cell elimination through their mutations on MHC class I [44]. It should also be noticed that the binding of tumor-produced soluble ULBP2 and soluble MICA to their receptor NKG2D may block the activation of NK cells [45]. This down-regulation of NKG2D on the tumor-penetrating NK cells and effector T cells in tumor patients may lead to restrained elimination of tumor cells lacking MHC class I, and further impaired recognition of tumor cells by lymphocytes and promoted escape from immunosurveillance. Several immunosuppressive receptors also control the function of effector CD8+ T cells. The lymphocyte-activation gene 3 protein (LAG-3), a co-inhibitory immune checkpoint receptor, has an important role in preventing autoimmunity through facilitating T cell exhaustion and reducing the activity of APCs [46]. The inhibition on LAG-3 significantly improved the tumor-killing efficacy of anti-PD-1 therapy as well as the survival of mice with fibrosarcoma [47]. T cell immunoglobulin mucin 3 (TIM-3), T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) and Fc receptor-like 6 (FCRL6) also serve as co-inhibitory signals to suppress tumor-infiltrating T cell function. It was reported that up-regulated TIM-3 expression caused a remarkable reduction in release of IL-2 and IFN-γ [48]. The inhibition of TIM-3 led to the restored production of cytokines of tumor-infiltrating T cells and reduced tumor growth in mice [48]. And treatment with antibodies targeting TIGIT was found to be effective in improving tumor suppression and cytotoxic T cell activity together with PD-L1 inhibitors [49]. FCRL6, similar to LAG-3, suppresses APCs, NK cells and antitumor T cells by binding to MHC class II [50]. Tumors with overexpression of FCRL6 showed significantly increased tumor evasion of immunosurveillance induced by PD-1 antibody, suggesting a potential role of MHC class II signaling in low response to ICI immunotherapy [51]. Additionally, the genetic alterations in Janus kinase 1/2 (JAK 1/2) signaling pathway induced by IFN-γ may lead to blocked STAT1 activation and thus resistance to tumor apoptosis as well as ICIs. It was indicated that mutations with dysfunction in JAK 1/2 signaling pathway, observed in 1 out of 23 patients, were associated with restrained response to anti-PD-1 treatments [52]. However, another study suggested that strong IFN signaling reduced the responses to anti-CTLA4, indicating a complicated mechanism of JAK 1/2 in tumor resistance that requires further investigations [53]. PTEN deficiency was found in about 30% of patients with melanomas [54]. It resulted in the production of immunosuppressive cytokines, activation of PI3K-AKT pathway, impaired T-cell infiltrating into tumors, inhibited autophagy and restricted tumor death in response to PD-1 blockade [54]. An interesting study on genome-scale chromatin changes during T cell differentiation under tumorigenesis revealed two dysfunctional chromatin states closely associated with the expression of surface proteins, CD101 and CD38. The tumor-specific CD8+ T cells with functional exhaustion may be in a fixed dysfunctional state after the initial plastic state, leading to non-responding exhaustion, relapse and resistance to reprogramming [55]. Besides deficiency of cytotoxic T cell production and activity, impaired T-cell memory generation was also proved to be responsible for the loss of response to ICIs. Proliferated effector memory T cells in tumors were observed in patients with clinical response to PD-1 blockade instead of the non-responders [56].

2.4 Strategies against resistance to ICIs

ICI treatment remains to be improved on many aspects in the future. Effective tumor targeting and infiltrating through tumor microenvironment could contribute to controlling serious adverse effects and toxicities due to off-target effects of ICIs. Delivery strategies including nanoparticle-mediated specific delivery of drugs and biomaterial scaffolds implanted into body have become research hotspots. The liposomal delivery system, for example, is based on lipid layer or bilayer conjugated with various of targeting antigens surrounding an aqueous core [57]. Due to its unique bio-affinity and flexibility in nanoparticle size and in modifications of surface structure, it has the potential to carry anti-tumor drug and achieve promising trafficking to tumor. It was reported that a novel liposomal delivery system incubated with doxorubicin and PD-1 antibody significantly inhibited tumor growth in mice [58]. And discovering new tumor targeting antigens in the future could further improve the targeting of ICIs and this nanoparticle carrier to tumor. For instance, aptamers like prostate specific membrane antigen (PSMA) improved targeting to prostate tumor in a siRNA delivery system [59]. Another antigen internalizing Arg-Gly-Asp peptide (iRGD) binding to integrin in tumor microenvironment has an essential role in tissue penetration through barriers into tumor parenchyma [60]. Various possible combinations between ICIs and other therapies remain to be investigated due to their potential in minimizing single drug resistance. A combination therapy with mitogen-activated protein kinase inhibitor and PD-L1 antibody induced much more significant tumor suppression in mice than either single agent treatment [61]. Another potential improvement of ICI clinical responses is by combinational ICI therapy with anti-angiogenic agents. Inhibitions on expression of angiogenic molecules like VEGF in TME promote the DC maturation and T cell penetration into tumor. Application of anti-VEGF antibody bevacizumab together with pembrolizumab achieved 56% of ORR [62]. The combination between bevacizumab and ICIs were shown to be promising in prolong the OS and response in some phase 1 studies [63, 64]. An observation in a phase 1 trial is that the utilization of axitinib (a VEGF receptor tyrosine kinase inhibitor) achieved impressive ORR of 73% and PFS of 20.9 months [65]. However, further examinations with more patients enrolled are still needed to confirm the safety and efficacy of these combinations. Similarly, the inhibition on many other available targets in inhibitory pathways of immune cycle may be effective against weakening resistance to ICIs. Targeting on LAG-3 is expected to suppress tumor growth by blocking the inhibitory signaling in effector T cells. Several ongoing phase 1 clinical trials ((NCT01968109 and NCT02460224) are trying to test the efficacy and safety of anti-LAG-3 antibody. An anti-TIM-3 drug Sym023 is being tested in a phase 1 trial (NCT03489343) to confirm its potential in treating metastatic cancer. The inhibitors on other targets like B7, TIGIT, TGF-β, JAK, IDO and CD47 also hold great promise of facilitate anti-tumor immune responses to ICIs and they are still in clinical trials. Though diverse combinations of multiple targets inhibiting are likely to increase overall response to immunotherapy, constantly observed immune-related adverse effects (irAEs) and toxicities during anti-CTLA4 or anti-PD1/PDL1 medications must be taken into consideration. Adverse events including vitiligo, pruritus, colitis and especially diarrhea were reported in 58.2% of patients treated with ipilimumab [14]. In a disproportionality analysis, respiratory, endocrine and hepatic disorders were identified to be the main toxicities emerged from patients with anti-PD1/PDL1 monotherapy [66]. Another trend for the development of ICIs and other immunotherapies is personalized therapeutic strategy based on discovering new effective biomarkers for predicting clinical responses. Assay on tumor infiltrating lymphocytes (TIL) was shown to be effective in predicting PD-1 blockade response of patients with squamous cell carcinoma [67]. Detecting CD8+ TIL density by immunostaining microscope analysis was proved to be effective in forecasting survival rate of patients with NSCLC [68]. High tumor mutational burden tested by whole-genome sequencing could also be a biomarker for immunotherapies such as combination therapy with ipilimumab and nivolumab [69]. The close association between up-regulated MHC class II production and restricted resistance to anti-PD-1 drugs suggested the potential of IFN γ signature in becoming a promising biomarker for ICI therapy [70]. Combination of different biomarker detections is beneficial to systematically evaluating activity of immune cells and TME situation.

3. Conclusion

Immunotherapies with different ICIs have achieved remarkable successes in cancer treatment. However, commonly observed initial or acquired resistance to ICIs remains a challenge to be solved. In this review, the status of the resistance in ICIs clinical application was discussed. The main findings focusing potential mechanisms of tumor resistance associated with different stages of anti-tumor immune cycle were also summarized, followed by the presentation of several potential strategies to improve clinical response to ICIs. By further understanding, the complicated roles of various TME immunosuppressive cells and co-regulatory signal pathways in the generation of resistance to ICIs, next-generation ICI therapy with improved specificity, efficacy and safety could be developed. Based on new targets, predictive biomarkers and comprehensive clinical data about immune tolerance, combinational, precise and personalized medications could be provided to cancer patients to achieve longer survival, better responses and even the goal to cure a majority of carcinomas in the future.

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