Mechanisms of resistance to immune checkpoint inhibitors and strategies to reverse drug resistance in lung cancer

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Abstract
In recent years, the research of immune checkpoint inhibitors has made a great breakthrough in lung cancer treatment. Currently, a variety of immune checkpoint inhibitors have been applied into clinical practice, including antibodies targeting the programmed cell death-1, programmed cell death-ligand 1, and cytotoxic T-lymphocyte antigen 4, and so on. However, not all patients can benefit from the treatment. Abnormal antigen presentation, functional gene mutation, tumor microenvironment, and other factors can lead to primary or secondary resistance. In this paper, we reviewed the molecular mechanism of immune checkpoint inhibitor resistance and various combination strategies to overcome resistance, in order to expand the beneficial population and enable precision medicine.

Keywords: Immune checkpoint inhibitors; Resistance; Mechanism; Combination therapy

Introduction
Lung cancer is the leading cause of cancer-related death worldwide. Currently, immune checkpoint inhibitors (ICIs) targeting programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) axis have become the most promising treatment in several kinds of cancer, especially in lung cancer. Although an unprecedented durable response rate has been observed in immunotherapies, the majority of patients do not benefit from the treatment and some patients relapse after a period of response. According to the most typical concept of practicing clinicians, mechanisms of resistance are divided into primary, adaptive, and acquired resistance. Primary resistance is defined as a clinical condition in which the tumor does not respond to immunotherapy strategies. The mechanism underlying the lack of response to immunotherapy may include adaptive immune resistance. Considering the evolutionary nature of immune/cancer cell interactions, this may be manifested as primary resistance, mixed reaction or acquired resistance. The definition of acquired resistance is a clinical scenario where a tumor initially responds to immunotherapy but recurs and progresses over time. At present, data are lacking about mechanisms involved in resistance to ICIs. Enhanced understanding of molecular and immunologic mechanisms of ICI response (and resistance) will not only identify novel biomarkers for prediction and/or prognosis, but ultimately guide optimal clinical combination of ICI. Here, we review the emerging data identifying novel mechanisms of innate and acquired resistance to ICI [Figure 1] and strategies to overcome cancer resistance.

Mechanisms of Resistance to ICIs

Primary and adaptive resistance

Antigen presentation and recognition

The most immediate cause of the tumor’s non-response to ICIs is lack of recognition by T cells because of absence of tumor antigen. The process of distinguishing tumor cells from normal cells depends on T-cell recognition of tumor-specific or tumor-associated antigens. Clinical studies show that neoantigens delivered through dendritic cells (DCs) or other means induce potent antigen-specific T cell response and favorable clinical responses in prostate cancer, melanoma, and glioblastoma patients. Combined with mass spectrometry and exome sequencing, the immunogenicity of neoantigens can be used to evaluate active T-cell responses.

Genetic instability due to alterations in DNA repair and replication genes can increase immunogenicity through high tumor mutational burden (TMB) with subsequent...
neoantigen formation. It was found that melanoma patients respond better to PD-1 therapy if tumor cells are enriched with BRCA2 mutations, which is an important homologous recombination DNA repair gene.\[13\] Alterations in additional DNA damage response genes have recently shown correlation with high TMB and improved clinical outcomes to ICIs in urothelial cancer.\[14\] Furthermore, tumors with deficiencies in DNA mismatch repair genes leading to microsatellite instability demonstrated high mutational burden with enhanced response to ICIs in a variety of tumors.\[15\]

Besides, antigen processing, presentation, and immune escape can also be affected by epigenetic modifications in tumor cells which change the expression of immune-related genes.\[16,17\] For example, histone deacetylase (HDAC) inhibitors have been reported to increase major histocompatibility complex (MHC) and tumor antigen expression, and shift gene expression to a pro-apoptotic milieu in cancer cells.\[18\] This suggests that reversing epigenetic modifications in tumor cells may enhance immune recognition and response.

**T cell priming and activation**

Abnormal Wnt/β-catenin signaling pathway can also lead to immunotherapy resistance.\[19\] High levels of β-catenin in mice were associated with reduced CD103+ DC in tumor microenvironment. The possible mechanism is that the abnormal WNT/β-catenin signaling pathway induces the expression of transcription inhibitor activating transcription factor 3, which inhibits the expression of CCL4 gene, a chemokine of CD103+ DC, thereby reducing the infiltration of CD103+ DC. The lack of antigen presenting cells (APCs) leads to the dysfunction of initial T cell activation and the decrease of infiltrating T cells, which ultimately affects the immune response. Among human melanomas shown to have a poorly infiltrated phenotype, those containing mutations affecting the β-catenin pathway lacked a CD103+ DC immune signature and were insensitive to anticancer immunotherapies.\[20\] In addition, the accumulation of CD103+ cross-presenting DCs in mouse tumors was shown to be dependent on the activation of intra-tumoral natural killer (NK) cells secreting the DC chemo-attractants chemokine (C-C motif) ligand (CCL) 5 and lymphotactin.\[21\] In several human-derived cancer cell lines, the presence of intra-tumoral CCL5 and lymphotactin transcripts is closely correlated with that of gene signatures of both NK cells and CD103+ DCs, and the presence of these cell populations is associated with favorable overall survival (OS).\[22\]

T cell specific antigen recognition provides the first signal of T cell activation, and the second signal comes from the interaction between the synergistic stimulus molecules expressed by APC and the corresponding receptors or ligands on the surface of T cells, the most important of which is the co-stimulatory molecule CD28-B7. Recent studies have shown that PD-1 inhibitor activated T cells still need the co-stimulation signal of CD28 to promote their proliferation and differentiation into killer T-cells.\[23\] Trials in mice found that blocking the interaction between CD28 and B7, or knocking out the CD28 gene, prevented T cells from responding to PD-1 treatment. The binding of B7 molecules on its surface with CTLA-4 can lead to the apoptosis of antigen-specific T cells, and the secretion of...
interleukin (IL)-10 induces T helper 2 type response, thus inducing antigen-specific immune tolerance.  

Many negative regulatory factors in tumor immune microenvironment, such as IL-10, vascular endothelial growth factor (VEGF), and transforming growth factor β (TGF-β), can drive the differentiation of many monocytes into M2-like tumor-associated macrophages (TAMs), which amongst their other suppressive actions, can also compete with local DCs for tumor antigens and consequently inhibit T cell priming. In addition, IL-10 and TGF-β can limit local T cell priming through the suppression of both DC function and the proliferative capacity of T cells. In addition, the TGF-β-driven activation of fibroblasts gives rise to a specific phenotype of immunomodulatory cancer-associated fibroblasts (CAFs). The release of TGF-β and IL-6, CAFs suppress the proliferation and trafficking capacity of antigen-presenting DCs, thereby interfering with tumor-directed T cell priming. In oral squamous cell carcinoma, tumor-secreted VEGF may promote the tumor immunologic escape by inhibiting the differentiation of immature DC from peripheral blood monocyte cells and increasing the levels of dysfunctional mature DC.  

**T cell trafficking and tumor infiltration**

Through the tight regulation of the local chemokine- and cytokine-gradient, CAFs also limit the attraction of T cells to the TME. Moreover, TGF-β CAFs can remodel the composition of the extracellular matrix (ECM), resulting in a dense ECM network that poses a physical barrier to T cell infiltration. Furthermore, CAFs can suppress the anti-tumor T cell response in the TME itself, through the up-regulation of immune checkpoint ligands on their cell surfaces.  

Chemokines regulate immune cell trafficking in tumors and are implicated in tumor development, progression, and angiogenesis. Most tumors shape local chemokine networks to promote their growth by recruiting stromal cells like TAMs, myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg), all associated with poor patient prognosis. Several recent studies have investigated the involvement of chemokines in T-cell recruitment to tumors. Tumor cells secrete ligands CCL5, CCL7, and C-X-C motif chemokine ligand 8, and bind to the receptor C-C motif chemokine receptor (CCR) 1 or C-X-C chemokine receptor 2 expressed on the MDSCs subtype to recruit MDSCs into the tumor microenvironment. Tregs selectively over-express the chemokine CCR4, and its specific ligand CCL12 is produced by tumor cells and tumor microenvironment to further recruit Tregs and suppress host immune response. In vitro experiments, anti-CCR4 monoclonal antibody inhibits Treg recruitment as well as promotes antibody-dependent cell-mediated cytotoxicity, further reducing the Treg population.  

In addition to promoting angiogenesis, VEGF functions as an immunosuppressive cytokine and is associated with resistance to ICIs. VEGF levels were found to be higher in anti-PD-1 therapy non-responders compared with responders. In mouse models, VEGF impeded commitment of lymphoid progenitors, reducing progression to the T-cell lineage. Additionally, VEGF signaling reduces trafficking and extravasation of cytolytic T lymphocyte (CTLs) into the TME while it promotes infiltration of Tregs through a selective endothelium. Furthermore, VEGF increases expression of inhibitory receptors, contributing to CTL exhaustion. Oncogenic signaling through the mitogen-activated protein kinase (MAPK) pathway results in the production of VEGF, IL-1, and IL-8, which inhibits the recruitment and function of T cells. BRAF gene is an important transduction factor of MAPK signaling pathway. In the mouse model, BRAF inhibitor significantly increased the amount of chimeric antigen receptor T cells (CAR-T)/T-cell receptor engineered T-cell infiltration and enhanced its anti-tumor effect.  

**T-cell killing activity within the tumor microenvironment**

The interferon-γ (IFN-γ) pathway is emerging as a key player in host immune response. It plays a dual role in anti-tumor immune response. IFN-γ is produced by tumor-specific T cells, it induces an effective anti-tumor immune response by the following ways: (1) its direct anti-proliferative and pro-apoptotic effects on tumor cells; (2) enhancing the expression of MHC and other molecules to increase tumor antigen presentation; and (3) recruiting other immune cells. However, continuous IFN-γ exposure can cause immunodeficiency of cancer cells, which leads to immune escape. One of the mechanisms by which tumor cells escape the effects of IFN-γ is to down-regulate the expression of or mutate related molecules involved in the IFN-γ signaling pathway, such as the downstream signaling molecules of the IFN-γ receptor: Janus kinase (JAK) 1/2 and the signal transducer and activators of transcription (STATs), and so on. It was reported that mutations or epigenetic silencing of molecules in this pathway lead to the loss of the anti-tumor effects both in cell line and animal models. Analysis of patients who did not respond to CTLA-4 antibody showed the high-frequency mutations of JAK2, IFN-γ receptor 1 and 2, interferon regulatory factor 1 (IRF1), and inhibited amplification of genes in this pathway, such as suppressor of cytokine signaling 1 and protein inhibitor of activated STAT 4. Mutations in this pathway also lead to the loss of PD-L1 expression upon IFN-γ exposure, resulting in tumor cells being genetically negative for inducible PD-L1 expression. In this case, it would be useless to block PD-L1 or PD-1 with antibodies. These patients may be the ones with initial resistance to anti-PD-1 treatment. Constitutively expressed inhibitory ligands such as PD-L1 on the surface of tumor cells may significantly inhibit the anti-tumor T cell response. Several studies have revealed a correlation between loss of phosphatase and tensin homolog (PTEN) in cancer and the up-regulation of PD-L1, implicating the role of PD-L1 in tumor immune evasion. PTEN is a tumor suppressor that negatively regulates the phosphatidylinositol-3-kinase (PI3K)/AKT pathway. The deletion of PTEN increases the expression of immunosuppressive cytokines, resulting in decrease of T cell infiltration and inhibition of autophagy in tumors, thereby reducing T-cell-mediated cell
death. The loss of PTEN relates with inferior outcomes with PD-1 inhibitor therapy. In murine models, selective PI3K β inhibitor improved the efficacy of anti-PD-1 and anti-CTLA-4 antibodies. This pathway is responsible for the regulation of cellular processes such as proliferation and survival. PD-L1 expression was also up-regulated in lung squamous cell carcinoma following the simultaneous depletion of PTEN and serine-threonine kinase 11.

Other mechanisms that have been shown to have a role in the constitutive up-regulation of PD-L1 include the transcription factor IRF-1 and mutations in the epidermal growth factor receptor (EGFR). IRF-1 is responsible for the regulation of cell proliferation, apoptosis, and immunity. The knockdown of IRF-1 using small interfering RNA resulted in the decrease in transcription and translation of PD-L1 in a lung carcinoma cell line. Similarly, activation of the EGFR pathway resulted in the increased expression of PD-L1 in lung cancer cell lines and tissues. Increased expression of markers of T-cell exhaustion, such as PD-1 and forkhead box P3 (FoxP3), was also observed in the tumor microenvironment. PD-1 blockade increased cytotoxic T-cell numbers as well as effector T-cell function, highlighting the role of the PD-1/PD-L1 axis in immune evasion and its manipulation as a therapeutic strategy. The increased expression of PD-L1 in tumor microenvironment resulted in decreased function of cytotoxic T cells and apoptosis, thus providing an immune escape mechanism for tumor cells.

Indoleamine 2,3-dioxygenase (IDO) was initially viewed as a promising target and biomarker, as high levels of IDO expression were associated with shorter progression free survival (PFS) and poorer outcomes. IDO induces the tryptophan required by cytotoxic T-cells, and induces the conversion of naïve T-cells to Tregs, thereby promoting a tolerogenic and immunosuppressive state. Unfortunately, recent clinical trials with IDO inhibitors have failed to show added efficacy on top of ICIs, raising the needs to re-evaluate its mechanisms and clinical trial design.

TAMs include M1 macrophages that promote anti-tumor immunity and M2 macrophages that promote tumor genesis and development. An association was proved between higher frequencies of TAMs and poor prognosis in many tumors. In the mouse model of lung adenocarcinoma, it is possible to inhibit tumor growth by depleting M2 macrophages through inactivation of CCL2 and/or CCR2 signal transduction. Similar results have been shown in various murine tumor models. Macrophages were suggested to play an important role in mediating therapeutic resistance. It has been reported that macrophages directly inhibit T cell response in hepatocellular carcinoma by B7-H1 and in ovarian carcinoma by B7-H4. In order to overcome the potential resistance mechanism of macrophages, the researchers found that by blocking colony stimulating factor 1 receptor (CSF-1R) in a mouse model of pancreatic cancer, the frequency of TAMs decreased, and the production of IFN-γ increased, leading to inhibition of tumor growth. Importantly, blocking PD-1 or CTLA-4 alone did not significantly inhibit tumor growth. However, combined treatment with CSF-1R blocker and PD-1 or CTLA-4 antibody can promote tumor elimination. Similarly, a synergistic effect of CSF-1R inhibitor and adoptive cell therapy (ACT) therapy has been shown in melanoma models. These data suggest that CSF-1R inhibitor blocked induced TAMs death, thus enhancing the efficacy of immunotherapy.

MDSCs are an important part of the tumor microenvironment and can inhibit the T cell effect and proliferation. More and more studies have demonstrated that the number of invasive MDSCs is associated with poor prognosis of patients and decreased efficacy of immunotherapy, including immune checkpoint therapy, adoptive T cell therapy, and DC vaccination. In addition, studies have shown that high expression of PI3K in MDSCs can increase inflammatory mediators and immunosuppressive factors. It was demonstrated that PI3K inhibitor (TG101348) can inhibit tumor growth in tumor-bearing mice. Indeed, in several tumors, selective PI3K inhibitors with ICIs promoted tumor regression and increased survival. The researchers also demonstrated that the combination of PI3K inhibitor and PD-1 inhibitor improved tumor regression and survival in tumor-bearing mice. In another study, compared with the combined treatment of two drugs (CTLA-4 inhibitor and PD-1 inhibitor), the triple-combination therapy (PI3K inhibitor, CTLA-4 inhibitor, and PD-1 inhibitor) showed stronger anti-tumor effect in tumor-bearing mice. Therefore, it is reasonable to speculate that PI3K inhibitor can promote the anti-tumor immune response and reverse the immune resistance caused by MDSCs.

Tregs play an important role in maintaining self-tolerance by secreting certain inhibitory cytokines such as IL-10, IL-35, TGF-β, or direct cell contact to suppress effector T cell (Teff) responses. Many studies indicate that several tumors are infiltrated by Tregs, A large number of animal experiments have shown that the consumption of Tregs in tumor microenvironment can enhance or restore anti-tumor immunity. The response to anti-CTLA-4 treatment was related to the ratio of Teff to Tregs, and the higher the ratio, the better the response to treatment. A retrospective study reported that the high expression of FoxP3+ Tregs was associated with better prognosis in patients treated with anti-CTLA-4 therapy. These data suggest that when immunotherapy fails to increase Teff and/or decrease Tregs to improve the ratio of Teff to Treg, tumors are likely to develop resistance to treatment, both initially and during disease recurrence.

Acquired resistance

At present, ICI treatment demonstrates a durable long-term response, but some patients develop tumor progression after remission, despite receiving continued therapy. Acquired resistance may be caused by the following reasons, such as changes in signaling pathways, tumor antigen presentation defects, and some new mutations.

Loss of antigen-presenting machinery components such as beta-2-microglobulin (β2M) and human leukocyte antigen (HLA) is an important mechanism to avoid antigen
processing and presentation by tumors. β2M is necessary for HLA class I molecules to fold and transport to the cell surface, and its genetic deficiency would result in lack of CD8+ T cell recognition. It was reported that some patients who initially responded to immunotherapies with IL-2 or tumor-infiltrating lymphocyte (TIL) ACT may develop acquired resistance through loss of β2M. In patients treated with PD-1 antibody, a new homozygous truncation mutation of β2M occurs in drug-resistant cells, resulting in lack of surface expression of HLA class I. Direct loss of the gene encoding the HLA-C*08:02 class I molecule was recently described in a colorectal tumor that progressed after initial response to T-cell transfer therapy. These T cells were HLA-C*08:02-restricted TILs, and thus this mutation directly allowed immune evasion by tumor cells. These examples together reinforce that immunotherapy failure was attributed to acquired defects in HLA class I molecules.

In the other two recurrence cases, a copy number neutral functional deletion mutation was found in JAK1 or JAK2, concurrent with loss of heterozygosity in β2M. These mutations in tumor cells lead to decreased sensitivity to IFN-γ, ultimately preventing IFN-γ-induced cell growth arrest. Upon exposure to IFN-γ (produced by activated T cells), JAK1/2 becomes activated and subsequently phosphorylates a tyrosine residue present on STATs. This JAK/STAT signaling pathway is responsible for cell proliferation, differentiation, cell migration, and apoptosis. IFN-γ also results in the up-regulation of PD-L1 on tumor cells, thus inactivating anti-tumor T cells. Some reports have described up-regulated expression of other T cell checkpoints at the time of acquired resistance, including T cell immunoglobulin mucin 3 (TIM-3), lymphocyte activation gene 3 (LAG3), and V-domain immunoglobulin suppressor of T cell activation (VISTA). By comparing the pathology before treatment with PD-1 inhibitor and the biopsy after drug resistance of uterine leiomyoma, it was found that drug-resistant tumor cells had unique double allelic PTEN gene deletion, and PD-1-positive T cell infiltration was reduced, suggesting that MAPK and PTEN gene mutation may be one of the mechanisms of acquired resistance by ICIs. TIM-3 is highly expressed in the tumor microenvironment, which can promote the apoptosis of T cells, mediate the proliferation of Tregs and inhibitory cells from bone marrow, and inhibit the functions of NK cells and DC cells, thus promoting the tumor immune escape. It was shown that the acquired resistance of anti-PD-1 monoclonal antibody in the treatment of non-small cell lung cancer (NSCLC) patients is due to the high expression of TIM-3 on cytotoxic CD8+ T cells and its binding to galectin-9 on MDCs. Studies have shown increased TIM-3 expression in TIL after the resistance with PD-1/PD-1 inhibitors. The binding degree of T cells to PD-1 antibody was also correlated with TIM-3 expression. Sequential TIM-3 blocker therapy significantly improved the median survival of mouse models resistant to PD-1/PD-1 inhibitors, and increased the number of tumor infiltrating CD8+ T cells. Analysis of immune cells in pleural effusion of patients with lung adenocarcinoma resistant to PD-1/PD-1 inhibitors showed that the expression level of TIM-3 in T cells was significantly higher than that of patients who did not receive PD-1/PD-L1 inhibitors or just underwent surgery. All the above studies suggested that abnormal activation of TIM-3 is one of important resistant mechanisms, and blocking the TIM3 signaling pathway is expected to improve the efficacy of PD-1/PD-L1 inhibitors.

LAG3 is an immune checkpoint receptor that suppresses T-cell activity when up-regulated. LAG3 (CD223) is a transmembrane protein receptor that has been identified on activated T-cells, regulatory T-cells, B-cells, NK cells, and plasmacytoid DCs. As with self-antigen, continued exposure to tumor antigens, such as in the tumor microenvironment, causes up-regulation of LAG3 and its inhibitory actions lead to T-cell exhaustion, thus rendering these cells ineffective at directing attacks towards tumors. VISTA suppresses the activity of T cells and works as an immune checkpoint regulator. In murine models, tumors that were found to express VISTA had an increased rate of growth due to the T cell suppression. Tumor cells that express PD-1 or CTLA-4 and treated with anti-PD-1/CTLA-4 antibodies were found to up-regulate VISTA to avoid immune surveillance. However, VISTA was found in almost all of NSCLCs and may be used as a new target of therapy.

Strategies to Reverse ICIs Resistance

Due to the complex resistant mechanisms of immunotherapy, there is still no standardized solution to this problem. Immunotherapy combined with immunotherapy, chemotherapy, radiotherapy, and targeted therapy is a strategy for the treatment of various cancers, including lung cancer. However, there are challenges related to identifying the appropriate population for combination therapy, balancing synergistic anti-tumor effects, and understanding adverse reactions associated with these treatments.

Combining with other ICIs

In theory, inhibition of CTLA-4 should increase the number of Teffs and reduce Tregs abundance in the tumor microenvironment, while anti-PD-1/PD-L1 therapy should prevent Teffs from being inhibited by malignant and stromal cells. CTLA-4 and PD-1 inhibitors can enhance the killing ability of T cells in different stages. The impact of combining these two therapies was first explored in melanoma and NSCLC. To date, immune checkpoints with promising results are being investigated as potential targets. These include immune checkpoints other than CTLA-4 or PD-1/PD-L1 antibodies. For example, TIGIT protein plays an important role in tumor immune escape and exists on a variety of immune cells, including Tregs, CD8+ T cells, and NK cells. In several mouse models of cancer, combining anti-PD-1 and anti-TIGIT antibodies increased immune responses.

LAG-3 is expressed on Teffs, Tregs, and DCs. Increased PD-1 and LAG-3 lead to T cell exhaustion and tolerance to self and tumor antigens. In a pre-clinical model, inhibiting the expression of PD-1 and LAG-3 eliminates the tumor. It was demonstrated that the overall response rate of
combination of LAG-3 and PD-L1 inhibition was 13% in patients relapsing after anti-PD-1 monotherapy. Blocking other immune checkpoints, such as B7S1, NKGA2, CD39, CD73, or the innate immune checkpoint CD47, also enhances anti-tumor responses induced by anti-PD-1/PD-L1 antibodies.

**Combining with chemotherapy**

Previous studies have shown that traditional chemotherapy drugs may activate the anti-tumor immune response in a variety of ways, including the induction of immunogenic tumor cell death, or disrupting tumor cells to evade immune surveillance. For example, tumor cells treated with anthracyclines activate TIL and DC through purine receptors or toll-like receptor 4, respectively. This changes these cells into “immunogenic tumor cells” and activates the anti-tumor immune response. Gemcitabine can also activate this immune effect by inducing apoptosis of tumor cells, enhancing the cross presentation of CD8+ T cells, and repairing antigen presentation defects in tumor invasive DCs. Therefore, the combination of ICI and chemotherapy may work together synergistically, but this effect is closely related to the type, dose, and regimen (simultaneous or sequential) of the chemotherapy drugs being used. KEYNOTE 189 and KEYNOTE 407, respectively explored the efficacy and safety of pembrolizumab combined with chemotherapy in first-line treatment in non-squamous and squamous NSCLC populations without sensitizing EGFR or ALK mutations, and this regimen has been approved by the FDA as first-line treatment.

**Combining with radiotherapy**

Radiotherapy has direct cytotoxic effects on tumor cells by releasing tumor antigens and mediating anti-tumor response. In addition, ionizing radiation can induce the expression of MHC molecules, immune-activated chemokines and cytokines, and enhance the diversity of T-cell receptor in tumor-specific T cells. Conventional radiotherapy combined with PD-1 inhibitors stimulates CD8+ T-cell mediated tumor clearance. Stereotactic body radiation therapy (SBRT), an emerging radiotherapy technique, may also enhance anti-tumor effects. An animal model study investigating melanoma and kidney cancer found that PD-1 inhibitor combined with SBRT could make the primary tumor disappear and result in an abscopal effect, which is known as eliciting anti-tumor activity in non-irradiated distant tumors. Pembrolizumab combined with SBRT significantly improved the median PFS and objective response rate (ORR) in advanced stage NSCLC patients, compared with those treated with pembrolizumab monotherapy. These results suggest a potential combination of ICIs with radiotherapy as a first-line treatment method.

**Combining with targeted therapy**

Targeted therapy not only kills tumor cells, but also induces immune effect in tumor cells, host immune system, and tumor microenvironment, which has synergistic anti-cancer effects when combined with immunotherapy. Previous studies have shown that the anti-tumor immune response and tumor angiogenesis pathways have mutual influence. VEGF increases the immunosuppressive cell populations (such as Tregs and MDSCs), limits the maturation of APCs and the function of Teffs, and impedes invasion and migration of tumor-specific T cells and other immune effector cells into the tumor microenvironment. Therefore, VEGF inhibitors enhance the anti-tumor immune effect by antagonizing these multiple pathways. ICIs can also mediate tumor angiogenesis that leads to tumor necrosis and mass invasion of mononuclear cells into the tumor. Pre-clinical and clinical studies have confirmed that immunotherapy may have a synergistic effect with VEGF inhibitors, such as bevacizumab. IMPower150 evaluated atezolizumab combined with chemotherapy and bevacizumab as first-line treatment for metastatic non-squamous NSCLC. Results from this study revealed that the median PFS and OS of the combined treatment group were significantly longer than the PFS and OS observed in the group that only receiving chemothera-apy technique, may also enhance anti-tumor effects. An animal model study investigating melanoma and kidney cancer found that PD-1 inhibitor combined with SBRT could make the primary tumor disappear and result in a...
lance. For example, IDO, a tryptophan-metabolizing enzyme produced by cancer cells, TAMs and MDSCs, diminishes T cell anticancer functions and enhances Treg activity. In a variety of tumors, the over-expression of IDO allows for the tumor evading immune monitoring and killing, and is associated with a poor prognosis. Thus, the IDO inhibitor can slow tumor growth and enhance the anti-tumor immune effect, which may have a synergistic effect along with immunotherapy. Pre-clinical models where both IDO and PD-1 were inhibited have demonstrated successful results. At present, the combination of ICIs and an IDO inhibitor has been carried out successively, as shown in KEYNOTE-654.

Other immune factor antagonists have also been gradually applied into treatment with immunotherapy. In murine models and clinical studies, antibodies blocking co-activated molecules such as CD137, CD134, CD357, and CD40 have obtained long-term survival and these blockers can enhance the anti-tumor efficacy of the PD-1 antibody.

Another area of interest is to inhibit TAM activity. Colony stimulating factor 1 (CSF-1) is a cytokine secreted by macrophage, maintaining M2 polarization and inducing TAM proliferation. An anti-CSF-1R antibody reprograms TAM polarization and works synergistically with checkpoint blockade in pancreatic cancer.

Combining with epigenetic modifiers

Epigenetic modifications on the DNA of tumor cells may change the expression of immune-related genes, affecting antigen processing, presentation, and immune evasion. Demethylating agents may enable re-expression of immune related genes and have potential therapeutic effects, especially when combined with immunotherapy. HDAC inhibitors increase expression of MHC and tumor-associated antigens, which work in synergy along with adoptive cell transfer therapy to improve anti-tumor responses in a murine melanoma model. In addition, hypomethylating agents increase CD80 expression on tumor cells and the number of tumor-infiltrating CD8+ T cells in a lymphoma model. These pre-clinical data suggest that it is possible to reverse epigenetic changes in cancer cells, which may enhance immune recognition and response to immunotherapy.

Combining with cancer vaccines

Cancer vaccines exhibit both antigenicity and immunogenicity. New tumor antigen epitopes are specifically expressed by related gene mutations in the tumor cells without immunologic tolerance, but with high immunogenicity. New tumor antigens of T cells effectively enhance anti-tumor ability. Two recent studies based on RNA and peptide were designed and produced individualized tumor vaccines used for patients with advanced melanoma. Results revealed that the tumor was effectively controlled, demonstrating the feasibility, safety, and immunogenicity of a personalized tumor vaccine. Moreover, vaccines achieved better clinical results with checkpoint immunotherapy of PD-1. So, the personalized tumor vaccine may open new opportunities for targeted immunotherapy for patients with malignant tumors.

Combining with CAR-T

CAR-T expresses antigen receptor of target cells on T cell surfaces through gene transfection technology. These cells recognize the antigen on the surface of tumor cells in an antigen specific and non-HLA dependent manner and have the ability to kill cells that are being targeted. Recent studies have shown a high success rate for the treatment of hematologic malignancies using CAR-T, particularly B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma. To translate this success in liquid cancers to solid cancers, CAR-T treatment will have to overcome two major obstacles. First, “on target/off tumor” toxicities must be improved by identifying new targets or by developing new targeting strategies such as dual targeting or rapid exchange of the target by using universal linkers. Second, in solid tumors, the tumor microenvironment affects the activation of CAR-T cells and inhibits their anti-tumor function. Researchers found that the combination of PD-1/PD-L1 antibody and CAR-T may result in resistance to the suppressive tumor microenvironment. In addition, the researchers designed CAR-T cells targeting both VEGF-2 and IL-2 to enhance the infiltration of CAR-T cells into the tumor. While both have serious immune-related adverse events, it will be interesting to see if combining ICIs may facilitate the use of CAR-T cell therapy in the treatment of solid tumors.

Conclusions

Recently, ICI has become a new breakthrough in the cancer treatment field. However, both primary and secondary resistance to these inhibitors poses great challenges to clinical practice. In-depth study investigating the mechanisms of resistance associated with ICIs and an exploration of how to overcome this resistance can not only increase our understanding of immunotherapy, but also greatly benefit the treatment of patients through the use of more accurate and personalized therapies. Uncovering predictive biomarkers, screening for the dominant population, exploring the use of ICIs combined with other treatments and the development of novel ICIs are effective strategies that may aid in overcoming resistance.

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Conflicts of interest

None.

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