Immune checkpoints and immunotherapy in non-small cell lung cancer: Novel study progression, challenges and solutions (Review)

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Abstract. Lung cancer is the most common type of cancer with the highest mortality rate worldwide. Non-small cell lung cancer (NSCLC) accounts for ~85% of the total number of lung cancer cases. In the past two decades, immunotherapy has become a more promising treatment method than traditional treatments (surgery, radiotherapy and chemotherapy). Immunotherapy has been shown to improve the survival rate of patients and to have a superior effect when controlling lung cancer than traditional therapy. However, only a small number of patients can benefit from immunotherapy, and not all patients who qualify experience long-term benefits. In the clinic, the objective response rate of programmed cell death protein 1 treatment without the prior screening of patients is only 15-20%. Immunotherapy is associated with both opportunities and challenges for patients with NSCLC. The current challenges of immunotherapy include the lack of accurate biomarkers, inevitable resistance and insufficient understanding of immune checkpoints. In previous years, several methods for overcoming the challenges posed by immunotherapy have been proposed, but combination therapy is the most suitable choice. A large number of studies have shown that the combination of drugs can significantly improve their efficacy, compared with monotherapy, and that some therapeutic combinations have been approved by the Food and Drug Administration for the treatment of NSCLC. Traditional Chinese medicine (TCM) is a traditional medical practice in China that can play an important role in immunotherapy. Most agents used in TCM originate from plants, and have the advantages of low toxicity and multiple targets. In addition, TCM includes a unique class of drugs that can improve autoimmunity. Therefore, TCM may be a promising treatment method for all types of cancer.

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1. Introduction

At present, the collective worldwide incidence and mortality rates of all cancers are high, and have become the second leading cause of death (1). Among all cancer types, lung cancer is the most common type of cancer, which accounted for 11.6% of all cancer cases in 2018 (2). Non-small cell lung cancer (NSCLC) accounts for ~85% of the total number of lung cancer cases (3). The 5-year survival rate of patients with advanced NSCLC is only ~15%, and the recurrence rate of advanced NSCLC following radical treatment is >40% (4). In the past two decades, an increasing number of therapies have been widely considered and studied to improve the survival

rate and quality of life for patients with advanced or meta-static NSCLC. These treatments include surgery, radiotherapy, chemotherapy, targeted therapy, immunotherapy and combined therapies. Among these treatment options, immunotherapy has become the optimum choice, particularly for patients with advanced or metastatic NSCLC. Immunotherapy can effectively control disease progression and improve survival rates (5), and the use of immune checkpoint inhibitors (ICIs) is an effective immunotherapeutic method (6). Thus, the aim of the present review was to investigate the developments in novel immune checkpoints and immunotherapy, with their associated challenges and potential solutions to these issues.

2. Overview of immune checkpoints in NSCLC

Immunotherapy is a type of treatment that uses ICIs to block the immune checkpoint signaling pathway and reactivate T cells, for the purpose of destroying tumors via the immune system (Fig. 1). The effects of immunotherapy are superior to those of other traditional therapies, rendering it an effective and innovative method for treating cancer (7). However, low response rates and immune-related adverse events (irAEs) have also been observed in patients treated with ICIs. Overcoming these challenges is necessary (8). At present, immune checkpoints, including cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lympho-cyte activation gene 3 (LAG-3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT), are a research hotspot (Table I) (9).

CTLA-4. In 1995, CTLA-4 was first discovered to deliver inhibitory immune response signals (10). Leach *et al* (11) demon-strated that blocking CTLA-4 enhanced the immune-mediated targeting of tumor cells, and that tumor cells had the ability to upregulate the expression of CTLA-4. CTLA-4 belongs to the immunoglobulin superfamily and its gene is localized to chromosome 2q33 in humans (12). The structure of CTLA-4 is homologous to that of CD28, with which it shares two ligands, CD80 and CD86 (13). Therefore, CTLA-4 and CD28 exert opposing functions. CTLA-4 induces inhibitory signals in T cells and blocks T cell responses by competing with CD28 for ligand binding (14). CTLA-4 also regulates T-cell activa-tion through several mechanisms, including the inhibition of T-cell proliferation, differentiation, IL-2 production and cell cycle progression. Therefore, CTLA-4 serves a significant role in immunotherapy, and has achieved satisfactory results in clinical treatment (15,16).

PD-1/PD-L1. PD-1 is a member of the CD28 superfamily involved in programmed cell death (17,18), that is preferentially expressed by T and B cells, but also expressed in other cell subsets, such as dendritic cells, natural killer (NK) cells and monocytes. PD-1 forms conjugates with PD-L1 and PD-L2, which belong to the B7 protein family, where PD-L1 is its primary ligand (19). PD-1 interacts with PD-L1 to inhibit the activation of T cells and promote immune escape, since Src homology region 2-containing protein tyrosine phos-phatase 2 inhibits kinases involved in T-cell activation (20).

Although anti-PD-1/PD-L1 inhibitors (that block the binding of PD-1 and PD-L1) have shown encouraging results in the clinic (21), certain challenges remain, including irAEs and low response rates (22).

LAG-3. LAG-3 (also known as CD223) was discovered in 1990 and includes Ig-like domains 1-4. Domain 1 contains an additional sequence of ~30 amino acids known as the 'extra loop' (23). LAG-3 is primarily expressed on CD4^{+} and CD8^{+} T cells, though plasmacytoid dendritic cells, regu-latory T cells (Tregs), activated B cells and NK T cells also express cell surface LAG-3 (24,25). Stable peptide-major histocompatibility complex class II (MHC-II) is considered to be a ligand of LAG-3 (26), and liver sinusoidal endothelial cell lectin (LSECtin), galectin-3 and fibrinogen-like protein 1 have also been reported as potential LAG-3 ligands (27,28). LAG-3 possesses a similar tumor immune escape mechanism to that of PD-1, and is considered the most important tumor treatment target after PD-1. At present, clinical trials to verify the efficacy of LAG-3 ICIs, alone and combined with other ICIs, are in progress.

TIM-3. TIM-3 is a member of the TIM family of immuno-regulatory proteins, first discovered in 2002 (29). TIM-3 is a type I transmembrane protein expressed on T cells, B cells, NK cells, dendritic cells (DCs) and monocytes (30). Ligands of TIM-3 have been reported to include galectin 9, phosphatidylserine, carcinoembryonic antigen-related cell adhesion molecule 1 (Ceacam1) and high mobility group protein B1 (31). The interaction between TIM-3 and its ligands (galectin-9 or Ceacam1) induces Tyr256 and Tyr263 phosphorylation in the intracellular domain of TIM-3, releasing BAG cochaperone 6 (BAG6) from the TIM-3 tail. The release of BAG6 allows the recruitment of Src kinases (including, but not limited to Lck and Fyn) and promotes the subsequent negative regulation of T cell receptor signaling (32,33). When TIM-3 is not bound by a ligand, BAG6 is bound to its unphosphorylated cytoplasmic tail, and maintains T-cell activation through Lck recruitment (9). TIM-3 is also co-expressed with PD-1, and the co-blockade of PD-1 and TIM-3 can exert synergistic effects, restoring T cell effector function and killing tumor cells (34).

TIGIT. TIGIT (also known as WUCAM, Vstm3 and VSIG9) belongs to a constantly expanding family of poliovirus receptor like proteins that plays a critical role in limiting immune functions (35). In 2009, TIGIT was first identified by three groups as an immune checkpoint that inhibits NK and T-cell activation. TIGIT is composed of an extracellular immunoglobulin variable domain, a type I transmembrane domain and two inhibitory motifs of the cytoplasmic tail; one immunoreceptor tyrosine-based inhibitory motif and one immunoglobulin tyrosine tail (ITT)-like motif (36,37). TIGIT has three ligands, CD112, CD113 and CD155, though it binds to CD155 with the highest affinity. In humans, TIGIT is expressed by activated CD4^{+} T and CD8^{+} T cells, Tregs, NK cells and follicular T helper cells. NK cytotoxicity is inhibited by ITT phosphorylation when TIGIT binds to its ligands in NK cells (38). Furthermore, TIGIT-mediated inhibition of effector T and NK cells is also achieved by interfering with
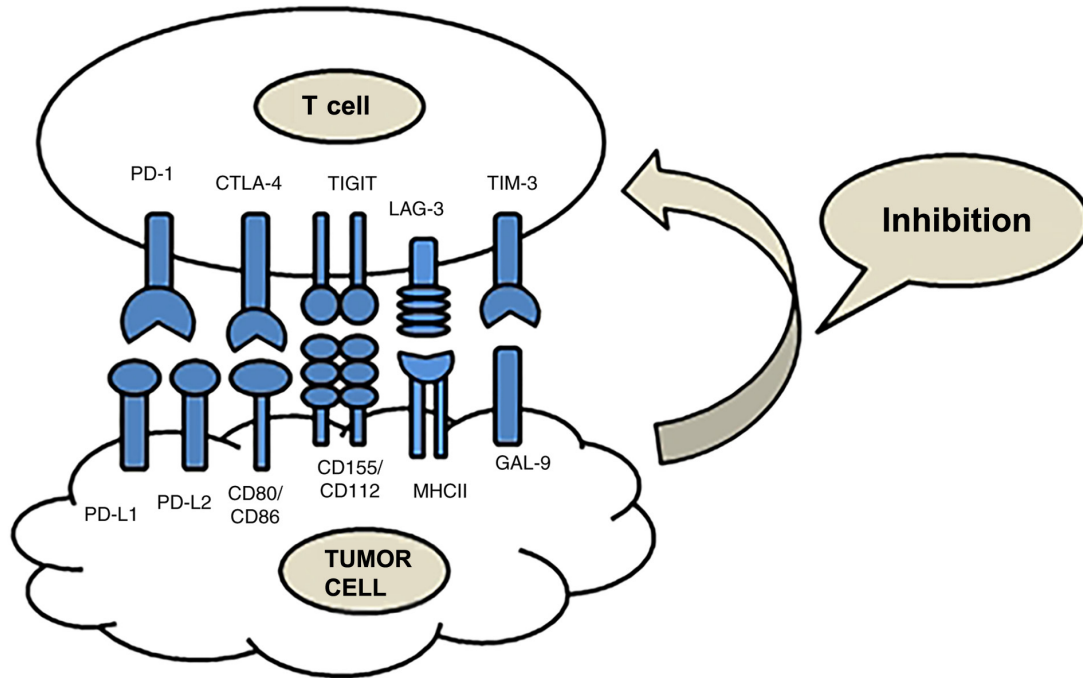
Figure 1. Function and ligands of immune checkpoints. PD-1 and CTLA-4 are classical, and TIM-3, LAG-3 and TIGIT are emerging immune checkpoints. These immune checkpoints are co-inhibitory and can suppress T cell activity. PD-1, programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; LAG-3, lymphocyte-activation gene 3; TIGIT, T cell immunoreceptor with Ig and ITIM domains.

DNAX accessory protein-1 co-stimulation, which directly delivers inhibitory signals to the effector cell (39).

3. Immunotherapy in NSCLC

In the current clinical treatment of NSCLC, immunotherapy is primarily centered around two checkpoint inhibitors that target CTLA-4 and PD-1/PD-L1 (40). CTLA-4 is the first ICI to be used in the clinic. In previous years, researchers have paid more attention to PD-1/PD-L1, leading to progress in basic and clinical research. Due to the high toxicity of iplimumab, an ICI of the CTLA-4 signaling pathway, the probability of irAEs increases (41). The drugs approved by the Food and Drug Administration (FDA) for the treatment of NSCLC include pembrolizumab, nivolumab, avelumab, atezolizumab and durvalumab. In addition, there are several novel ICIs in the developmental and clinical research stages (42,43). In conclusion, immunotherapy is associated with both benefits and challenges. It is therefore crucial to overcome the difficulties in expanding the number of individuals who are able to receive treatment, and to improve the efficacy of immunotherapy.

4. Challenges of immunotherapy in NSCLC

Although immunotherapy presents a promising treatment option, not all patients can benefit from it, and the benefits may not be long-lasting. In the clinic, the objective response rates (ORRs) of PD-1 treatment without the prior screening of patients is only 15-20%, thus only a proportion of patients with NSCLC are suitable for, and benefit from, immunotherapy (44). The primary reason for the low response rate is that suitable patients are not accurately selected prior to treatment. Therefore, it is essential to identify biomarkers that can predict the efficacy of immunotherapy in patients (45). Of the patients that do benefit from immunotherapy, the majority develop resistance, which has a marked impact on future treatment. Therefore, selecting suitable biomarkers and overcoming resistance is necessary for improving therapeutic effects (46).

Biomarkers

PD-L1 expression in tumors. PD-L1 is a potential tumor biomarker, and its predictive value varies in different tumors (47). Pembrolizumab has been approved by the FDA as a first-line treatment for patients with NSCLC with PD-L1 expression ≥50%, and without EGFR or anaplastic lymphoma kinase (ALK) gene mutations (48). A meta-analysis revealed that the higher the expression level of PD-L1, the greater its benefit in patients using anti-PD-1/PD-L1 (49). However, patients with squamous NSCLC are treated with nivolumab regardless of PD-L1 expression level (50). As a biomarker, PD-L1 expression is not omnipotent. At present, PD-L1 detection is widely employed in the clinic, and can be used as a clinical auxiliary or supplementary diagnostic tool to predict the efficacy of immunotherapy (51,52); however, it is not without its challenges.

PD-L1 is a continuous variable, the levels of which can be altered by induction under constant conditions, for example, by cisplatin. Therefore, accurately detecting the level of PD-L1 is challenging (53). Furthermore, surgical resection and biopsy can impact PD-L1 expression, due to the heterogeneity of the tumor (54). The expression of PD-L1 in surgically-removed sections and lung biopsies was analyzed by SP142 immunohistochemistry (IHC) in 160 patients with NSCLC. The results showed that the expression of PD-L1 in lung biopsies was not

Table I. Summary of immune checkpoints.

Checkpoint	Chromosomal location	Number of amino acids	Binding partner	Binding partner expression location	Receptor expression	Trial	Drugs
PD-1	2q37.3	288	PD-L1 (B7-H1) PD-L2 (B7-DC)	Cancer cells, APCs, tumor MDSCs	TILs, effector T cells, T cells, regulatory B cells, NK cells	CheckMate-017 CheckMate-057 KEYNOTE-010 KEYNOTE-021 KEYNOTE-024 KEYNOTE-189 IMpower-131 IMpower-150 Pembro-RT CT02008227 NCT02125461 NCT02395172	Nivolumab Pembrolizumab Atezolizumab Durvalumab Avelumab PDR001 REGN2810 Y3300054 Tislelizumab MGA012 MEDI4736 SHR-1210 AB122
CTLA-4	2q33	239	B7-1 (CD80) B7-2 (CD86)	APCs Tumor MDSCs	Effector T cells, Tregs	CheckMate227 CheckMate-568	Ipilimumab Tremelimumab
LAG-3	12p13.32	498	MHC-II Galectin-3 LSECtin A-synuclein FGL1	APCs	Effector T cells, Tregs, B cells, NK cells, DCs	NCT03250832 NCT02460224 NCT01968109 NCT02966548 NCT03005782 NCT03459222	LAG525 TSR-033 BMS-986016 REGN3767
TIM-3	5q33.2	302	Galectin-9 Ceacam-1 HMGB-1 PtdSer	APCs Cancer cells	Effector T cells, B cells, Tregs DCs, NK cells, Monocytes	NCT03099109 NCT03744468 NCT02608268 NCT02817633	LY3321367 BGB-A425 MBG453 TSR-022
TIGIT	3q13.31	244	CD155 CD112	APCs Cancer cells	T cells, NK cells	AB154 MTIG7192A BGB-A1217	Domvanalimab Tiragolumab BGB-A1217

PD-1, programmed death 1; PD-L1, programmed cell death ligand-1; PD-L2, programmed cell death ligand-2; CTLA-4, cytotoxic T-lymphocyte antigen-4; TIM-3, T cell immunoglobulin and mucin domain 3; LAG-3, lymphocyte activation gene-3; TIGIT, T cell immunoglobulin and ITIM domain; APC, antigen presenting cell; MDSC, myeloid-derived suppressor cell; LSECtin, liver sinusoidal endothelial cell lectin; MHC-II, major histocompatibility complex-II; FGL1, Fibrinogen Like 1; PtdSer, Phosphatidylserine; Tregs, regulatory T cell.

consistent with that in the surgically-removed sections (overall inconsistency rate, 48%), and the expression of PD-L1 in the biopsies was generally lower than that in the sections (55). Finally, the difference between IHC assays also has important implications on PD-L1 detection, as results are inconsistent among different assays. Currently, four IHC assays have been approved by the FDA (28-8, SP263, SP142 and 22C3), and selecting the most suitable one for each patient is crucial. In addition, the scoring system for PD-L1 expression has not been standardized (56,57). In conclusion, clinical detection of PD-L1 demands standardization and improved accuracy.

Tumor mutation burden (TMB). TMB is a potential biomarker for immunotherapy, which is defined as the total number of detected somatic gene coding, base substitution, gene insertion or deletion errors in every million bases (Mb). The principle of TMB in predicting the efficacy of immunotherapy is that tumors with a high TMB may express a greater variety of antigens and have stronger immunogenicity, thus increasing their recognition by, and the killing effect of, cytotoxic CD8^{+} T cells. Therefore, tumors with a high TMB are suitable for immunotherapy (58). In terms of clinical trials, CheckMate-568 revealed that patients with a TMB of >10 mutations (mut)/Mb had higher ORR and longer progression-free survival (PFS) than those with a TMB of <10 mut/Mb, regardless of PD-L1 expression level. The results showed that TMB could be used as a prospective biomarker for patients treated with nivolumab combined with

ipilimumab (59). However, the use of TMB as a biomarker has certain limitations.

In terms of detection methods, whole genome sequencing or whole-exome sequencing (WES) are the standard methods for calculating the TMB (58); their disadvantages include the need for high sample quality, long detection times and high cost, and as such, their wide application in clinical practice is limited (60). A promising and convenient alternative is next-generation sequencing (NGS) (61). In a study by Samstein *et al* (62), integrated mutation profiling of actionable cancer targets (MSK-IMPACT™) was used to sequence the genome. The results showed that for the majority of tumors treated with ICIs, patients with a higher TMB exhibited a higher survival rate (62). The relevant data also showed that the results of quantification of TMB detected by NGS and WES were correlated (63). The FDA has approved two NGS panels (FoundationOne®CDx and MSK-IMPACT) to evaluate TMB. Although NGS has its advantages, in-depth research is required in order to improve accuracy in clinical practice before it can become the standard method for TMB determination (64). Secondly, there is no fixed standard TMB cut-off value. Generally speaking, a TMB of >20 mut/Mb is considered high, whereas a TMB of <10 mut/Mb is considered low. Therefore, it is necessary to determine the optimal cut-off value of each tumor type in more prospective clinical studies and clinical practice (65).

Additionally, 30% of cancer patients face further challenges, including the inability to obtain tumor tissue, insufficient tumor tissue samples and tumor tissue content not meeting the requirements for detection (66). Therefore, a blood-based assay was developed to measure blood tumor mutation burden (bTMB). Blood detection is safer, less costly, and blood samples are easier to obtain than tumor biopsies. Related studies have confirmed that the bTMB can predict the efficacy of atezolizumab in patients with advanced NSCLC (67). However, other studies have shown that the bTMB cannot evaluate the results of immunotherapy (PFS and ORR). Patients with a low bTMB may also benefit from immunotherapy (68). In addition, recent studies have reported that the maximum somatic allele frequency combined with bTMB has a higher predictive effect than bTMB alone in patients with advanced NSCLC treated with atezolizumab (69). bTMB is therefore not a mature biomarker, and further experimentation is required for clinical verification. Although both TMB and bTMB can predict the efficacy of immunotherapy, there are still several obstacles that need to be overcome, including the specification of the detection platform, and the lack of standardization in the evaluation of TMB and bTMB.

At present, there is no single biomarker that can accurately predict the efficacy and prognosis of immunotherapy. In a clinical setting, PD-L1 and TMB are the most commonly used biomarkers, but there are certain limitations regarding their clinical use. It is therefore crucial to continue investigating existing, and identify novel, biomarkers. For example, tumor-infiltrating lymphocytes (TIL), epithelial-to-mesenchymal transition/inflammation signature score, intestinal flora, microsatellite instability high/deficient mismatch repair, gene expression signatures and tumor-specific genotypes are potential biomarkers currently being explored (70,71). However, the identification of a 'perfect' biomarker is unlikely; therefore, assessing the suitability of patients for immunotherapy based on the comprehensive evaluation of multiple indicators is currently the most effective strategy. A study has shown that a combination of human leukocyte antigen (HLA) class I, CD8+ T cell infiltration, PD-L1 expression and tumor mutational load is a promising biomarker for predicting the efficacy of anti-PD-1 in the treatment of NSCLC (72). However, this combination still requires considerable clinical verification to confirm its predictive ability. Therefore, the focus of future studies should be not only on identification of novel biomarkers, but also the investigation of the most effective combination of various existing biomarkers.

Immunotherapy-induced resistance. With the development of immunotherapy, the global use of ICIs has increased; however, the development of drug resistance remains a considerable challenge (73). Resistance can be categorized as acquired or primary, with the probability of primary resistance at ~60% (74). Most patients develop resistance following immunotherapy, which reduces its anticancer effects. Therefore, overcoming resistance is important in improving immunotherapeutic efficacy. Existing literature suggest that the current understanding of resistance mechanisms is limited; the potential mechanisms of resistance (based on existing data) are summarized herein.

Firstly, tumor immunogenicity and TMB are associated with the mechanism of resistance. The necessary conditions for effective immunotherapy include tumor expression of the appropriate antigens and the generation of tumor antigen-specific T cells (75). Tumors with a high immunogenicity (NSCLC, renal cell carcinoma and human melanoma) are more sensitive to immunotherapy (76,77). However, tumors with a low TMB produce fewer tumor antigens and have poor immunogenicity, which negatively impacts the activation of effector T cells, further leading to resistance (78). Prostate cancer has poor immunogenicity and low expression of PD-L1, which is the primary reason for drug resistance (79). Therefore, tumor immunogenicity and TMB are closely associated with the mechanism of resistance. Secondly, immunotherapy restores T cell function by blocking immune checkpoints. However, if tumor cells cannot be identified by T cells, drug resistance ensues. The expression of tumor cell MHC-I is necessary for identification of tumor cells by T cells, and MHC-I loss or downregulation results in tumor immune escape. β_2 m is an integral part of the HLA class I molecule, and is necessary for antigen presentation. Mutations in β_2 m limit the recognition of CD8+ T cells, which results in T cell failure (80). Therefore, a promising solution is to generate synthetic long peptides from tumor-associated antigens, as well as DNA and RNA, in order to develop novel vaccines. The associated mechanism is that the vaccine is transported to MHC-I and MHC-II molecules of antigen-presenting cells, thus promoting CD8 and CD4 T cell responses to overcome resistance (81). In addition, radiotherapy can lead to the independent upregulation of MHC-I, recover antigen presentation and overcome resistance (82). Finally, the pathway of immunosuppression is not only associated with PD-1/PD-L1, but also other pathways with similar functions, such as those of TIM-3, LAG-3, CTLA-4 and B- and T-lymphocyte attenuator (BTLA). In the event of the combined action of these pathways, blockade of one may not be sufficient, as the others pathways will likely compensate for the loss of immunosuppressive signals. Therefore, the combination of multiple ICIs may improve their therapeutic effects (83). In clinical and mouse models, the expression of TIM-3 was

Table II. First-line treatment of NSCLC.

Study	Immunological pathway	Study design	Phase	Condition or disease	Objective response rate, %	Median progression-free survival, months	Median overall survival, months
CheckMate-227	CTLA-4/PD-1	Nivolumab plus ipilimumabvs. chemotherapy	III	Squamous or non-squamous	45.3	7.2	NR
CheckMate-568	CTLA-4/PD-1	Nivolumab plus ipilimumab	II	Advanced/metastatic NSCLC	30	4.2	NR
Keynote-021	PD-1	Pembrolizumab plus pemetrexed carboplatin vs. pemetrexed carboplatin	II	Non-squamous	56.7	24	NR
Keynote-189	PD-1	Pembrolizumab platinum-pemetrexed vs. placebo-platinum pemetrexed	II	Non-squamous	47.6	8.8	NR
IMpower-131	PD-L1	Atezolizumab + carboplatin+ paclitaxel vs. atezolizumab+ paclitaxel vs. atezolizumab+ carboplatin + nab-paclitaxel vs. carboplatin + nab-paclitaxel	III	Squamous	49	6.3	14
IMpower-150	PD-L1	Atezolizumab plus carboplatin plus paclitaxel vs. Atezolizumab plus bevacizumab plus carboplatin plus paclitaxel vs. Bevacizumab plus carboplatin plus paclitaxel	III	Non-squamous	69.3	8.3	19.2
Pembro-RT	PD-1	Pembrolizumab-SBRT vs. Pembrolizumab	III	Metastatic NSCLC	36	6.6	15.9

PD-1, programmed death 1; PD-L1, programmed cell death ligand-1; CTLA-4, cytotoxic T-lymphocyte antigen-4; NSCLC, non-small cell lung cancer.

increased following anti-PD-1 resistance, and the combination of anti-TIM-3 and anti-PD-1 was found to improve survival rates (84,85). Therefore, the combination of multiple ICIs may improve tumor control and overcome resistance.

5. Future solutions and research directions for immunotherapy in NSCLC

Although its affects remain controversial, obtaining a deeper understanding of immunotherapy is necessary for its improvement. Future research directions can be divided into a basic and a clinical aspect. With regard to basic research, key directions may include the identification of novel targets, the effective combination of various biomarkers, and the investigation of resistance mechanisms. Based on current basic research findings, the purpose of clinical research is to combine existing treatment methods to obtain the optimum therapeutic effect. Several studies are currently in progress, which have achieved promising results (Table II), and are described in the following sections.

Combination of multiple treatment options in clinical research Combination of multiple ICIs for NSCLC therapy. PD-1/PD-L1 is one of the signaling pathways of several immune checkpoints, but various other immune checkpoints perform similar functions. The anti-tumor effect can be improved using a combination of multiple ICIs. The combination of anti-PD-1 and anti-CTLA-4 has been widely studied and shown to yield positive results. CheckMate 012 showed that the efficacy of nivolumab + ipilimumab as a first-line treatment was superior to that of nivolumab alone (86). The phase III clinical study CheckMate 227 confirmed that the efficacy of nivolumab + ipilimumab as a first-line treatment was superior to that of traditional chemotherapy. It was also shown that, in patients with a high TMB, the median PFS of the nivolumab + ipilimumab group was longer than that of the chemotherapy group (7.2 vs. 5.5 months) (87). Furthermore, CheckMate 568 revealed an association between the efficacy of nivolumab + ipilimumab as a first-line treatment, and the expression of PD-L1 and TMB. The results indicated that patients with a TMB of >10 mut/Mb had a higher ORR and longer PFS, regardless of PD-L1 expression, providing evidence for the use of TMB as a potential biomarker (5). In addition to the combination of anti-PD-1 and anti-CTLA-4, a number of novel immunotherapy combinations are in the experimental stages.

ICIs combined with chemotherapy. Chemotherapy is a traditional treatment for advanced cancer, which can increase tumor antigen presentation, enhance the activity of effector T cells, and increase the expression of PD-L1 in tumors (88). In addition, the regulatory effect of a variety of chemotherapeutic drugs on immune function has been reported. For example, cisplatin upregulates PD-L1 expression in tumor cells through the PI3K/AKT signaling pathway (53), and pemetrexed can promote the activation of NK cells, increasing the production of IFN-γ (89). Also, docetaxel selectively reduces the number of Tregs and prevents immune suppression (90). Therefore, chemotherapy and immunotherapy have a synergistic effect, suggesting that combination therapy has a superior effect to monotherapy.

A number of studies have shown that as a first-line treatment, the therapeutic benefits of immunotherapy combined with chemotherapy are greater than those of chemotherapy alone. KEYNOTE-021 studied the difference in efficacy between pembrolizumab + pemetrexed-carboplatin (PC) and PC alone. The results showed that the ORR of the combination group was 56.7%, while that of the PC group was 30.2%. Compared with PC alone, PFS and overall survival (OS) following combination therapy also improved significantly (91). Patients were divided into three groups based on first-line treatment: Atezolizumab + carboplatin + paclitaxel, atezolizumab + bevacizumab + carboplatin + paclitaxel (ABCP) and bevacizumab + carboplatin + paclitaxel (BCP) as. In the ITT-WT population (patients with EGFR or ALK alterations were excluded), the mPFS of the ABCP group was significantly higher than that of the BCP group (8.3 vs. 6.8 months). In the WT population which had high expression of an effector T cell (Teff) gene signature in the tumor (Teff-high WT) cases, the mPFS of the two groups was 11.3 and 6.8 months. The results showed that adding atezolizumab to bevacizumab + chemotherapy for non-squamous metastatic NSCLC improved OS and PFS, regardless of PD-L1 expression. In December 2018, the FDA approved atezolizumab + carboplatin + paclitaxel + bevacizumab as a first-line treatment option for patients with advanced metastatic NSCLC without EGFR and ALK mutations (92).

ICIs combined with radiotherapy. For patients with advanced NSCLC, radiotherapy is one of the most conventional and effective treatments, and improves survival rate by increasing the control of primary tumour (93). Radiotherapy can increase tumor antigen release, enhance antigen presentation and promote T-cell infiltration into the tumor tissue, thus enhancing the systemic anti-tumor immune response and altering the tumor microenvironment (94). Radiotherapy can induce immunogenic cell death, enhance the TIL repertoire and upregulate MHC and PD-L1 expression (95,96). In immunotherapy, the expression of PD-L1 plays an important role in predicting the therapeutic effect. A previous study revealed that radiotherapy increases the expression of PD-L1 in tumor cells by activating the PI3K/AKT and STAT3 signaling pathways. In addition, radiotherapy is less toxic, and thus a more favorable option for combined immunotherapy (97).

At present, there are few NSCLC clinical studies on radiotherapy combined with ICIs. PEMBRO-RT is a recent multi-center phase II randomized clinical trial, the purpose of which was to determine whether the use of stereotactic body radiotherapy before pembrolizumab treatment increases the anti-tumor response of patients with metastatic NSCLC. The patients in the experimental arm were treated with pembrolizumab following stereotactic body radiotherapy at a single tumor site, while the control arm were treated with pembrolizumab only. The results showed that the ORR at 12 weeks in the experimental arm was significantly higher than that of the control arm (36 vs. 18%). The mPFS of the experimental arm was 6.6 months, and that of the control arm was 1.9 months. The median OS was 7.6 in the control arm, and 15.9 months in the experimental arm. Although the experimental results showed that the ORR following combination therapy was significantly higher than that of the control arm, the results did not reach the expected standard. In addition, different patient PD-L1 expression levels may also have effeced the experimental results. Therefore, these findings require further experimental confirmation (98). However, based on the existing experimental outcomes, immunotherapy combined with radiotherapy is superior to radiotherapy alone, and does not increase the number of adverse reactions.

ICIs combined with Traditional Chinese Medicine (TCM). In patients with NSCLC, combined immunotherapy can compensate for the deficiencies of immunotherapy alone, allowing increased benefit to a greater number of patients. Combined immunotherapy appears promising, but the current thinking cannot be limited by the existing programs when investigating this research area. TCM has a long history of use in China (99). During the novel coronavirus outbreak in 2020, the majority of hospitals in China used a combination of western and TCM to treat patients, with successful results (100). China has also achieved encouraging results in providing TCM treatment for patients in other countries, which has become an important player in

global disease treatment (101). The tumor environment is an important consideration when treating tumors, as changes therein may directly affect tumor cell proliferation rate, and subsequently, the effects of immunotherapy and occurrence of resistance (102). For the treatment of cancer, TCM has the potential to improve the tumor environment for increased treatment efficacy. Most TCM agents are derived from plants, and thus, have the advantages of low toxicity and multiple targets(diversity of compounds). In addition, TCM includes a specific class of drugs for the improvement of self-immunity, which improve the tumor environment; as such, the primary purpose of systematic treatment for patients with metastatic NSCLC is to reduce the burden of cancer symptoms, and to improve survival, while also maintaining patient quality of life (103). Currently, traditional treatments and immunotherapy cannot achieve satisfactory results in patients with metastatic NSCLC. Therefore, the use of TCM combined with these other treatment types is a promising option for patients with NSCLC, and research into the role of TCM in immunotherapy is ongoing.

Astragalus polysaccharide (PG2) is the active component of the dried roots of *Astragalus membranaceus*. A study showed that PG2 increased the M1/M2 macrophage polarization ratio of H441 and H1299 cells. It also increased the T cell-mediated antitumor immune response by promoting the functional maturity of DCs (104). Adding PG2 to cisplatin, a conventional chemotherapeutic drug, can synergistically increase the antitumor effect (105). According to the above theory, the effective results of immunotherapy combined with PG2 in NSCLC were expected (106). *Phytolacca acinosa* polysaccharides I (PAP-1) is a compound of *Phytolacca*, which has been proven to affect immune functions in mice. It can also enhance the production of IL-2 and NK cytotoxic factor, and exerts antitumor activity (107). It has also been reported that total glucosides of paeony can regulate the expression of PD-1 and PD-L1 in peripheral blood monocytes (108). Thus to conclude, TCM has the potential to positively influence immunotherapy. In addition, various other TCM agents can regulate immunity and antitumor effects. At present, research on the antitumor effects of combining TCM with immunotherapy is being conducted, the findings of which may provide patients with further treatment options and superior benefits.

6. Conclusions

For future clinical trials in cancer, several challenges need to be overcome, including irAEs, low patient response rates and tumor cell resistance to treatment. Although immunotherapy is currently the best option, more promising treatments are expected to arise from ongoing basic and clinical research. Until then, solutions to these problems can be found through the continuous identification of immune checkpoints, and the creation of more favorable combination treatment methods. The research and application of immunotherapy have broad prospects; the discovery of new immune checkpoints and continuous attempts at combination treatments, including that of TCM and immunotherapy, may well be hotspots of future research on NSCLC.

Availability of data and materials

No applicable.

Authors' contributions

PYY and ELHL conceived and designed the study. LRM and JXL wrote the paper. RZL, LT, JSY and AS revised the paper for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

No applicable.

Patient consent for publication

No applicable.

Competing interests

The authors declare that they have no competing interests.

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