Integrative Cancer Therapies
Volume 18: 1–13

DOI: 10.1177/1534735419890020
journals.sagepub.com/home/ict
SAGE

Current Clinical Progress of PD-1/PD-L1 Immunotherapy and Potential Combination Treatment in Non–Small Cell Lung Cancer

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Abstract

Conventional methods in treating non–small cell lung cancer contain surgery, chemotherapy, radiotherapy, and targeted therapy, which have various defects. Recently, with the deeper research on tumor immunity, immunotherapy has made the breakthrough in the treatment of cancers. Especially developments of programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) inhibitors bring the therapy into a new stage. This review mainly focuses on introducing existing monoclonal antibodies containing nivolumab, pembrolizumab, atezolizumab, avelumab, and durvalumab, along with 3 ordinary biomarkers such as PD-L1 expression, tumor mutation burden, and microsatellite instability. By understanding the resistance mechanism of anti-PD-1/L1 blockade, research is further improving the survival benefit and expanding the benefit population. So, PD-1/PD-L1 inhibitors begin to be combined with various therapeutic strategies clinically. Discussion and comparison of their effectiveness and safety are also comprehensively reviewed. Meanwhile, we explore the potential, the impact, and mechanisms of combining traditional Chinese medicine with immunotherapy.

Keywords

non–small cell lung cancer, immunotherapy, PD-1/PD-L1 inhibitor, biomarker, combinational therapy, Chinese herbal medicine, review

Submitted July 12, 2019; revised October 12, 2019; accepted October 29, 2019

Introduction

Lung cancer, which is the most common malignancy in the clinic, is a main cause of death worldwide. Non–small cell lung cancer (NSCLC), including squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, accounts for over 85% of lung cancer cases and has attracted substantial attention as well as pharmaceutical investment.[1] Nowadays, treatments of NSCLC mainly includes surgery, chemotherapy, radiotherapy (including hypofractionated or stereotactic radiotherapy), and targeted therapy (especially drugs targeting antiangiogenesis as well as the epidermal growth factor receptor [EGFR] mutations). Surgical treatment is to remove the cancerous tissue while cleaning the nearby lymph nodes, and it is only suitable for early stages. Resection rates for stages I and II range from 49% to 77%.[2,3] Chemotherapy is a method of using conventional chemotherapeutic agents to increase antigenicity via immunogenic cell death of tumor cells as well as to augment antitumor immunosurveillance in the tumor microenvironment (TME), but chemotherapy also damages the normal tissue cells of the body and it has the problem of resistance.[4] Similarly, radiotherapy is used as a treatment for irradiating tumors and killing tumor cells with different energy rays. Over time, stereotactic body radiation therapy (SBRT) has

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become a replacement for conventional radiotherapy, providing high doses of radiation in a small fraction with great precision.[5] However, patients with SBRT have a higher incidence of lung toxicity than patients who have not received it.[6] Targeted therapy that specifically targets drugs have been identified at a carcinogenic site, allowing tumor cells to die without affecting surrounding normal tissue cells. Tyrosine kinase inhibitors (TKIs) for patients harboring epidermal growth factor receptor (EGFR) mutations have remarkably improved clinical results. Antiangiogenic agents targeting the vascular endothelial growth factor (VEGF) and VEGF receptor are also useful methods to treat NSCLC. Nevertheless, tumor-targeting therapy also has a large defect, that is, the targeted drug is often only effective for tumors at specific mutation sites, and tumor cells often have multiple mutation sites.[7]

These above-mentioned treatments all have various disadvantages, and fewer than 5% of patients are alive 5 years later; the median survival is ~10 months.[8] Thus, there is a compelling need to develop therapies for enhancement of antitumor effect in those with NSCLC. In recent years, with the further study of the antitumor immunity, especially when the 2018 Nobel Prize in Physiology or Medicine Prize was awarded to American scientist James P. Allison and Japanese scientist Tasuku Honjo in recognition of their pioneering contributions made in tumor immunotherapy, a promising therapeutic approach about using immune checkpoint inhibitors (ICIs) that reverse cancer immunosuppression for the treatment of NSCLC has been discovered.[7] Particularly successful examples are drugs that interfere with the programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) pathway that many tumors are able to hijack to avoid immune surveillance and editing. To be specific, PD-1 is highly expressed on the surface of activated T-cells in response to inflammation or infection, while PD-L1 is expressed on the surface of tumor cells. When PD-1 is linked to its ligand PD-L1, the complex pathway acts to suppress the immune response by inhibiting the cytotoxic T-cell response. Tumor cells can use this pathway to promote immunosuppression, thereby evading antitumor activity. PD-1 or PD-L1 inhibitors disrupt this inhibitory T-cell signaling, thereby reactivating the antitumor activity of specific cytotoxic T-cells.[9-11] At the same time, cancer biomarkers are important to immunotherapy because they are characteristic biochemical indicators of tumor pathology or treatment processes that can be measured objectively and contribute to screening, diagnosis, and prediction.

Despite some success, the majority of cancer patients who receive PD-1/PD-L1 blockades will not respond, and only small proportion of patients (<30%) may have benefit from immune checkpoint monotherapy. Thus, this fact has led investigators to prioritize more efficacious combinatorial therapies.[12] This review chiefly summarizes 5 anti-PD-1/L1 inhibitors as well as 3 main biomarkers; then we highlight the combination of PD-1/L1 inhibitors with the existing cancer treatments, including targeted therapy, chemotherapy, radiotherapy, and other immunotherapy in NSCLC due to the current limitation of monoclonal antibodies. Because some Chinese herbal formulas or single compounds can decrease the toxicity and may improve overall survival (OS), we also want to explore the effect and mechanisms of combining Chinese herbal medicines with anti-PD-1/PD-L1 antibody for NSCLC patients.

Current Clinical Available PD-1/PD-L1 Antibodies for NSCLC Therapy

Since the first immunological checkpoint inhibitor was approved by the Food and Drug Administration (FDA) for lung cancer, numerous clinical trials are underway. There are currently 5 monoclonal anti-PD-1/PD-L1 antibodies, nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab, approved in advanced NSCLC by the US FDA.

First, nivolumab is a humanized immunoglobulin (Ig) G4 PD-1 ICI antibody.[13] It was initially approved in squamous cell lung cancer irrespective of PD-L1 expression, and the subsequent approval in non–squamous cell lung cancer was also specified.[14] Two similar phase III trials, CheckMate-017 and CheckMate-057, comparing nivolumab against a standard second-line chemotherapy agent, docetaxel, have demonstrated the benefit of nivolumab. Second, pembrolizumab is a humanized IgG4 antibody against PD-1.[13] It was approved in platinum-refractory advanced NSCLC by the FDA in 2015 and is the only checkpoint inhibitor currently approved for first-line advanced NSCLC.[14] Furthermore, it was originally approved in those with high PD-L1 expression (Tumor Proportion Score ≥50%), and the FDA modified their approval for it as second-line therapy to include those with PD-L1 >1% based on the results of the KEYNOTE-001 and KEYNOTE-010 trials.[9,15] In 2017, pembrolizumab plus chemotherapy such as carboplatin has been approved for the first-line treatment of metastatic/advanced non–squamous non–small lung cancer regardless of PD-L1 expression and with no EGFR or ALK genomic tumor aberrations. Third, atezolizumab is a humanized engineered IgG1 monoclonal antibody targeting PD-L1, being the first and currently the only anti-PD-L1 antibody approved in NSCLC. It is efficient and safe for second-line treatment of both advanced squamous and adenocarcinoma histologies irrespective of PD-L1 status. The OAK trial highlighted the efficacy of atezolizumab in second-line treatment of NSCLC, suggesting that there would be an interest of using this anti-PD-L1 inhibitor in postprogression prolongation of survival.[14,16] Moreover, durvalumab, as a humanized IgG1 anti-PD-L1 antibody, is still in ongoing studies in the first-line metastatic NSCLC therapy. One of the trials, PACIFIC, exhibited positive progression-free survival (PFS) results,

demonstrating that it can be used for maintenance treatment of patients with locally advanced (stage III) unresectable NSCLC without disease progression following platinum-based chemoradiation therapy.[14,17] Last but not least, avelumab is a fully human IgG1 antibody, also still in ongoing studies. Avelumab monotherapy is currently being compared with docetaxel as first-line therapy in patients with PD-L1 expressing in the phase III JAVELIN Lung 100 trial and as second-line therapy in the phase III JAVELIN Lung 200 trial, showing improved OS.[18]

As we all know, traditional chemotherapeutics usually induce adverse events (AEs) such as fatigue, cough, and diarrhea, while anti-PD-1/L1 inhibitors have less. However, when using this immunotherapy, immune-related AEs including colitis, pneumonitis, hepatitis, endocrinopathy, skin toxicity, and arthralgia are commonly encountered. Corticosteroids are the mainstay for management of immune-related AEs, and the use of them does not necessarily change the efficiency of anti-PD-1/L1 inhibitor therapy.[19,20] Currently, a large number of completed or ongoing clinical trials are carried out across the world for evaluating the efficacy and safety of multiple ICIs as monotherapy, and partial results are presented in Table 1.[15,17,21-27] Thus, from the results, we can find that compared with regular chemical agents, anti-PD-1/PD-L1 agents have higher response rates as well as improved PFS and OS. Furthermore, patients who were treated with atezolizumab had much higher median OS than with atezolizumab, pembrolizumab, and nivolumab. PFS is generally positively correlated with OS and has been used as an alternative indicator of OS in some studies. From those clinical trials referred to in Table 1, patients treated with durvalumab had higher PFS than with pembrolizumab, showing the good effect in durvalumab. At the same time, the frequency of AEs of grade 3 or 4 about ICIs is mostly much lower than chemical agents (Table 1).

Current Available Valid Biomarkers to Predict Responses to PD-1/PD-L1 Therapy and Their Limitations

Despite the success of ICIs, not all patients have long-term responses and the response varies between different patients. Considering irreversible autoimmune toxicities, accurate patient selection will become more crucial. So there remains an urgent need to find reliable biomarkers to help determine patients who will benefit from ICIs. Nowadays PD-L1 expression by immunohistochemistry (IHC), overall tumor mutational burden (TMB) along with microsatellite instability (MSI) have emerged as the 3 most commonly used clinical biomarkers.

PD-L1 Expression by Immunohistochemistry

It is well known that PD-L1 expression on tumor cells predicts responsiveness to PD-1 inhibitors, and overexpression of it by IHC staining has been linked with higher response rates and better results. Hence, we can conclude that the higher the expression of PD-L1 on tumor cells, the better the curative effect is, which can guide clinical decision-making. Currently, 5 clones including 22C3, 28-8, SP142, SP263, and 73-10 are being used for PD-L1 IHC testing (Table 2).

The Dako 22C3 pharmDx is the only IHC kit assay that has obtained "companion" diagnostic status by the US FDA for the therapeutic method with pembrolizumab.[28] Meanwhile, the Dako 28-8 pharmDx has gained the European Conformity–mark certification. It also gains the US FDA approval as a "complementary" diagnostic tool for the use of nivolumab.[29] At the same time, the Ventana SP142 assay is CE-marked and is approved as "complementary" diagnostic instrument by the US FDA for atezolizumab.[30] Furthermore, the Ventana SP263 assay has also gained the CE certification and US FDA "complementary" test only for patients with urothelial carcinoma who can get benefit from durvalumab treatment.[31,32] Finally, the Dako 73-10 assay is commercialized to support avelumab therapy, and it is still in development.[33] All indications, assessment conditions, and PD-L1 positivity criteria are exhibited in detail in Table 2.

Each PD-L1 diagnostic testing assay uses its own platform, antibody, custom reagents, and scoring criteria to calculate Tumor Proportion Score on tumor cells. Meanwhile, each antibody binds to a specific epitope, and each assay has a unique cutoff to forecast the drug response, indicating that the interchangeability of assays might be limited. Honestly speaking, there is no gold standard assay that can precisely predict PD-1 blockade response. Thus, several cross-validation studies such as the Blueprint project are ongoing, and they will be key to achieve standardization among different IHC assays. Although it remains a major challenge, it is pivotally important to assist clinicians to select the best treatment options for patients.

Overall Tumor Mutational Burden

Tumor mutation burden is defined as the total number of detected errors in somatic gene coding, base substitution, gene insertion, or deletion per million bases. It is a quantitative biomarker that reflects the total number of mutations carried by tumor cells. Nonsynonymous somatic mutations change the amino acid sequence of proteins that are encoded by affected genes, forming neoantigens that can help the immune system to recognize tumors and stimulate the increase in the number of anticancer T-cells as well as the ability of antitumor response.[36-38] TMB is assayed for FoundationOne samples, and results are listed as follows: TMB-low corresponds to equal or less than 5 mutations per megabase, while TMB-high corresponds to equal or greater than 20 mutations per megabase.[39]

In several clinical trials with PD-1/L1 inhibitors, data about the potential of TMB have been assessed by whole

Table 1. Select Anti-PD-1/L1 Clinical Trials in NSCLC.

Therapeutic Antibody	Trial	Phase	Sample Size (n)	Patient Population	Treatment Arms	Efficacy Endpoint(s) Results	Grade 3 or 4 Adverse Events	Reference	Other Phase III or IV Clinical Trials of Each Antibody
Nivolumab (anti-PD-1)	CheckMate-017 (NCT01642004)	III	272	Previously treated metastatic squamous NSCLC	Nivolumab versus docetaxel	Median OS 9.2 months versus 6.0 months; HR = 0.59; $P < .001$	7% versus 55%	21	NCT02041533
	CheckMate-057 (NCT01673867)	III	582	Previously treated metastatic non–squamous NSCLC	Nivolumab versus docetaxel	Median OS 12.2 months versus 9.4 months; HR = .73; $P = .002$	10% versus 54%	22	
Pembrolizumab (anti-PD-1)	KEYNOTE-010 (NCT01905657)	II/III	1034	Previously treated, PD-L1 positive, metastatic NSCLC	Pembrolizumab 2 mg/kg versus pembrolizumab 10 mg/kg versus docetaxel	Median OS (2 mg/kg) 10.4 months versus 8.5 months; HR = 0.71; $P = .0008$ Median OS (10 mg/kg) 12.7 months versus 8.5 months; HR = 0.61; $P < .0001$	13% versus 35%; 16% versus 35%	15	NCT03134456 NCT02220894 NCT02864394 NCT03302234 NCT02504372 NCT02775435 NCT02578680
	KEYNOTE-021 (NCT02039674)	II	120	Previously untreated metastatic NSCLC	Pembrolizumab + carboplatin + pemetrexed versus carboplatin + pemetrexed	ORR 55% versus 29%; median PFS 13 months versus 8.9 months; HR = 0.53; $P = .01$	39% versus 26%	23	
	KEYNOTE-024 (NCT02142738)	III	305	Previously untreated, PD-L1–positive, metastatic NSCLC	Pembrolizumab versus platinum-based chemotherapy	Median PFS 10.3 months versus 6.0 months; HR = 0.5; $P < .001$	26.6% versus 53.3%	24	
Atezolizumab (anti-PD-L1)	OAK (NCT02008227)	III	850	Previously treated metastatic NSCLC	Atezolizumab versus docetaxel	Median OS 13.8 months versus 9.6 months; HR = 0.73; $P = .0003$	15% versus 43%	25	NCT02813785 NCT02367781 NCT02409342 NCT02486718 NCT02367794 NCT03191786 NCT02409355 NCT02657434 NCT03456063
	IMpower150 (NCT02366143)	III	1202	Previously untreated metastatic NSCLC	Atezolizumab + bevacizumab + CP versus bevacizumab + CP	Median PFS 8.3 months versus 6.8 months; HR = 0.62; $P < .0001$	25% versus 19%	26	
Durvalumab (anti-PD-L1)	PACIFIC (NCT02125461)	III	713	Locally advanced unresectable NSCLC, after chemoradiotherapy	Durvalumab versus placebo	Median PFS 16.8 months versus 5.6 months; HR = 0.52; $P < .001$	29.9% versus 26.1%	17	NCT02352948 NCT03003962 NCT02453282 NCT02273375 NCT02542293 NCT03164616
Avelumab (anti-PD-L1)	JAVELIN Lung 200 (NCT02395172)	III	792	Previously treated, PD-L1–positive, metastatic NSCLC	Avelumab versus docetaxel	Median OS 11.4 months versus 10.3 months; HR = 0.90; 1-sided $P = .16$	10% versus 49%	27	NCT02576574

Abbreviations: CP, carboplatin + paclitaxel; HR, hazard ratio; NSCLC, non–small cell lung cancer; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Table 2. Summary of PD-L1 Monoclonal Antibodies and Technical Aspects for Evaluation and FDA's Approval in NSCLC.

PD-L1 mAb Clone	Ab Host Species	Automated Platform	Checkpoint Inhibitor (Target)	FDA Status	Indication	Reference	Definition of Positivity
28-8	Rabbit	Dako	Nivolumab	Complementary	Second-line NSCLC	29, 34	TC >1% (minimum of 100 TC)
SP142	Rabbit	Ventana	Atezolizumab	Complementary	Second-line NSCLC	30	TC >50% or IC >10% (minimum of 50 TC with associated stroma)
22C3	Mouse	Dako	Pembrolizumab	Companion	Second-line and first-line NSCLC	28	TC >1% (minimum of 100 TC)
SP263	Rabbit	Ventana	Durvalumab	FDA approval only for urothelial carcinoma	Locally advanced NSCLC	31, 32, 35	TC >25% (minimum of 100 TC)
73-10	Rabbit	Dako	Avelumab	FDA approval	Still in development	33	TC >1% (minimum cells are not defined)

Abbreviations: Ab, antibody; companion, provides information critical to the effective use of the corresponding drug or biological product within the approved label; complementary, provides additional information on how to use the drug, but it is not required; FDA, US Food and Drug Administration; IC, immune cell; mAb, monoclonal antibody; NSCLC, non–small cell lung cancer; PD-1, programmed cell death receptor-1; PD-L1, programmed cell death ligand-1; TC, tumor cell.

exome sequencing traditionally. For the first time, Rizvi et al reported that higher mutation load was associated with longer PFS, improved objective response, and durable clinical benefit in advanced NSCLC patients treated with pembrolizumab.[40] Similarly, in the randomized phase III trial (CheckMate-026), which compared nivolumab with chemotherapy in first-line NSCLC, results showed that patients with high mutational burden had higher objective response rate (ORR) and longer PFS.[41]

However, at present, whole exome sequencing is not widely available because of its costly and time-consuming characteristic.[14] Although TMB is an intrinsic objective measurement of tumor, the intratumoral heterogeneity and dynamic change over time make it imperfect. Sometimes patients derive benefit despite "low" mutational burden, while many patients do not benefit despite "high" mutational burden.[41] In addition, because of different techniques used to measure TMB, it is necessary to standardize assay methods.

Microsatellite Instability

Microsatellites are tandem repeats of units of 1 to 6 base pairs that are widely found in the genome. In the entire genome of the tumor, there are some microsatellites with many small genetic mutations, which means deficient DNA mismatch repair, resulting in some microsatellites being unstable. This phenomenon is MSI, and tumors can be further developed through the MSI pathway due to mismatched gene repair defects.[42] MSI testing has been widely used in patients with colorectal cancer, and exploration in other tumors continues. MSI may also plays a role in endometrial, ovarian, skin, brain, and upper gastrointestinal tumors.

Fluorescent multiplex polymerase chain reaction, genome- or exome-wide sequencing, next-generation sequencing, and IHC for mismatch repair proteins are used for testing MSI.[43] The 1997 National Cancer Institute consensus meeting advised to test a core panel of 5 markers: BAT26, BAT25, D5S346, D2S123, and D17S250. At the same time, the MSI-high was defined in the rule about there were over 30% of the markers showing instability in other marker groups or 2 or more of the 5 markers of the core team showing instability.[44]

Colorectal cancer is a relatively mature disease in MSI research. In comparison to patients having non-MSI colorectal cancers, especially when the tumors are at the early stage, we can know that patients with high MSI have more favorable survival and the survival advantage of MSI gradually weakens as the tumor stage advances.[45]

In any case, the purpose of "precise" medical treatment is to accurately find the cause of the disease and the target of treatment. MSI testing effectively achieves accurate medical treatment for specific types of cancer patients and improves the cure rate.

To sum up, in addition to the 3 biomarkers described above, recent studies have identified several potential biomarkers such as lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin domain–containing-3 (TIM-3), TIGIT, tumor infiltrating lymphocyte,[46] and so forth. In general, it is obvious that one single biomarker cannot be used to predict the benefit of immunotherapy completely. Further work needs to be carried out to explore more biomarkers and understand their mechanisms.

Classical Drug-Resistant Mechanisms of Anti-PD-1/L1 Blockades

In 2016, immunotherapy was named the largest scientific breakthrough in the world by *Science* magazine in the United States. PD-1/PD-L1 monoclonal antibodies have successfully subverted traditional anticancer patterns. However, not all

patients benefit from it, or they do not work at all, or they can only maintain a short-term effect mainly because of resistance. Thus, it is urgent for us to understand mechanisms of the resistance to PD-1/L1 inhibitors.

Ascierto et al found that the LAMA3 gene expression activity of tumors that were ineffective against PD-1 immunotherapy was increased by about 2000-fold, and the activity of the CXCR2 gene was also increased 4-fold through sequencing the entire exome.[47] In another study, it has been shown that substances produced by CXCR2 inhibited T-cell function, while T-cells were major anticancer immune cells.[48] The team of Professor Antoni Ribas explored the effect of JAK1/JAK2 gene function loss on the body's immune antitumor response from in vitro cell experiments. Results indicated that the JAK1/JAK2 gene mutation directly led to the insensitivity of tumor cells to the killing effect of interferon, thereby promoting the resistance of tumor cells to PD-1 inhibitors.[49] Similarly, the β-2 microglobulin gene (B2M), as a component of the major histocompatibility complex (MHC)-I molecule, played an important role in the immunogenic antigen presentation process. Mutations in the B2M gene might block CD8 T-cell recognition and were also the cause of anti-PD-1 resistance.[50]

In addition to classical mechanisms such as neoantigen loss,[51] and which have referred to above, HLA loss,[52] TGF-β overexpression,[53] along with increased TIM-3, TIGIT, and LAG-3 expression[46] are related to resistance mechanism. More intriguingly, the research team at the University of Pennsylvania, through careful and rigorous work, discovered that some cancer patients were not sensitive to PD-1/PD-L1 antibodies for the simple reason that their tumors were too big. The larger the tumor was, the more Ki-67-positive killer T-cells would exist in the blood, which induced the resistance.[54]

In short, PD-1 antibody can only block the "hole" of PD-1. After that, the tumor will evade the immune system by other means. So, there are many limitations to apply PD-1/PD-L1 monoclonal antibodies for curing NSCLC. What we need to do now is to chase and intercept the roads at various intersections.

Clinical Combination Strategies to Treat NSCLC

There is a large consensus among doctors and researchers that both PD-1 and PD-L1 inhibitors are only approximately 20% effective in treating lung cancer. Therefore, in order to expand the beneficiary population, combination therapy is a major trend. Not only can the combination of different treatments produce a large antitumor synergy, but also it is able to reduce the dosage of each treatment as well as to avoid the emergence of drug resistance.[55] Promising clinical and preclinical evidence for combined therapy in NSCLC has been reported (Table 3).[56]

Combining PD-1/L1 Inhibitors With Radiotherapy

In the past, when NSCLC patients were unable to undergo surgical resection of tumor lesions, segmental radiation therapy was performed. With the development of new radiotherapy techniques, SBRT and stereotactic ablative radiotherapy (SABR) technology appear with characteristics of great precision and high doses of radiation.[57] Studies have found that SBRT can induce the expression of MHC-I, inflammatory factors, costimulatory molecules, heat-shock proteins, immunoregulatory factors, and cell adhesion molecules, which results in promoting antigen presentation and enhancing the immune system's response to tumors.[58] During radiotherapy, immunotherapy can also upregulate the immune system and increase the interaction of cytotoxic lymphocytes (CTL) with cancer cells.[59] Thus, radiotherapy has become one of the ideal options for synergistic effects in combination with PD-1/PD-L1 blockades.

The method of combining SABR with PD-1 inhibitors can make the original tumor almost completely disappear. At the same time, the distant lymph nodes and metastases also disappear. This phenomenon is called the "abscopal effect," which explains the rationale behind their usage in brain metastasis.[59,60] Currently, in the treatment of NSCLC, clinical trials evaluating the enhanced effect of radiotherapy on immunotherapy are underway (Table 3).[56] Results show that combination of immunotherapy with radiotherapy has improved ORR, PFS, and OS compared with ICIs alone. However, a higher frequency of pulmonary toxicity exists in combination therapy.[61]

Combining PD-1/L1 Inhibitors With Chemotherapy

Current chemotherapeutic agents such as cisplatin, carboplatin, taxanes, and pemetrexed are able to increase antitumor immune responses by a variety of mechanisms. Chemotherapy enhances CD8+ T-cells, mutational load, neoantigen heterogeneity, the maturation of APC, and a higher PD-L1 expression on tumor cells, augmenting tumor antigen presentation via immunogenic cell death of tumor cells and the MHC class I. At the same time, it downregulates immunosuppressive cells including CD4, CD25, and regulatory T-cells in the TME.[4,62-66]

Currently, there are limited data on clinical trials of PD-1/PD-L1 antibody combined with chemotherapeutic drugs in the treatment of NSCLC. For instance, the KEYNOTE-021 study was a phase II research studying the activity of pembrolizumab with carboplatin and paclitaxel or platinum-doublet chemotherapy alone on 123 untreated patients with stage III B or IV non–squamous cell NSCLC. Results showed that the ORR of the combination therapy group and the chemotherapy-alone group were 55% and

Table 3. Ongoing Combination PD-1/L1 Trials in NSCLC.

Other Strategy With PD-1/L1 Inhibitors	References
SBRT (stereotactic body radiation therapy)	NCT02888743, NCT02407171, NCT02608385, NCT02400814, NCT02444741, NCT02831933, NCT03004183, NCT02492568, NCT02904954, NCT02599454, NCT03035890, NCT03110978, NCT03050554
Chemotherapy	Phase 1: NCT01840579, NCT02998567, NCT02451930, NCT01454102, NCT03064854, NCT02309177
EGFR-TKIs	Phase 1/2 or 2: NCT02382406, NCT02422381, NCT02733250, NCT02574078, NCT02039674, NCT01903993, NCT02250326, NCT02574598, NCT02581943, NCT02591615, NCT02684461, NCT02716038, NCT02967133, NCT02944396, NCT03041181, NCT03057106, NCT03081689, NCT03083808 Phase 3: NCT02477826, NCT03134872, NCT02367781, NCT02578680, NCT02657434, NCT02775435, NCT02864251, NCT02008227, NCT02367794, NCT02813785, NCT02366143, NCT03117049, NCT02013219, NCT01998126, NCT02088112, NCT02364609, NCT02947386, NCT02143466, NCT02454933, NCT02630186, NCT02924233, NCT02323126, NCT02574078, NCT01454102, NCT02900664
VEGF-antiangiogenic drugs	NCT02681549, NCT02366143, NCT02574078, NCT01454102, NCT03117049, NCT02443324, NCT02501096, NCT02856425, NCT03006887, NCT02484404, NCT03083041
CTLA-4 inhibitors	Tremelimumab (Phase 3): NCT02352948, NCT02453282, NCT02542293 Ipilimumab (Phase 2): NCT03083691, NCT03001882, NCT02350764, NCT02659059, NCT03091491 Ipilimumab (Phase 3): NCT02477826, NCT02785952, NCT02864251, NCT02869789, NCT02998528
Anti-LAG-3 antibody	NCT01968109
Anti-TIM-3 antibody	NCT02608268
IDO1 inhibitors	NCT02327078, NCT02318277, NCT02298153

Abbreviation: NSCLC, non–small cell lung cancer.

29%, respectively ($P = .0016$); the median PFS was significantly prolonged in the combination group (13.0 months vs 8.9 months; hazard ratio [HR] = 0.53, $P = .010$). Referring to the adverse effects, in the combination treatment group, 39% of patients had grade 3 or higher AEs, while in the chemotherapy-alone group, 26% of patients had.[23] Similarly, in a clinical phase I study of nivolumab and 3 platinum-based chemotherapy drugs (CheckMate012), 56 stage IIIB or IV NSCLC patients were enrolled. Grouped by tumor histological types, patients received different doses of nivolumab in combination with gemcitabine-cisplatin (squamous cell carcinoma), pemetrexed-cisplatin (non–squamous cell carcinoma), and paclitaxel-carboplatin (both histologies). In the group of nivolumab (10 mg/kg) combined with PT-DC, the OS time was 11.6 to 19.2 months, which was significantly longer than that of the platinum monotherapy group ($P < .01$). There was also a significant prolongation of OS in nivolumab (5 mg/kg) combined with paclitaxel-carboplatin. Among them, 45% of patients had a grade 3/4 treatment-related adverse reaction.[67] In addition, the combinations of atezolizumab or durvalumab with different chemotherapy have also been tested, exhibiting improved ORR and more severe treatment-related AEs.[68,69]

In a word, combining chemotherapy with ICIs has a stronger improvement in outcomes for advanced NSCLC patients than using ICIs alone, but adverse side effects are much more serious. It is worth noting that the use of hormones before chemotherapy has become a standardized treatment for alleviating adverse reactions such as nausea and vomiting, but the use of hormones may prevent ICIs from functioning.[70] Therefore, the treatment of adverse reactions is a major challenge in the future.

Combining PD-1/L1 Inhibitors With Targeted Therapy

Although targeted drug therapy takes effect rapidly, it inevitably produces resistance; immunotherapy, such as PD-1/PD-L1 inhibitors, has a slow onset, but it can play a long-lasting role due to the generation of memory cells. Hence, targeted therapies for tumor angiogenesis and intrinsic driver genes in combination with immunotherapy may be a

direction for the treatment of NSCLC with clear genetic mutations.

Epidermal growth factor receptor is the most common driver gene in NSCLC, and around 50% of Asian NSCLC patients, especially those with lung adenocarcinoma, have mutations in the EGFR gene.[71] EGFR gene mutation is able to upregulate the expression of PD-L1 in tumor cells; EGFR-TKI can downregulate the expression of PD-L1 by inhibiting the signaling pathway of nuclear transcription factor-κB (NF-κB).[72,73] Chen et al found that PD-1/L1 inhibitors can significantly reduce the viability of H1975 cells that are resistant to gefitinib,[72] suggesting that immunotherapy combined with TKI may be an alternative for the treatment of patients with EGFR gene mutation in NSCLC, especially those who are resistant to EGFR-TKI. Clinically, a total of 21 patients with NSCLC who had not received chemotherapy for stage IIIB/IV EGFR mutations were enrolled in the trial NCT01454102. Nivolumab was given with erlotinib for a median follow-up time of 71.9 weeks. In this study, researchers found that there were 4 cases of grade 3 to 4 treatment-related adverse reactions, mainly including elevated aspartate transaminase and alanine aminotransferase. Nivolumab combined with erlotinib resulted in a clinical ORR of 19% and the 24-week PFS rate of 47%. Of the 20 patients with erlotinib-acquired resistance, 3 (15%) had partial tumor remission and 9 (45%) had stable response. The above-mentioned interim data indicate that nivolumab combined with erlotinib can provide sustained and safe clinical benefit for patients with NSCLC who are EGFR-mutant, especially those with TKI resistance.[74]

The growth and metastasis of tumors are inseparable from their angiogenesis. The regulatory molecules that mediate tumor angiogenesis are mainly VEGF, platelet-derived growth factor, and fibroblast growth factor.[75] The VEGF pathway not only acts on the vascularization process of the tumor but also mediates the inhibitory immune microenvironment, thereby allowing tumor cells to escape immune surveillance. This, therefore, provides a theoretical basis for the combination of checkpoint inhibitors and antiangiogenic drugs. Researchers recently did a phase III IMpower150 study of bevacizumab (monoclonal antibody against VEGF) and atezolizumab (anti-PD-L1 antibody) in patients with advanced non–squamous NSCLC. Results showed that patients who accepted the atezolizumab and bevacizumab plus chemotherapy had a longer PFS compared with those who accepted bevacizumab plus chemotherapy (8.3 vs 6.8 months, $P < .0001$). Similarly, the ORR was 64% versus 48%.[76]

Much clinical research about PD-1/PD-L1 inhibitors in combination with targeted therapy is being gradually carried out in NSCLC (Table 3).[56]

Combining PD-1/L1 Inhibitors With Other Immunotherapies

CTLA-4 is the earliest discovered immunological checkpoint that is expressed only on T-cells and inhibits T-cell responses when it binds to B7 on antigen-presenting cells. The CTLA-4 inhibitor ipilimumab enhances immune system function by releasing checkpoints and by using antibody-dependent cytotoxicity to eliminate inhibitory T-cells inside the tumor.[77] One clinical IB phase, multicenter study (NCT02000947) showed that different doses of durvalumab plus tremelimumab were administered to 102 patients with NSCLC who had not accepted immunotherapy before enrollment. The median follow-up time was 18.8 weeks; 36% of patients developed AEs, and finally 28% of patients discontinued due to treatment of AEs; 63 patients had an ORR of 17%. The study found that durvalumab (20 mg/kg) + tremelimumab (1 mg/kg) is the maximum dose that can be tolerated. Based on the results of this study, this tolerated dose gradient will be expanded for clinical phase III studies in the future. This clinical trial also concluded that the antitumor effect of the combination does not depend on the degree of PD-L1 expression and might, therefore, provide a new treatment option for those patients with negative PD-L1 expression.[78]

In addition, anti-LAG-3 protein, anti-TIM-3 antibody as well as anti-indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor are novel immune checkpoint blockades. LAG-3 antibodies may reverse T-cell depletion and then activate antitumor immune responses. A preclinical study showed that a single LAG-3 antibody could not restore T-cell function, but combined with the PD-1 inhibitor, it could inhibit the growth of ovarian tumors.[79,80] TIM-3 and PD-1 are coexpressed in tumor-infiltrating CD8 + T-cells in mice, making it unable to produce interleukin-2 (IL-2), tumor necrosis factor, and interferon-γ. Using these 2 checkpoint inhibitors maximally inhibited tumor growth compared with using one inhibitor alone.[81,82] IDO1 is highly expressed in a variety of cancers, inhibiting T-cell function through metabolites and then promoting immune escape. Preclinical data supported the hypothesis that blocking IDO1 may be an antitumor strategy. In a number of homologous mouse models, inhibition of IDO1 leads to tumor control.[83,84]

In brief, based on the results of current immunotherapy research, the combination of most immunotherapy is based on PD-1/PD-L1 inhibitors, so the choice of another immunosuppressant "companion" is particularly important. They selectively act on the TME and tumor-draining lymph nodes to exert synergistic antitumor effects with PD-1/PD-L1 inhibitors.

In conclusion, many combination drug regimens have entered the stage of clinical trials and achieved satisfactory initial results. However, there are still some problems that

need to be deeply considered. For instance, the mode of combination administration (simultaneous or sequential administration) is particularly important. At the same time, selection of doses in combination therapy and treatment of serious adverse reactions should also be attended to. Patients receiving combination therapy are likely to experience delayed response to treatment, with a process in which the tumor first increases and then becomes smaller. This "pseudoprogression" is determined as disease progression; therefore, it is necessary to discuss and develop new standards to evaluate the effectiveness of the combined therapy.

New Insight: Potential Cancer Therapy With Herbal Extracts or Formulas Related to the Immune System

In the development of traditional Chinese medicine, many effective herbs have been well documented to treat cancers. They have advantages of improving patients' functional status, clinical symptoms, immune function, and survival rate. At the same time, they induce fewer adverse reactions and are suitable for long-term treatment. To be specific, several herbal extracts mainly focus on treating NSCLC by the immune system. For instance, triptolide, extracted from *Tripterygium wilfordii* Hook F, inhibits NF-κB activation to have antitumor effects.[85] At the same time, ginsenoside Rg3, isolated from ginseng, can decrease the PD-L1 expression as well as restrain NF-κB p65, being deemed as a new agent in chemotherapy refractory NSCLCs.[86] On the other hand, dendritic cells (DCs) added with ginseng polysaccharides decrease the expression of Th2 cytokines and increase the expression of Th1 cytokines, playing a major role in patients' immune function.[87] Meanwhile, *Astragalus* polysaccharide combined with vinorelbine and cisplatin activate mouse B-cells and macrophages. They also prolong survival time.[88] In addition, traditional Chinese medicine compounds have characteristics of multitarget influence. Pang et al[89] found that Bu-Fei decoction achieved antitumor effect by interfering with the relationship between cancer cells and tumor-associated macrophages through suppressing the expression of PD-L1 and IL-10. Furthermore, Supplementing Qi and Nourishing Yin decoction (SQNY) can significantly increase the number of mature DCs in the spleen tissue, thereby activating T lymphocytes, producing an effective immune response, and prompting the body to actively exert anticancer effects. At the same time, SQNY can improve the effect of chemotherapy drugs on intestinal microecology of lung cancer mouse model and can make certain changes in the proportion of gut microbiota, which may be related to enhancing the antitumor immune function of the body.[90] Thus, traditional Chinese herbs and compounds play important roles in the cancer treatment.

Since anti-PD-1/PD-L1 agents have good curative effect on NSCLC patients, some researchers combine them with herbal extracts to study the rate of AEs. For example, combining *Viscum album* L extracts with ICIs has been studied to evaluate the safety profile of ICIs combined with herb extracts. Although *Viscum album* L application does not alter ICI-induced AE rates, it is a good attempt that should encourage more research in the future.[91]

In a word, the unclear effective components of herbs or compounds and unclear mechanism of action of combination therapy limit the widespread application and promotion of traditional Chinese medicine in the treatment of advanced NSCLC. So, more research needs to be carried out in order to explore new strategies that are both effective and safe.

Conclusion and Perspectives

To sum up, the treatment of lung cancer has a long way to go. And the emergence of immunotherapy, especially checkpoint inhibitors such as PD-1/PD-L1 antibody, has brought good news to patients with advanced NSCLC. But there are still some problems that have to be dealt with. For example, first, we should understand the dosing, frequency, and sequential treatment algorithm to use combination strategies as well as discover other effective combination strategies. It is also significant for us to avoid combinations that lack efficacy and lead to increased toxicity. Second, in addition to biomarkers that we referred above, novel and more precise immune predictive biomarkers are supposed to be found for patient selection. Third, improving the accuracy of combination therapy with detailed analysis of different stages of NSCLC, patients' age and TME as well as different kinds of metastatic cancers is necessary.

In recent years, there appear more and more advanced technology and novel methods being used to treat NSCLC. First of all, the National Comprehensive Cancer Network guidelines recommend broad-spectrum gene sequencing for patients to detect rare driver mutations and seek to participate in other targeted therapies. In multivariate analysis, patients who receive this technology are more likely to receive immunotherapy. Moreover, the birth of imaging mass cytometry has a profound impact on the research of tumor immunity. We can identify the immune cells in the tumor tissue and know what subgroup they belong to, and also know their location in the tissue, including which cells surround it. More important, the gut microbiota can affect tumor development through a variety of ways: mainly by regulating the host's innate immune system and increasing DC function to treat tumors; regulating the host metabolism; or directly contacting with tumor tissues. Especially *Bifidobacterium* in the gut microbiota of mice has anticancer effects and can promote the efficacy of anti-PD-L1 immunotherapy. Besides, network pharmacological studies

discover the mechanism of interference of Chinese compound formula or Chinese herb extracts on the "pathogenic network" from the perspective of overall bionetwork balance, and then predict the pharmacodynamic active ingredients and multitargets of Chinese herbal medicines to prevent and treat cancers. They may be involved in the regulation of cell signal transduction pathways, potential pharmacological mechanisms, and prescription compatibility. Finally, animal or cell experiments can be done to reveal the modern pharmacological mechanisms of herbal prevention and treatment, and develop new antitumor auxiliary agents. All in all, using novel research methods to treat NSCLC is of great importance and integrating Chinese medicine with Western medicine treatment is a breakthrough point.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is funded by The Science and Technology Development Fund, Macau SAR (File No. 0096/2018/A3) and The Faculty Research Grant Projects of Macau University of Science and Technology (File No. FRG-19-028-FC).

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