A brand new era of cancer immunotherapy: breakthroughs and challenges

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Abstract

Immunotherapy has opened a new era in cancer treatment. Drugs represented by immune checkpoint inhibitors have led to important breakthroughs in the treatment of various solid tumors, greatly improving the survival rate of cancer patients. Many types of immunotherapeutic drugs have become widely available; however, their efficacy is variable, and relatively few patients with advanced cancer experience life-altering durable survival, reflecting the complex and highly regulated nature of the immune system. The research field of cancer immunotherapy (CIT) still faces many challenges in pursuing the broader social goal of "curing cancer." Increasing attention has been paid to strengthening the understanding of the molecular or cellular drivers of resistance to immunotherapy, actively exploring more effective therapeutic targets, and developing combination therapy strategies. Here, we review the key challenges that have emerged in the era of CIT and the possible solutions or development directions to overcome these difficulties, providing relevant references for basic research and the development of modified clinical treatment regimens.

Keywords: Neoplasm; Immunotherapy; Resistance; Combination therapy

Introduction

After one hundred years of development, notable breakthroughs have been made in the field of cancer immunotherapy (CIT), significantly improving the survival rate of cancer patients. Many types of immunotherapeutic drugs are increasingly used, including tumor vaccines, cellular immunotherapeutic agents, immunomodulatory drugs that target T cells, oncolytic virotherapy, and immune checkpoint inhibitors (ICIs), all of which are gradually applied to patients with stage I–IV tumors. Among them, ICI therapy, represented by programmed cell death 1 (PD-1)/PD ligand 1 (PD-L1) inhibitors, has resulted in notable breakthroughs in the treatment of a variety of solid tumors. Besides, anti-tumor immunotherapeutics directed at multiple targets and mechanisms are under active development, such as lymphocyte-activation gene 3 (LAG-3; CD223) antagonists and CD3 immunomodulators. However, despite the successful application of multiple immunotherapeutic agents in a wide range of human cancers, their efficacy remains limited and variable,[1] with relatively few patients with advanced cancer having experienced life-altering durable survival, reflecting the complex and highly regulated nature of the immune system. First, the tumor in itself is complex, adaptive and heterogeneous. Second, the tumor immune microenvironment (TIME) is a complex system with a wide and diverse range of influencing factors.[2] In the TIME, there is a complex interaction network involving tumor and various non-tumor cells, including fibroblasts, macrophages, B/T lymphocytes, and antigen-presenting cells (APCs), that affects the entire body. Furthermore, drug resistance, which involves complex genetic, metabolic, inflammatory, and neovascularization mechanisms, in addition to others, remains an important bottleneck for the application of CIT. However, the complex mechanisms remain incompletely understood, and there is a lack of effective predictive biomarkers,[3,4] making it difficult to accurately grasp the effect of immunotherapy. The successful application of CIT must be defined as the ability to achieve a durable response and increased survival in patients with advanced or early-stage cancer. Although CIT may be beneficial for individual patients, the corresponding research fields still face numerous challenges in pursuing the broader social goal of "curing cancer." Increasing attention has been paid to strengthening the understanding of the molecular or cellular drivers of resistance to immunotherapy, actively exploring more effective therapeutic targets, and developing combination therapy strategies.

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Overcoming Cancer Immunotherapy Bottlenecks

Strengthening the understanding of the molecular or cellular drivers of resistance to immunotherapy

Clinical practice has shown that even if specific patients have a good response to ICIs, a large proportion (>50%) of them do not respond to these drugs, and there is heterogeneity in the degree of response among different tumor lesions in the same patient.[3] These limitations pose numerous challenges for immunotherapy. Most patients either do not initially respond to immunotherapy or initially respond to treatment and subsequently develop resistance to treatment, which is known as primary resistance or acquired resistance, respectively.[3] Adaptive immune resistance is another newly proposed mechanism that distinguishes immunotherapy from traditional chemoradiotherapy or targeted therapy, whereby a tumor can be recognized by the immune system, but can then evade immunity by adapting to the immune attack. Here, owing to the dynamic regulation of the TIME and the interaction between immune and cancer cells, adaptive resistance can manifest as primary resistance, mixed responses, or acquired resistance.[3] Intrinsic mechanisms of tumor immune resistance include changes in antitumor immune response pathways (eg, aberrant expression of tumor antigens, changes in antigen presentation mechanisms) and changes in tumor cell signaling pathways that lead to an inhibitory immunosuppressive microenvironment. External factors include the local tumor microenvironment (TME) (eg, immunosuppressive cells, molecules, and abnormal neovascularization in the TME) and host-related factors (such as age, gender, hormone status, diet, intestinal flora), which can synergize with tumor cells to promote their growth and resistance to ICIs.[5]

Knowledge of the TIME enhances the understanding and exploration of the mechanisms underlying resistance to CIT. With the progress of technology, the theoretical perception of the complexity and diversity of the immune context of the TME and its influence on responses to therapy are constantly being updated and changed. Studies have identified distinct subclasses of the TIME that influences tumorigenesis and responses to treatment.[6] Different immunophenotypes of the TME have different molecular and pathological characteristics and can also reflect different immune response effects. The pattern of tumor immune infiltration can be broadly classified into immune-inflamed, immune-excluded and immune-desert.[7] In addition, one study performed an extensive immunogenomic analysis of >10,000 tumors, comprising 33 cancer types, by utilizing data compiled by the Cancer Genome Atlas. The authors identified six TIME subtypes, namely, wound healing, interferon-gamma (IFN-γ)-dominant, inflammatory, lymphocyte-depleted, immunologically quiet, and transforming growth factor-beta (TGF-β)-dominant, that are characterized by differences in macrophage or lymphocyte markers, the Th1:Th2 cell ratio, the degree of intratumoral heterogeneity, and the degree of neoantigen load, among others.[8] Multiple modes of control (transcription, microRNAs, copy number, and epigenetic processes) of intracellular and extracellular networks are involved in tumor-immune cell interactions, across and within immune subtypes.[8]

The degree of response to immunotherapy also varies in different immunophenotypes, and targeted treatment strategies can be selected based on the molecular characterization of different immunophenotypes. It can target many aspects of TIME through multiple channels, relieve immunosuppression, and achieve the benefit of immunotherapy, including the deletion of immunosuppressor cells (eg, regulatory T cells [Tregs], myeloid-derived suppressor cells [MDSCs], tumor-associated macrophages [TAMs]), antagonism of suppressive cytokines or metabolites (eg, TGF-β, interleukin [IL]-10, indoleamine 2,3-dioxygenase [IDO], arginase), modification of chemokine expression profiles (eg, using oncolytic virus, chemokine [C-C motif] ligand [CCL]6, CCL16), and activation of innate immune pathways (eg, stimulator of IFN genes [STING] agonists). The use of suitable animal models and innovative and high-dimensional technologies such as multiparameter tomography and single-cell RNA sequencing can further characterize and parse the unique TIME classes and subclasses that exist within a tumor. Further stratification of patients based on tumor and TIME type will better predict overall survival (OS) or responses to immunotherapy, providing a broad data set that helps in the identification of new drug targets. An increased understanding of the molecular or cellular drivers of resistance to immunotherapy should lead to a wider application of this type of therapy in cancer treatment.

Considering that ICI is associated with durable responses in only a minority of patients, combination strategies and multimodal approaches are needed to improve clinical outcomes and reduce or overcome the development of drug resistance. At the same time, new molecular mechanisms of efficacy and drug resistance need to be gradually elucidated to explore new therapeutic targets and develop new drugs, and finally expand the scope of clinical application of CIT.

Exploring more effective therapeutic targets and combination therapy strategies

Continuous exploration of novel therapeutic targets or combination strategies is a powerful means to improve/activate anti-tumor immune responses and thus eliminate tumors. Measures should be taken to overcome drug resistance in different segments of the immune response according to the immunophenotypic characteristics. At the same time, different targets and combination modes of drugs, should be considered. Currently, in addition to traditional radiotherapy and chemotherapy, there are multiple promising therapeutic targets and novel combination strategies.

Strategies for combining ICIs with conventional radiotherapy and chemotherapy

Immunotherapy is most commonly used in combination with traditional radiotherapy and chemotherapy, and several studies have shown that this synergy holds promise for the future. A study showed that basic radiotherapy with pembrolizumab immunotherapy can significantly improve response and outcome in patients with metastatic lung cancer,[9] but these results need to be verified in a randomized phase III trial. Several studies have shown

that systemic chemotherapy can reduce the number of B lymphocytes; notably, however, no relevant effect on T lymphocytes, natural killer (NK) cells, or their subsets was observed. This supports that combining T-cell-dependent immunotherapy and chemotherapy may achieve additive or synergistic therapeutic effects. A study pooled and analyzed individual patient data from Keynote-021 Cohort G, Keynote-189, and Keynote-407.[10] The authors reported that pembrolizumab plus chemotherapy could improve OS and progression-free survival (PFS) compared with chemotherapy alone, which led to this combination being used as a standard first-line treatment for patients with advanced non-small cell lung cancer (NSCLC).

Next-generation inhibitory receptors (IRs) and corresponding combination therapy strategies

A combination of receptor blockers can improve the function of $CD8^+$ T cells and NK cells, reduce Treg-mediated suppressive effects,[11] and overcome immunotherapeutic resistance mediated by different IRs. In addition to PD-1/PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), inhibitors targeting other immune checkpoint molecules, such as LAG-3, T-cell immunoglobulin mucin receptor 3 (TIM-3), and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), are under continuous development.[11] For the optimal design of ICI-combination therapies, it is necessary to have a better understanding of the similarities and differences in co-inhibitory pathways as well as their synergistic mechanisms.

LAG-3 is commonly co-expressed with PD-1, and plays a role in inhibiting T cell proliferation and immune activity. A variety of combination regimens based on different LAG-3-targeting drugs are currently available, such as relatlimab (BMS-986916, anti-LAG-3) combined with nivolumab and ipilimumab; LAG525 (IMP701) combined with spartalizumab (anti-PD-1); TSR-033 (anti-LAG-3) combined with TSR-042 (anti-PD-1) or TSR-022 (anti-TIM-3); and anti-LAG-3 drugs alone (INCAGN02385, FS118, and MGD013), all of which are under preliminary exploration.[12] The Keynote-495 (NCT03516981) study explored the use of pembrolizumab combined with lenvatinib or MK-4280, a highly selective humanized monoclonal antibody used to block the interaction between LAG-3 and its ligand, myosin heavy chain (MHC)-II, as a first-line treatment for advanced NSCLC patients. Interim analysis of the TACTI-002 study (NCT03625323) of eftilagimod alpha (IMP321) in combination with pembrolizumab as a first-line treatment in advanced NSCLC indicated a positive response. A phase 2 study is ongoing. TIM-3, a marker of inactivation and exhaustion of $CD8^+$ T cells in various tumor types, is normally co-expressed with PD-1 on the surface of $CD8^+$ T cells, and the upregulation of TIM-3 has been observed to correlate with PD-1 inhibitor resistance both *in vitro* and in the clinic.[13,14]

Trials combining anti-PD-1/anti-PD-L1 therapies with agents targeting TIM-3 (NCT03099109) and LAG-3 (NCT03005782 and NCT01968109) are already ongoing. The simultaneous blockade of the LGA-3 and TIM-3 or MHC-II and LAG-3 pathways improves the immune response of tumor cells that are resistant to CTLA4 and PD-L1, with quadruple blockade having the best response.[15] A variety of monoclonal antibodies against TIGIT are available, such as MK-7684, tiragolumab (MTIG7192A), and etigilimab (OMP-313M32).[12] Other potential IRs/targets include B7 family-related ligands, such as B7-H3, B7-H4, and B7-H5 (VISTA), for which antibodies are undergoing preliminary studies, with positive results.[12,16]

Next-generation immune agonists and corresponding combination therapy strategies

NTRK-214, a CD122-preferential IL-2 pathway agonist, can achieve pleiotropic immune activation through the IL-2 pathway, preferentially activating anti-tumor-specific T cells and NK cells in TIME, and increasing the expression of PD-1 on the surface of these immune cells, which provides a theoretical basis for binding to PD-1 inhibitors.[17] The results of the PIVOT-02 study showed that NTRK-214 in combination with nivolumab achieved good efficacy in the treatment of advanced solid tumors,[17] indicating that NTRK-214 can be combined with ICIs as dual immunotherapy for a range of advanced solid tumors. In the latest data, efficacy was observed regardless of baseline PD-L1 status and tumor-infiltrating lymphocytes (TILs),[18] suggestive of a therapeutic potential for patients with poor prognostic risk factors for response to PD-1/PD-L1 blockade. Early-phase clinical studies of NKTR-214 in combination with pembrolizumab/atezolizumab in advanced solid tumors are ongoing (2018 ASCO TPS3115). In addition, immunotherapy combined with agonistic antibodies targeting stimulatory checkpoint molecules, including CD27, CD40, OX40, glucocorticoid-induced tumor necrosis factor related protein, and inducible T cell co-stimulator, can modulate the TIME, and some of these have entered clinical trials. For example, OX40 can promote the activation and proliferation of $CD8^+$ T cells, inhibit Treg activation, and mediate the antibody-dependent cell-mediated cytotoxicity effect, suggesting that it has a synergistic effect with PD-1/PD-L1 inhibitors.[19]

Bispecific antibody represented by the PD-L1/TGF-β antibody

A bispecific antibody (bsAb) can bind two different epitopes or antigens at the same time, so as to achieve a variety of functions with synergistic effect. This type of antibody has received much attention in recent years. Bintrafusp alfa (M7824) (MSB0011359C) is a bifunctional molecule that targets both PD-L1 and TGF-β and was demonstrated to effectively inhibit tumor growth and metastasis by antagonizing both PD-L1 and "trapping" TGF-β.[20] One study has shown that M7824 can be used in the second-line treatment of advanced NSCLC owing to its good clinical efficacy (NCT02517398).[17] Compared with a combination of two protein inhibitors, M7824, a single molecule targeting two pathways, has fewer side effects and reduces the complexity of clinical development; moreover, the inhibitory effect of M7824 on the TGF-β signaling pathway is limited to within the TME, further reducing the side effects. INTREPID LUNG 037

(NCT03631706), a clinical trial for M7824 *vs.* pembrolizumab as first-line treatment, is ongoing. A variety of immune bsAbs are currently undergoing clinical trials in China, including KN046 and AK104 that target PD-L1/CTLA-4; A-337 and M307 that target CD3/epithelial cell adhesion molecule (EpCAM); SHR-1701 that targets PD-1/PD-L1/TGF-β; and IBI-318 that targets PD-1/PD-L1.

ICIs in combination with antiangiogenic agents

Recently, it has been shown that stimulation of immune cell function or immune reprogramming helps to normalize tumor blood vessels. The mutual regulation between the tumor vasculature and immunity can form a reinforcement loop that reorganizes the TIME, thereby inducing long-lasting anti-tumor immunity. In addition, evidence suggests that proangiogenic factors can modulate immune responses by reducing T cell infiltrating into the TME and by systemic effects on the function of immunoregulatory cells.[21] This indicates that ICIs plus antiangiogenic agents may synergistically enhance anti-tumor immune responses. The JVDF study has preliminarily demonstrated the anti-tumor activity of ramucirumab,[22] an IgG1 vascular endothelial growth factor receptor 2 (VEGFR-2) antagonist, in combination with pembrolizumab. Mouse colorectal cancer models and phase Ib clinical studies suggest that the lenvatinib/PD-1 inhibitor combination has a good anti-tumor therapeutic effect.[23] Bevacizumab (anti-VEGF), which prolonged PFS from 6.1 months with ICIs to 11.7 months with combination therapy, reversed myeloid-induced immunosuppression,[24] and a similar incremental benefit was reported for an ICI combined with a small-molecule VEGF receptor tyrosine kinase (RTK) inhibitor.[25] There are also two ongoing clinical trials: the phase III LEAP-006 study (pembrolizumab in combination with pemetrexed plus platinum-based chemotherapy with or without lenvatinib, NCT03829319) and the LEAP-007 study (pembrolizumab with or without lenvatinib, NCT03829332).

Immunomodulatory drugs acting on the TIME

The adenosine pathway mediates immunosuppression at multiple levels and modulates resistance to PD-1/PD-L1 inhibitors. Inhibitors targeting the ecto-5′-nucleotidase (CD73) or recombinant adenosine A2A receptor (ADORA2A) can produce favorable anti-tumor effects in preclinical models. The anti-tumor efficacy of CPI-444, an anti-ADORA2A drug, with and without anti-PD-L1 treatment, was identified in mouse tumor models, and was associated with increased T-cell activation and CD73 expression, as well as with the induction of a *Th1* gene expression profile.[26] Clinical studies of multiple adenosine pathway-related targets are currently underway, such as oleclumab targeting CD73 (NCT02503774), and CPI-444 (NCT02655822) and PBF-509 (NCT02403193) targeting ADORA2A. In addition, the high expression of IDO is associated with decreased TIL and increased Treg numbers. Anti-PD-1 therapy can upregulate the expression of IDO1,[27] which may partly explain why anti-PD-1/PD-L1/IDO inhibitor combination therapy has shown efficacy in small-scale studies.[28] However, the phase III clinical ECHO-301 (Keynote-252) study was declared a failure. Further studies on related signaling pathways and mechanisms of resistance are still needed to identify new coping strategies. The cytokine IL-1β can promote the infiltration of immunosuppressive cells into the TME, thereby enhancing tumor growth. Canakinumab is a fully human IgG monoclonal antibody with high affinity and specificity for IL-1β, and there are four ongoing international multicenter phase III clinical studies of canakinumab that are related to lung cancer. These studies are assessing the efficacy and safety of pembrolizumab plus platinum-based doublet chemotherapy with or without canakinumab in the first-line treatment of advanced lung cancer (CANOPY-1, NCT03631199); the second-line treatment of advanced lung cancer after progression of immunotherapy with chemotherapy (CANOPY-2, NCT03631199); neoadjuvant therapy (CANOPY-N, NCT03968419); and post-operative adjuvant therapy (CANOPY-A, NCT03447769) for NSCLC patients without epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) mutation. Immunomodulatory agents that act on the immune microenvironment can promote TIME reprogramming by affecting both tumor and immunity, and synergize with other immunotherapeutic drugs; however, more studies are needed to confirm their efficacy.

Individualized tumor vaccines in combination with ICIs

Antigenic determinants (neoantigens) derived by T cells targeting mutations can drive anti-tumor responses. The combination of neoantigen-based individualized cancer vaccines with ICIs and/or anti-angiogenic drugs can exert stronger anti-tumor effects, which is also an effective method of conquering "cold tumors." The NT-001 study showed that the NEO-PV-01 vaccine combined with nivolumab had good tolerance and anti-tumor activity in patients with advanced NSCLC[29]; NT-002 was a single-arm, phase Ib clinical study to analyze the efficacy and safety of NEO-PV-01 combined with pembrolizumab plus pemetrexed/carboplatin in the treatment of patients with advanced non-squamous, NSCLC. The preliminary results of a phase I/II clinical trial (NCT02955290) analyzing the CIMAvax-EGF (epidermal growth factor) vaccine combined with a PD-1 inhibitor in the treatment of NSCLC were presented at the annual meeting of the American Association for Cancer Research in 2019, and showed that the efficacy of combination therapy was satisfactory in patients with low PD-L1 expression in tumors and a poor response to nivolumab. A phase II clinical trial of the vaccine is ongoing (NCT02955290). A clinical trial of CIMAvax-EGF *vs.* pembrolizumab for first-line treatment is ongoing in patients with advanced NSCLC with high PD-L1 expression. A study (ChiCTR1900022986) developed a personalized neoantigen/cancer testis antigen nanovaccine combining nanotechnology with a selection of neoantigens containing patient-specific mutations based on whole-exome sequencing and RNA sequencing of tumor-specific mutations; preliminary results indicated that it can induce neoantigen-specific T-cell responses, and merits further studies in a larger population range. RO7198457 is a personalized cancer vaccine based on messenger RNA (mRNA). In the latest phase Ib clinical trial ($n = 132$), patients with various types of advanced

solid malignancies who were treated with RO7198457 in combination with the PD-L1 inhibitor, atezolizumab, showed clinical benefits.[30] Therefore, individualized neoantigen-specific immunotherapy combined with ICIs has promising prospects for clinical application.

The combination of cellular immunotherapy and ICIs

The integration of cellular immunotherapy into current immunotherapy may be a novel strategy for antineoplastic therapy. Mechanistically, the success of antibody-mediated checkpoint blockade requires a relatively high mutational load and the presence of TILs, while adoptive cell therapies can utilize autologous lymphocytes that have been isolated from the tumor itself or blood and manipulated *in vitro* to enhance their activity by expressing specific T-cell receptors or chimeric antigen receptors (CARs; synthetic receptors that retarget T cells to tumor surface antigens) against target antigens.[31] Therefore, the adoptive transfer of tumor-targeted T cells may fill the immunotherapy gap in patients with less immunogenic or "non-inflammatory" tumors. A study described a PD-1 dominant negative receptor that mediates enhanced T-cell functional persistence when co-transduced with second-generation CARs.[32] This combination strategy (co-stimulation and checkpoint blockade) directly counteracts PD-1-mediated inhibition in the presence of tumor PD-L1 expression to enhance T cell function, resulting in long-term disease-free survival after low-dose infusion of CAR-T cells. Ultimately, an ideal combination of co-stimulation combined with concurrent or adjuvant co-inhibitory blockade would maximize the potency of CAR-T cells; the selected co-stimulatory and co-inhibitory pathways could be tailored to the specific characteristics of the tumor and patient. Oyer *et al*[33] found that combining PM21-NK cells and anti-PD-L1 therapy improved NK cell function and significantly prolonged survival in an animal model of aggressive ovarian cancer. Lin *et al*[34] investigated the safety and efficacy of pembrolizumab in combination with allogeneic NK cell therapy *vs*. pembrolizumab alone in previously treated patients with advanced NSCLC. The results showed that the combination therapy significantly improved the patient survival outcome and increased the levels of both immune cells and cytokines in their bodies. SNK01 is a novel non-genetically modified autologous NK cell therapy with enhanced cytotoxicity that has been found to kill several types of lung cancer cells and is being studied in a randomized phase I/IIA clinical trial. These preliminary results mentioned above have shown that ICIs combined with cellular immunotherapy have good efficacy and safety, and warrants a prospective study in a larger sample.

New targets for resistance to PD-L1 inhibitors under development

With the increased understanding of the mechanisms of drug resistance, immunotherapeutic strategies targeting different molecular or cellular mechanisms of resistance and combination therapy strategies targeting specific or novel targets can improve/activate anti-tumor immune responses. Sitravatinib is a selective kinase inhibitor that potently inhibits RTKs, including rearranged during transfection (RET), TAM (TYRO3, Axl, MER) family receptors, and split family receptors (VEGFR-2, KIT). The strong inhibitory effect of sitravatinib on TAM and split family receptors can help patients overcome resistance to ICIs, potentially enhancing the antigen-presentation capacity of dendritic cells (DCs), impairing Tregs and MDSCs, and converting TAMs in the TME into immunopotentiating type 1 macrophages.[35] Sitravatinib is currently being evaluated in a phase Ib extension trial in NSCLC and other tumor patients with *RET*, chr4q12 (a genomic loci), and Casitas B-Lineage Lymphoma mutations. Sitravatinib in combination with nivolumab is also being evaluated in NSCLC patients who progressed after ICI treatment, while a phase III clinical study of sitravatinib plus nivolumab *vs*. docetaxel in advanced non-squamous NSCLC patients that has progressed after ICI plus chemotherapy is ongoing (NCT03906071). Entinostat (ENT) is an oral histone deacetylase inhibitor that can reduce the number and function of MDSCs and Tregs, induce a cascade of inflammation-promoting responses in the TME, enhance antigen presentation, and increase the anti-tumor effect of effective T cells and NK cells. Preclinical models have shown that ENT synergizes with anti-PD-1 inhibitors, thereby exerting durable therapeutic effects in NSCLC patients who have failed PD-1/PD-L1 inhibitor therapy. In the phase Ib/II ENCORE-601/Keynote-142 study assessing the efficacy and safety of ENT in combination with pembrolizumab in multiple tumor types, exploratory analyses showed that high monocyte levels at baseline were indicative of a better PFS benefit. The mRNA levels of sialic acid-binding Ig-like lectin 15 (siglec-15, *S15*) were shown to be low in normal human tissues and various immune cells, but high in macrophages.[36] Sialic acid linked by α-2,6 glycosidic bonds on the surface of lung cancer cells promotes S15-positive mononuclear macrophages to secrete the immunosuppressive cytokine, TGF-β, thereby directly inhibiting T cell activity. *In vitro* analysis showed that there was no correlation between PD-L1 and S15 expression, and that they may even be mutually exclusive,[36] which explains the role of the siglec/sialoglycan axis in the process of immune escape in tumors treated with PD-1/PD-L1 inhibitors. Drugs developed against siglec to inhibit immune escape, such as NC318 monoclonal antibody, are undergoing clinical trials.

Various combination strategies are summarized in Supplementary Table 1, http://links.lww.com/CM9/A527.

How to Achieve Precision in Combination Therapy

ICIs combined with multiple drug therapy can better achieve the purpose of presenting antigens and relieving immunosuppression, thereby increasing the infiltration of immune cells, maintaining the killing and memory function of T cells, and ultimately achieving long-term tumor control. Immunotherapy can be combined with a variety of therapies such as chemotherapy, radiotherapy, targeted therapy, or other immunotherapy, with more than 1000 combination regimens in clinical trials.[37] The overall goal of combination therapy is to maximize synergistic effects, avoid overlapping toxicities, and minimize the potential for overlapping resistance. Combined regimens may

include anti-PD-1/PD-L1 agents along with others that can remove negative regulators; activating, initiating, and/or creating new immunity in patients without a strong immune response; increasing MHC-I molecules; increasing T cell survival; and/or driving T cell memory. However, many challenges remain to be solved before these combination therapies can become the clinical standard. These include indications, target populations, combination sequences, medication times, medication doses, efficacy evaluation criteria, the prediction and management of treatment-related toxicities, and the identification of practical biomarkers. First, appropriate measures should be taken according to the immunophenotypic characteristics to overcome drug resistance in different links of the immune response. Second, different drug combination patterns should be considered to achieve maximum benefit. For example, treatment with a TGF-β inhibitor has been shown to promote the proliferation of tumor angiogenesis factor and the expression of matrix metalloproteinase-9, and the latter can negatively regulate PD-L1 expression on the surface of tumor cells, thereby reducing anti-PD-1 efficacy.[38] Therefore, anti-PD-1 and TGF-β inhibitors should be administered sequentially rather than simultaneously to optimize efficacy. Similarly, preclinical studies have demonstrated that the regimen of a PD-1 inhibitor in combination with an OX40 antibody is not effective; however, the efficacy can be significantly improved when the OX40 antibody is used first, followed by the PD-1 inhibitor, suggesting the importance of the order of combination therapy.[19] Furthermore, it should be recognized that although numerous clinical studies were carried out,[37] the translation from mechanism to clinical practice is not necessarily effectively achievable, that is, promising strategies do not necessarily correlate with success in clinical studies. The question of how to improve the success rate of research rather than wasting resources should be actively explored in the future.

In the era of precision medicine, biomarker testing is the cornerstone of precision therapy. Although no biomarker with sensitivity and specificity of 100% has been found for immunotherapy, precision therapy studies based on PD-L1, TMB, TILs, and gut microbes are still ongoing. Precision treatment according to different PD-L1 expression levels has been validated by numerous clinical studies. In the case of lung cancer, the Keynote-024 study showed a benefit of pembrolizumab as a first-line treatment for lung cancer patients with PD-L1 ≥50%[39]; Keynote-042 further confirmed the benefit of immunotherapy for patients with PD-L1 ≥1%[40]; Keynote-010 expanded pembrolizumab to second-line treatment for NSCLC with PD-L1 ≥1%[41]; and Keynote-189 confirmed that patients could benefit from pembrolizumab combined with chemotherapy regardless of the PD-L1 expression level.[42] Second, the exploratory Keynote-158 study showed that TMB, as a novel marker, can predict the efficacy of pembrolizumab in post-third-line treatment of solid tumors.[43] On June 16, 2020, the US Food and Administration approved pembrolizumab for the treatment of solid tumors with a TMB of ≥10 mutations/Mb that have progressed after the failure of prior therapy. In addition, immunotherapy may be ineffective in patients with *EGFR* and *ALK* mutations, among others, and some will experience serious adverse events and even hyperprogression.[44] However, overall, the mechanisms regulating the immune response are very complex, and the effect of immunotherapy and adverse events cannot be completely predicted by a biomarker alone. At present, most of the clinical trials on immunotherapy, combined with other treatment methods, are still ongoing, and optimal combinations, timing, and drug doses are still being explored. Therefore, we cannot know exactly which treatment scheme should be adopted for which patient group. In clinical practice, we recommend that all patients conduct whole-genome or whole-exome sequencing to characterize all their gene mutations as much as possible for initial screening of immune combination therapy strategies. Nevertheless, we can make a preliminary judgment and screening based on well-characterized biomarkers, while different CIT categories are selected according to their gene expression patterns. Patients with high expression of IRs such as PD-L1, CTLA-4, LAG-3, and TIM-3 can be treated with different combinations of the corresponding inhibitors; patients whose T cells, NK cells, or DCs cannot be activated after immunotherapy can be combined with the corresponding immune agonist therapy; bsAb may be considered in patients with high expression of both PD-L1 and TGF-β, CD3, EpCAM, etc. Meanwhile, patients with high expression of VEGF/VEGFR-2 or FGFR can be combined with anti-angiogenic drugs. Patients with high expression of CD38, CD39, CD73, or IDO can be combined with the corresponding target inhibitors to regulate TME metabolism. For patients with cold tumors, combined cellular immunotherapy, therapeutic vaccines, and other methods can be considered. During treatment, a high-throughput platform for the continuous analysis of peripheral blood can be used to monitor efficacy and predict secondary drug resistance. With the continuous exploration of molecular mechanisms of drug resistance and efficacy, there has been an increase in research and development of new targets and new drugs, which is expected to bring survival benefits to more patients. In the future, the study of immunotherapy will continue to focus on more refined stratification, aiming to determine the optimal benefit population and optimal matching medication of immunotherapy in terms of age, tumor stage, and number of treatment lines and biomarkers, truly realize individualized precise treatment, and maximize the anti-tumor effect of immunotherapy while reducing adverse events.

Challenges and Future Directions of CIT

CIT is flourishing, and there are numerous clinical studies underway for different mechanisms. There are several major research directions in the future. First, clinical research methods and CIT endpoints should be optimized. New clinical trial designs are needed to rapidly prioritize and accelerate the development of combination regimens.[45] These trial "platforms" include umbrella trials focused on tumor histology, which contain multiple agents and treatments determined based on pre-specified biomarkers;[46] and basket trials, to organize clinical trials around genomic alterations or other intrinsic tumor characteristics, including those related to the immune system.[47] Overall, these clinical trial designs can facilitate

faster and more confident decisions and also enable the sequential addition of therapeutic agents to the protocol or, conversely, successively deconstruct the components of complex protocols. Second, given the diversity and complexity of current monotherapy or combination immunotherapy regimens, and with the development and continuous improvement of multiplex immunohistochemical technology, high-throughput sequencing, and microarray technology, the use of biomarkers (including markers related to the genome or a specific phenotype of tumor cells; immune cells or immune molecules in TME; and circulating blood or systemic factors, among others) to screen patient groups that may benefit the most have become a research trend to overcome drug resistance and effectively improve therapeutic efficacy. For mutually independent predictive markers, their combined detection can expand the population likely to benefit from ICIs, while for the markers interacting with each other, a comprehensive bioinformatics-based prediction model can be established based on the different impact weights of each factor to improve the accuracy of screening beneficiary populations. However, how to better use the interrelationship network of various markers is an aspect that needs to be considered when establishing the comprehensive prediction model. In addition, the combined prediction of multiple factors should be comprehensively evaluated and validated, so that it can achieve the best cost-effectiveness and more effectively serve the clinical immunotherapy of tumors. In the future, by extracting large samples, multidimensional features, and constructing multivariate scoring models using machine learning/big data analysis,[48,49] it may be possible to obtain the most effective and comprehensive biomarkers or models, and construct a new framework for the precise treatment of tumors.

Finally, improvements in drug development and novel drug delivery platforms should be developed. They could deliver different immunotherapeutic drugs, such as cytokines, checkpoint inhibitors, agonistic antibodies, edited T cells, and tumor vaccines, to specific parts of the human body by a variety of different drug delivery methods, such as *in vivo* nanoparticle transport to immune cells, *in vitro* T cell functionalization using nanoparticles, controlled release systems, biomaterial implant stents, injectable biomaterial stents, and transdermal drug delivery systems, thereby enhancing therapeutic effects and reducing adverse events.[50] In recent years, the rapid development of nanocarrier systems has enabled drugs to cross physiological barriers and safely and sustainably reach the target position, and a large number of exploratory studies have been carried out to assess the possible application of these systems in CIT.[51,52] The application of biofilm nanomedicine delivery systems in CIT can result in the effective protection of the biological activity of immune-related molecules such as tumor antigens and achieve their long-acting circulation in the blood and targeted delivery to tumor sites, and can also solve many difficulties and challenges faced by current CIT strategies through targeted modification. This suggests that it has great potential for clinical translation. For example, studies have used genetic engineering to express PD-L1 on 293T cell membranes, followed by the extraction of vesicles loaded with 1-methyltryptophan (1-MT) immune agonists to form membrane-coated nanoparticles.[53] This not only restores T cell immune activity by occupying PD-L1 but also breaks the immune silencing of DCs by using 1-MT to bind to immunosuppressive dioxygenase molecules (IDO) expressed on the surface of DCs, thereby initiating anti-tumor immune responses. A polymeric carrier consisting of disulfide-crosslinked polyethyleneimine and dermatan sulfate can reportedly deliver small interfering RNAs targeting PD-L1 to effectively reduce PD-L1 expression in melanoma cells and inhibit the growth of melanoma in immunocompetent and immunodeficient mouse models.[54] Choo *et al*[55] used exosome-mimetic nanovesicles derived from M1 macrophages (M1NVs) to repolarize M2 TAMs into M1 macrophages that release proinflammatory cytokines and induce anti-tumor immune responses. Compared with M1NV or anti-PD-L1 therapy alone, injection with the combination of the two into mice further reduced tumor size, demonstrating the enhancement of ICI-anti-tumor efficacy by nanotechnology. Liu *et al*[56] constructed nanovesicles comprising hybrid tumor cell and DC membranes that could simulate the function of APCs and directly activate T cell anti-tumor immune responses through the various specific molecules present on the surface. In addition, nanopreparations can regulate or remodel the immunosuppressive TME through a variety of mechanisms, such as relieving the factors that influence immunosuppression, strengthening the intensity of the autoimmune response, remodeling the pathological structures of tumors that affect the efficacy of immunotherapy, providing new ideas and methods for the study of CIT strategies.

Conclusion and Future Prospective

CIT has become a validated, crucial, and promising therapy for the treatment of cancer patients, and has revolutionized the prospects for the diagnosis and treatment of malignant tumors. Indeed, an increasing number of patients have achieved meaningful and lasting clinical benefits. However, despite these impressive therapeutic advantages, the research field of CIT still faces many challenges in pursuing the broader social goal of "curing cancer." Here, we reviewed the key challenges that have emerged in the CIT era and the possible solutions or development directions to overcome these difficulties, providing relevant references for basic research and the development of modified clinical treatment regimens. At present, combination therapy is still in the exploratory stage, with the efficacy and superposition of toxicity still requiring confirmation in subsequent experimental studies; however, overall, combination therapy may benefit numerous patients, and merits extensive further investigation.

In future studies, first, serial sampling and longitudinal evaluation of fresh human specimens (tumor, blood, serum, and microbiome) during treatment are required, so as to clarify the heterogeneous mechanisms of drug resistance in combination with a comprehensive analysis of multiple factors; alternatively, it is necessary to adopt new technologies, such as whole-genome sequencing, single-cell sequencing, and epigenetic analysis, to identify

the characteristic drug resistance sites or sub-clones. With the continuous in-depth exploration of the mechanisms of resistance, immunotherapy may be applied to the treatment of a wider range of cancers. Second, with the development of a variety of high-tech technologies, effective biomarkers should be explored to screen patients based on different tumor characteristics and different microenvironment phenotypes. Finally, the research directions of cancer treatment modes are multidisciplinary (such as surgery, internal medicine, radiotherapy) and the combination of multiple drugs, which can develop the best individualized treatment plan according to the condition of each patient. Coping with these challenges requires the joint efforts of clinicians and scientists performing basic research, and the focusing of resources to accelerate the understanding of the complex interactions between cancer and immunity with the aim of developing improved treatment options for cancer patients and promoting the advancement of CIT.

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Conflicts of interest

None.

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