# Combination strategies of immunotherapy in non-small cell lung cancer: facts and challenges

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**Abstract**

Immunotherapy has dramatically altered the treatment of non-small cell lung cancer. Currently, the emergence of combination strategies in immunotherapy has brightened the prospects of improved clinical outcomes and manageable safety profiles in the first/second-line settings. However, sub-optimal response rates are still observed in several clinical trials. Hence, alternative combination models and candidate selection strategies need to be explored. Herein, we have critically reviewed and commented on the published data from several clinical trials, including combined immunotherapy and chemotherapy, anti-angiogenic agents, epidermal growth factor receptor/anaplastic lymphoma kinase tyrosine kinase inhibitors, radiotherapy, and other immune checkpoint inhibitors.

**Keywords:** Immunotherapy; Programmed death 1/programmed death ligand 1; Immune checkpoint inhibitors; Non-small cell lung cancer

## Introduction

Lung cancer is the most commonly encountered cancer and is a leading cause of cancer-related deaths worldwide,[1] with approximately 80% of the cases being non-small cell lung cancer (NSCLC). In the past decade, immune checkpoint inhibitors (ICIs) such as programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1) inhibitors have dramatically altered the therapeutic strategy of NSCLC.[2] Based on previous clinical trials, pembrolizumab and nivolumab (PD-1 inhibitors) and atezolizumab (PD-L1 inhibitor) have been approved by the Food and Drug Administration (FDA) for the first/second-line treatment of metastatic NSCLC, while durvalumab (PD-L1 inhibitor) has been approved for stage III unresectable NSCLC.

PD-L1 is an immunosuppressive molecule expressed by tumor cells (TCs). PD-1/PD-L1 axis induces T-cell apoptosis and inhibits anti-tumor immunity in the tumor microenvironment (TME),[3,4] thereby accelerating the infiltration of cancer cells. Agents targeting PD-1/PD-L1 are designed to treat NSCLC by regulating anti-tumor immunity.

Despite the advances in immunotherapy, acquired resistance and limited efficacy are observed during single ICI therapy.[5] Recent studies have established that traditional therapies such as chemotherapy and/or targeted therapy alter the immunogenic patterns.[6] Therefore, efforts have been made to combine different strategies of ICIs with chemotherapy, anti-angiogenic therapy, targeted therapy, or therapy using agents targeting other immune checkpoints to enhance the treatment efficacy. This review has summarized the available data on combination therapy using ICIs, with an emphasis on candidate selection and adverse events [Table 1].

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## Immunotherapy Combined with Other Treatments

### *Efficacy of ICIs when combined with chemotherapy*

Accumulating evidence has indicated that chemotherapy augments the immunologic effects of ICIs via various molecular mechanisms [Figure 1].[7] Chemotherapy can induce immunogenic cell death, which releases danger-associated molecular patterns and tumor-associated antigens to recruit antigen-presenting cells.[8] Thus, tumor infiltrating CD8+ T-cells are cross-primed, whereas myeloid derived suppressor cells and regulatory T cells are downregulated.[9-11] This complex regulation of immune cells (ICs) is accompanied by an increase in PD-L1 expression or tumor antigen presentation.[12] Based on these findings, several clinical trials have been conducted to investigate the efficacy and safety of ICIs when combined with platinum-based chemotherapy.

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**Table 1: Several results from clinical trials evaluating the combination strategies of anti-PD-(L)1 agent.**

| Agents | Study | Line | Subtype | Treatment arms | ORR (*P*) | PFS (months) | PFS HR (95% CI, *P*) | OS (months) | OS HR (95% CI, *P*) |
|---|---|---|---|---|---|---|---|---|---|
| Pembrolizumab | KEYNOTE-021 | First-line | NSq | Cohort G: Pembro + pemetrexed-carboplatin<br>Pemetrexed-carboplatin | 58% *vs.* 33% | 24.5 *vs.* 9.9 | 0.54 (0.35–0.83) | 34.5 *vs.* 21.1 | 0.71 (0.45–1.12) |
| | KEYNOTE-189 | First-line | NSq | Pembro + pemetrexed-cisplatin/carboplatin<br>Pemetrexed-cisplatin/carboplatin | 48.0% *vs.* 19.4% (<0.001) | 9.0 *vs.* 4.9 | 0.48 (0.40–0.58, <0.001) | 22.0 *vs.* 10.7 | 0.56 (0.45–0.70, <0.001) |
| | KEYNOTE-407 | First-line | Sq | Pembro + carboplatin-paclitaxel/nab-paclitaxel<br>Carboplatin-paclitaxel/nab-paclitaxel | 62.6% *vs.* 38.4% | 8.0 *vs.* 5.1 | 0.57 (0.47–0.69, <0.001) | 17.1 *vs.* 11.6 | 0.71 (0.58–0.88, <0.001) |
| | PROLUNG | Second-line | NSq + Sq | Pembro + docetaxel<br>Docetaxel | 42.5% *vs.* 15.8% (0.01) | 9.5 *vs.* 3.9 | 0.24 (0.13–0.46, <0.001) | – | – |
| | KEYNOTE-598 | First-line | NSq + Sq | Pembro + ipilimumab<br>Pembro + placebo | 42.4% *vs.* 42.4% | 8.2 *vs.* 8.4 | 1.06 (0.86–1.30, 0.72) | 21.4 *vs.* 21.9 | 1.08 (0.85–1.37, 0.74) |
| | KEYNOTE-799 | First-line | A: NSq + Sq<br>B: NSq | A: Pembro + pacilitaxel-carboplatin + radiotherapy<br>B: Pembro + pemetrexed-cisplatin + radiotherapy | 69.6%<br>70.5% | – | – | – | – |
| Nivolumab | CheckMate 227 | First-line | NSq + Sq | Nivo + ipilimumab<br>Platinum-based chemotherapy | 36.4% *vs.* 30.2%* | – | – | 17.1 *vs.* 14.9* | 0.79 (0.67–0.93, 0.033)* |
| | CheckMate 9LA | First-line | NSq + Sq | Nivo + ipilimumab + chemotherapy<br>Chemotherapy | 38.2% *vs.* 24.9% | 6.7 *vs.* 5.0 | 0.68 (0.57–0.82) | 15.6 *vs.* 10.9 | 0.66 (0.55–0.80) |
| Atezolizumab | IMpower130 | First-line | NSq | Atezo + carboplatin-nab-paclitaxel<br>Carboplatin-nab-paclitaxel | 49% *vs.* 32% | 7.0 *vs.* 5.5 | 0.64 (0.54–0.77, <0.0001) | 18.6 *vs.* 13.9 | 0.79 (0.64–0.98, 0.033) |
| | IMpower131 | First-line | Sq | Atezo + carboplatin-nab-paclitaxel<br>Carboplatin-nab-paclitaxel | 49.7% *vs.* 41.0% | 6.3 *vs.* 5.6 | 0.71 (0.60–0.85, 0.0001) | 14.2 *vs.* 13.5 | 0.88 (0.73–1.05, 0.1631) |
| | IMpower132 | First-line | NSq | Atezo + pemetrexed-cisplatin/carboplatin<br>Pemetrexed-cisplatin/carboplatin | 47% *vs.* 32% | 7.6 *vs.* 5.2 | 0.60 (0.49–0.72, <0.0001) | 17.5 *vs.* 13.6 | 0.86 (0.71–1.06, 0.1546) |
| | IMpower150 | First-line | NSq | Atezo + carboplatin-paclitaxel<br>Bevacizumab-carboplatin-paclitaxel | 63.5% *vs.* 48.0% | 8.3 *vs.* 6.8 | 0.62 (0.52–0.74, <0.001) | 19.2 *vs.* 14.7 | 0.78 (0.64–0.96, 0.02) |
| Durvalumab | MYSTIC | First-line | NSq + Sq | Durva + tremelimumab<br>Platinum-based chemotherapy | 34.4% *vs.* 37.7% | 3.9 *vs.* 5.4 | 1.05 (0.72–1.53, 0.71) | 11.9 *vs.* 12.9 | 0.85 (0.61–1.17, 0.20) |
| | PACIFIC | First-line | NSq + Sq | Durva + chemoradiotherapy<br>Placebo + chemoradiotherapy | 28.4% *vs.* 16.0% (<0.001) | 16.8 *vs.* 5.6 | 0.52 (0.42–0.65, <0.001) | Not reach *vs.* 29.1 | 0.69 (0.55–0.86, 0.20) |

*PD-L1 TPS ≥1%. CI: Confidence interval; HR: Hazard ratio; NSq: Non-squamous; ORR: Objective response rate; OS: Overall survival; PD-(L)1: Programmed death (ligand) 1; PFS: Progression free survival; Sq: Squamous; TPS: Tumor proportion score.

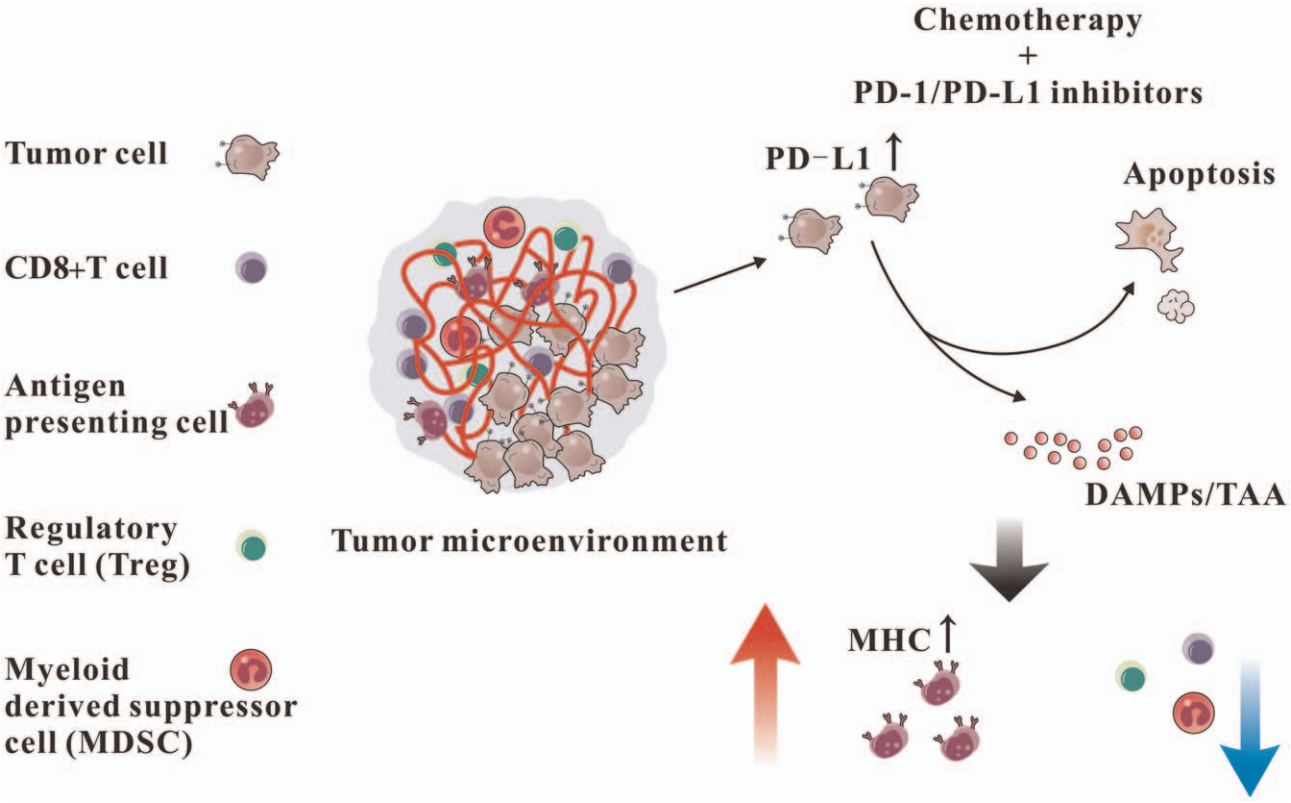


**Figure 1:** Effect of chemotherapy on the modulation of tumor microenvironment. DAMPs: Danger-associated molecular patterns; MHC: Major histocompatibility complex; PD-1: Programmed death 1; PD-L1: Programmed death ligand 1; TAA: Tumor-associated antigen.

KEYNOTE-021,[13] a multi-center, open-label, randomized clinical trial was designed to compare the efficacies of pembrolizumab plus carboplatin-pemetrexed, and carboplatin-pemetrexed. The findings revealed that pembrolizumab with carboplatin-pemetrexed was the most effective combination. After a median follow-up period of 49.4 months, cohort G demonstrated significant improvement with pembrolizumab plus carboplatin-pemetrexed *vs*. carboplatin-pemetrexed in objective response rate (ORR) (58% *vs*. 33%) as well as progression-free survival (PFS) (24.5 *vs*. 9.9 months; hazard ratio [HR]: 0.54; 95% confidence interval [CI]: 0.35–0.83). However, the overall survival (OS) of the two groups was comparable.[14] Based on this trial, the FDA accelerated the approval of pembrolizumab in combination with carboplatin plus pemetrexed for the first-line treatment of non-squamous NSCLC. KEYNOTE-189[15] validated this finding in previously untreated metastatic non-squamous NSCLC lacking epidermal growth factor receptor (*EGFR*) or anaplastic lymphoma kinase (*ALK*) mutations. Crossover to pembrolizumab was allowed at progression. The latest updated analysis of a median follow-up of 23.1 months revealed that the OS was significantly prolonged in the pembrolizumab-combination group when compared with the placebo-combination group at 22.0 *vs*. that at 10.7 months, respectively (HR: 0.56; 95% CI: 0.45–0.70).[16] Meanwhile, both studies performed exploratory analysis based on the level of PD-L1 expression, which indicated that pembrolizumab combined with chemotherapy exhibited better performance regardless of PD-L1 expression. Similar OS improvement was observed for squamous NSCLC patients treated with pembrolizumab plus chemotherapy over placebo plus chemotherapy (17.1 *vs*. 11.6 months) in KEYNOTE-407.[17]

Atezolizumab is another PD-L1 monoclonal antibody offering substantial clinical benefit when combined with chemotherapy. The clinical trial IMpower130[18] was designed to evaluate the efficacy and safety of atezolizumab plus chemotherapy and compare it with chemotherapy alone as a first-line therapy for metastatic non-squamous NSCLC. The latest results suggested that combining atezolizumab with chemotherapy enabled significant improvement in both OS (18.6 *vs*. 13.9 months) and PFS (7.0 *vs*. 5.5 months), except in the liver metastases subgroup and *EGFR*/*ALK* mutation subgroup. Furthermore, 45% of the patients in the combination group suffered from immune-related adverse events (irAEs), most of which were grade 1 or 2. Two other trials, IMpower131[19] and IMpower132,[20] highlighted the superiority of the combination therapy, asserting that atezolizumab combined with carboplatin and nab-paclitaxel (A + CnP) or carboplatin/cisplatin and pemetrexed (APP) significantly improved the PFS but not OS in first-line squamous and non-squamous NSCLC settings when compared with platinum-based chemotherapy.

Immunotherapy combined with chemotherapy has been immensely successful in the first-line setting. Some combinations provided benefits for patients with disease progression after traditional platinum-based chemotherapy. A phase II trial, PROLUNG,[21] demonstrated that,

when compared to docetaxel alone, pembrolizumab combined with docetaxel improved the ORR (42.5% *vs.* 15.8%) and PFS (9.5 *vs.* 3.9 months) in NSCLC patients who had previously undergone chemotherapy, including those with *EGFR* mutations. KEYNOTE-789 is an ongoing clinical study comparing the efficacies of pembrolizumab plus platinum-based chemotherapy and platinum-based chemotherapy alone after the progression of first-line targeted tyrosine kinase inhibitors (TKIs) in advanced NSCLC patients with *EGFR* mutation. The data have not yet been officially published, and we look forward to the final performance.

### Efficacy of ICI combinations

CheckMate 012[22] was a phase I study designed to assess the efficacy and safety of nivolumab plus ipilimumab for NSCLC. Patients receiving different doses of nivolumab plus ipilimumab as first-line therapy were compared in all the PD-L1 expression cohorts. The results implied that the performance of ICI combination therapy was superior, particularly in the cohorts with positive PD-L1 expression. A retrospective analysis of CheckMate 012 uncovered that a higher tumor mutation burden (TMB) assessed by whole exome sequencing (WES), which was independent of PD-L1 levels, was correlated with superior ORR and PFS in the immunotherapy-combination arm.[23] In another phase III trial, CheckMate 227,[24] stage IV or recurrent NSCLC patients were randomly assigned in a 1:1:1 ratio. Patients with PD-L1 ≥1% received nivolumab plus ipilimumab, nivolumab, or chemotherapy alone, whereas those with PD-L1 <1% received nivolumab plus ipilimumab, nivolumab plus chemotherapy, or chemotherapy alone. The median OS was observed 17.1 months with nivolumab plus ipilimumab and 13.9 months with chemotherapy alone. A 3-year update revealed that patients with PD-L1 ≥1% derived survival benefit from nivolumab plus ipilimumab *vs.* chemotherapy (HR: 0.79; 95% CI: 0.67–0.93); this improvement in health-related quality of life was also observed in patients treated with nivolumab plus ipilimumab.[25,26] CheckMate 9LA further investigated whether the addition of a limited course (two cycles) of chemotherapy to this combination would enhance the benefit. Recently, it revealed prolonged OS in the experimental group than that in the control group (15.6 *vs.* 10.9 months).[27]

On the other hand, KEYNOTE-598 compared the efficacy of pembrolizumab plus ipilimumab *vs.* that of pembrolizumab alone for NSCLC with PD-L1 tumor proportion score ≥50%. As a result, the addition of ipilimumab to pembrolizumab did not improve the overall efficacy with the median PFS of 8.2 and 8.4 months for pembrolizumab-ipilimumab and pembrolizumab-placebo treatments, respectively. In addition, the combination therapy was associated with greater toxicity than monotherapy.[28] Another three trials (MYSTIC, NEPTUNE, and ARTIC) have evaluated the combination of durvalumab and tremelimumab (either completed or ongoing). Recently, the open-label, phase III randomized MYSTIC[29] trial compared durvalumab with or without tremelimumab and chemotherapy as the first-line therapies for metastatic NSCLC patients (except in the case of *EGFR* or *ALK* alterations). A total of 1118 patients were randomized, with OS and PFS being the primary endpoints. It was disappointing that this study did not meet the primary endpoints of prolonged OS or PFS with the use of immunotherapy combinations. Even though the results are yet to be officially published, a recent press release from AstraZeneca revealed that this combination strategy did not meet its endpoint and that it failed to show improved survival in NEPTUNE[30] as well. Another trial, ARTIC,[31] also failed to improve the clinical benefit in the combination arm.

Past studies with ICI combinations mentioned earlier provided completely different bipolar outcomes, which has raised some questions, for example, whether it is "less is more" or "the more the better." The combination of nivolumab and ipilimumab suggested advantages over monotherapy at multiple endpoints, while the combination of pembrolizumab and ipilimumab or durvalumab and tremelimumab indicated more serious AEs. As a result, some questions are raised, for example, whether there are any differences between different PD-(L)1 monoclonal antibodies; how can one select from different options for the advantaged population; and how can further extend research in this field.

### Efficacy of ICIs when combined with anti-angiogenics

Anti-angiogenic agents block the vascular endothelial growth factor (VEGF), thereby exerting immunomodulatory effects, including promotion of the infiltration of T cells in the tumor, acceleration of dendritic cell maturation, and prevention of the immunosuppressive TME.[32] Current studies focusing on the cross-talk between the tumor and its microenvironment have provided opportunities for combining ICIs and anti-angiogenics.

The clinical trial IMpower150[33] demonstrated improved PFS and OS with the use of atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP) when compared with bevacizumab plus carboplatin plus paclitaxel (BCP) in chemotherapy-naïve patients independent of the PD-L1 expression. Similarly, the LEAP-006[34] trial evaluated the first-line lenvatinib (multiple-receptor TKI of VEGFR 1–3, fibroblast growth factor receptor 1–4, platelet-derived growth factor receptors α, c-kit, and rearranged during transfection proto-oncogene [RET]) with pembrolizumab plus chemotherapy for metastatic non-squamous NSCLC. This trial is ongoing and expected to enroll approximately 714 patients across countries. WJOG11218L/APPLE[35] is another ongoing study that aims to explore the efficacy of atezolizumab with platinum-pemetrexed and with/without bevacizumab for non-squamous NSCLC.

Notably, the ABCP regimen in IMpower150 also provides clinical benefits for patients with *EGFR* mutations. These data together suggest that an understanding of the PD-1/PD-L1 axis and its associated regulatory proteins can be leveraged to improve the treatment efficacy, including for those who do not respond well to ICIs monotherapy. In the future, for patients harboring sensitive *EGFR* mutations, we could explore the options of combination therapy and determine suitable treatment options.

### Efficacy of ICIs combined with TKIs

In the treatment-naïve NSCLC patients with *EGFR* or *ALK* alterations, EGFR/ALK TKIs remain the standard treatment. Most prospective clinical trials have also excluded patients with *EGFR* mutations or *ALK* rearrangements. Therefore, only limited randomized data were available in this regard.

KEYNOTE-021[36] tested the efficacy of pembrolizumab in combination with TKIs as the first-line therapy for advanced NSCLC patients containing sensitizing *EGFR* mutation. The study unearthed that the combination treatment group exhibited longer median PFS than the group receiving first generation EGFR-TKIs or osimertinib (19.5 *vs*. 11.0 *vs*. 19.2 months). The CheckMate 012[37] trial involved the *EGFR*-mutant subgroup treated with nivolumab and erlotinib. A total of 21 NSCLC patients, 20 of whom were pretreated with TKIs, were enrolled. According to the interim data, the ORR was 15% and five had treatment-related grade 3 toxicities. Similar safety data have been reported for the durvalumab plus gefitinib (grade 3–4 toxicities, 20%)[38] and atezolizumab plus erlotinib (grade 3–4 toxicities, 39%)[39] cohorts in TKI-naïve patients.

The phase 1b TATTON study evaluating the combination of durvalumab with osimertinib in patients with *EGFR* mutation was terminated because of the occurrence of interstitial pneumonia (38%). Owing to toxicity concerns, the phase 3 CAURAL trial comparing the combination of durvalumab and osimertinib with osimertinib alone in *EGFR* T790M positive NSCLC patients was also prematurely closed.

Another study, CheckMate 370, explored the efficacy and safety of nivolumab combined with crizotinib in NSCLC patients with *ALK* rearrangements. Severe hepatic toxicities were observed in the nivolumab plus crizotinib cohort (38%).[40] However, atezolizumab combined with alectinib only resulted in tolerable side effects related to the skin (18.9%).[41] Based on the outcomes of these clinical trials, it could be inferred that the combination of ICIs and TKIs is associated with a high incidence of toxicities. Overall, the efficacy and safety of the combined use of ICIs and EGFR/ALK-TKIs remain controversial.

### Efficacy of ICIs combined with radiotherapy

Radiotherapy can kill cancer cells while simultaneously triggering pro-inflammatory mediators that release and increase ICs infiltration, thereby turning immunologically "cold" tumor into a "hot" one.[42] Based on this mechanism, radiotherapy, through its immuno-modulating effect, represents a promising combination alternative with ICIs.

PACIFIC[43] study compared durvalumab as a consolidation therapy with placebo in patients with unresectable stage III disease with non-progression after ≥2 cycles of platinum-based concurrent chemoradiation therapy (CCRT). Updated OS data (median follow-up duration: 33.3 months) demonstrate the long-term clinical benefit with durvalumab when compared with placebo (not reaching *vs*. 29.1 months; HR: 0.69; 95% CI: 0.55–0.86).[44] LUN 14-179 is another study that evaluated pembrolizumab as a consolidation therapy after CCRT in stage-III NSCLC patients. The primary endpoint, which is the time to metastatic disease or death, reached 30.7 months after the median follow-up of 32.2 months, which was significantly longer than the control period of 12.0 months.[45] Similar to the PACIFIC study and the LUN14-179 study, the KEYNOTE-799 demonstrated promising anti-tumor activity and tolerable toxicity of pembrolizumab plus CCRT in patients with unresectable stage-III NSCLC. This study was unique in bringing the time of immunotherapy forward from the maintenance to the synchronization treatment of CCRT.[46]

Presently, on the basis of these researches, the exploration of ICIs plus radiotherapy treatment in NSCLC continues to move forward. The efficacy and safety of this combined immunotherapy strategy are expected to be verified in more phase III studies.

### Efficacy of ICIs when combined with other agents

Apart from combinatory therapies with chemotherapy, anti-angiogenics, other ICIs, TKIs or radiotherapy, emerging compounds which have been specifically designed to modulate the co-stimulatory/inhibitory receptors currently in combination with PD-(L)1 have been studied in combination therapy with ICIs. According to their targets, drugs have been classified into lymphoid inhibitor, lymphoid stimulators, and non-lymphoid inhibitors, non-lymphoid stimulators.[47]

T-cell immunoglobulin- and mucin-domain-containing molecule 3 (TIM-3) is a co-signaling molecule expressed by several ICs, which were verified to be associated with resistance to PD-1 inhibitors. Therefore, some clinical trials (NCT03099109, NCT03307785, NCT03311412, etc) are presently exploring TIM-3 inhibitor in combination with PD-(L)1 inhibitors for NSCLC.[48] Other lymphoid inhibitors including lymphocyte activation gene 3 protein inhibitor (NCT02460224, NCT02966548, NCT03849469, etc), T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain inhibitor (NCT02794571, NCT03628677, NCT03119428, etc),[49] and adenosine signal receptor blockade (A2AR, A2BR) (NCT04262856, NCT02403193, etc) were also tested for the safety and efficacy with PD-(L)1 inhibitor in NSCLC.

Indoleamine 2,3-dioxygenase (IDO) is a cytosolic enzyme. The upregulation of the IDO expression was observed after PD-L1 treatment, which indicated a candidate mechanism of resistance to ICIs.[50] IDO inhibitor belongs to non-lymphoid inhibitor. Several phase I clinical trials are investigating the efficacy and safety of IDO inhibitors, both in monotherapy and in combinatorial strategy in NSCLC (NCT02327078, NCT03085914, NCT02862457, NCT02178722, etc).[51] Clinical trials on combination therapies with lymphoid stimulators including OX40 agonists (NCT02410512, NCT03241173, etc) and glucocorticoid-induced tumor necrosis factor receptor-related

gene agonists (NCT02740270), and non-lymphoid stimulators including anti-semaphorin 4D (SEMA4D) (NCT03268057), and NKTR-214 (NCT02983045, NCT03138889) are underway.

In addition to these immune modulators, the use of the combination of anti-PD-(L)1 therapy with other agents is also an attractive approach for NSCLC treatment. Chimeric antigen receptor (CAR)-T-cell therapy, as novel agents of immunotherapy, shows great promise for NSCLC. Currently, autologous CAR-T cells targeting PD-L1 and CD80/86 are being tested for the treatment of recurrent NSCLC in a phase I study (NCT03060343). Anti-PD-L1 CAR-T-cell therapy has also been used in metastatic PD-L1-positive NSCLC (NCT03330834).[52] Histone deacetylase inhibitors can enhance tumor immunogenicity through various mechanisms. A past study demonstrated that the combination of pembrolizumab plus vorinostat was associated with a considerably higher ORR than pembrolizumab monotherapy (NCT02638090).[53] JASPER (NCT03308942) is a phase-II study that has explored the combination of anti-PD-1 and niraparib (a poly adenosine diphosphate [ADP] ribose polymerase inhibitor) in metastatic NSCLC.[54] It showed that niraparib plus pembrolizumab induced durable response in NSCLC without new adverse events.

These studies represent the most promising therapies. Although these new therapies are still in the early phases of exploration, there is a need to undertake additional preclinical and clinical studies and be cautious in drawing conclusions.

## Comments and Future Challenges

### *Patient selection*

#### PD-L1

Although immunotherapy has been successful in the treatment of NSCLC, the response rate remains suboptimal with ICIs. More than half of the unselected patients fail to benefit from immunotherapy.

To date, assessing PD-L1 expression by immunohistochemistry (IHC) is the only diagnostic test for ICIs that has been approved by the FDA. The expression of PD-L1 was determined by evaluating the percentage of TCs and ICs exhibiting a certain membrane expression intensity. In clinical trials, it was noted that PD-L1 expression is not an ideal biomarker for immunotherapy. Some patients with highly positive PD-L1 expression failed to benefit from ICIs, whereas about 10% of NSCLC patients with negative PD-L1 expression demonstrated reasonable response to ICIs.[55-57] Similarly, NSCLC patients with PD-L1-negative tumors might benefit equally or more from a combination regimen,[16,25] but some patients with PD-L1 tumor proportion score (TPS)≥50% did not acquire improved efficacy.[58] This finding could be attributed to variable PD-L1 IHC assays (22C3, 28-8, SP142, and SP263 for pembrolizumab, nivolumab, atezolizumab, and durvalumab, respectively) and platforms (Dako and Ventana). The Blueprint project[59,60] was designed to examine the consistency of different IHC assays, and revealed that the 22C3, 28-8, and SP263 assays were highly aligned but not SP142. In addition, surgically resected specimens and biopsies showed inconsistent PD-L1 expression.[61]

The use of PD-L1 as a marker in immunotherapy has some challenges. For the clinical application of PD-L1 testing, there are strict requirements regarding the quality and source of specimens. Moreover, no antibody has so far been approved by the National Medical Products Administration for clinical testing and there is also a lack of standards for PD-L1 IHC specifications. Therefore, owing to interobserver difference, technological limitations, and tumor heterogeneity, a more appropriate diagnostic and reporting process is urgently needed for more extensive PD-L1 testing.

#### TMB and neoantigens

In recent years, TMB has been considered as another candidate biomarker since some somatic mutations generate tumor neoantigens that can be better recognized and captured by the immune system through protein translation. This view was supported by the findings of CheckMate 026[62] and CheckMate 227.[24] After the failure of CheckMate026, the researchers retrospectively stratified the population based on TMB and found that it was a potential biomarker for improved outcomes from nivolumab treatment. TMB analysis might be useful in candidate selection for immunotherapy plus chemotherapy combination. Nivolumab plus ipilimumab combination was also considered as a possible treatment algorithm for NSCLC with high TMB levels (≥10 mut/Mb). However, the application of TMB is controversial. Only 57.7% of the samples were adequate for TMB testing in Checkmate 227 trial.[63] Furthermore, TMB partially reflects tumor neoantigens, which is more closely related to the response rate of the treatment. The Memorial Sloan Kettering Cancer Center team studied the clinical and genomic data of 1662 advanced cancer patients treated with ICIs and 5371 patients not treated with ICIs. They found that even though high TMB is a predictor of efficacy, it is not a prognostic factor.[64] In addition, the long time and high costs associated with TMB and the decision regarding the use of whole-exome sequencing (WES) or panel detection are issues that need to be addressed in future research.

Recently, a novel blood-based assay evaluating TMB in plasma (bTMB) was retrospectively assessed in OAK and POPLAR trials.[65] It was discerned that bTMB reproducibly identified patients with significantly improved PFS in second-line atezolizumab treatment. In the B-F1RST trial of atezolizumab as well as in the MYSTIC[29] trial of durvalumab plus tremelimumab, bTMB was noted to possess clinical value and was found to be more feasible than TMB.

Some studies based on the affinity of new antigens to major histocompatibility complex (MHC) or the similarity of new antigens to known antigens and the machine learning of second-generation sequencing have introduced new antigen prediction models.[66,67] Intra-tumoral heterogeneity (ITH), an uneven distribution of genomic diversifi-

cation in tumor, was also confirmed as a biomarker for immunotherapy efficacy prediction. However, these potential biomarkers are yet to be tested in clinical practice.

Owing to the complexity of tumor-immune interactions, intra-tumoral heterogeneity, and the cutoff value for "high" TMB definition, TMB alone does not explicitly identify all NSCLC patients who are likely to benefit from immunotherapy. The dynamic monitoring of TMB or neoantigen fitness models directly based on MHC binding affinity might be the future trend in response prediction.

### Tumor infiltrating lymphocytes (TILs)

TILs are infiltrating ICs in the TME that mediate anti-tumor immune response.[68] According to ICs infiltration, tumors can be divided into inflamed and non-inflamed types. The presence of TILs, high levels of interferon (IFN) γ-producing CD8+ T-cells, and positive PD-L1 expression in TILs are characteristics of inflamed tumors.[69] Several researchers have reported that TILs are predictive biomarkers in immunotherapy. Tumeh *et al*[70] established the correlation between the efficacy of PD-1 inhibitor and TILs in melanoma. In serially sampled tumors, patients responding well to PD-1 inhibitor showed upregulated levels of intratumoral CD8+ T-cells rather than CD4+ T-cells, which is directly correlated with a reduction in tumor size. In the OAK trial, PD-L1 expression in TILs was determined to be an independent predictor of benefitting from atezolizumab.[71] Teng *et al*[72] stratified tumors into four types based on the expression of TILs and PD-L1 as follows: type I: TILs+ PD-L1+; type II: TILs+ PD-L1−; type III: TILs− PD-L1+; and type IV: TILs− PD-L1−. Preliminary studies have reported that type I is more likely to benefit from PD-1/PD-L1 inhibitors. Notably, TILs have not been proposed as a biomarker for choosing the combination regimen of immunotherapy. Currently, a preclinical study has found that TILs reflect radiation-induced DNA damage in hepatocellular carcinoma.[73] In lung cancer mice models, radiotherapy could also enhance the function of TILs, which was strengthened in combination with anti-PD-L1.[74] In the future, more supporting data are still needed.

### Genomics feature

Microsatellite instability and mismatch repair (MMR) deficiency are also emerging as indicators for treatment with ICIs. Genetic alterations encoding MMR proteins such as MutS Homolog 2 (MSH2), MutS Homolog 6 (MSH6), and postmeiotic segregation increased 2 (PMS2), which lead to DNA replication errors, have been proven to influence the response to immunotherapy.[75] Similarly, other DNA repair alterations such as DNA polymerase epsilon (POLE) and DNA polymerase delta (POLD) are also associated with the clinical benefit of immunotherapy. So far, as for immunotherapy combination, there is still a lack of direct evidence demonstrating the relationship between genomics feature and treatment efficacy.[76]

On the contrary, serine/threonine kinase 11/serine-threonine kinase B1 (*STK11/LKB1*) and Janus kinase 1/2 (*JAK1/2*) mutations suggest primary resistance to ICIs. Skoulidis *et al*[77] reported that ORR to PD-1 inhibitors differed among the *STK11/LKB1* (KL), *TP53* (KP), and *KRAS*-only (K-only) subgroups of *KRAS*-mutant lung adenocarcinoma. In the Checkmate-057 clinical trial, the KL group had shorter PFS ($P < 0.001$) and OS ($P = 0.002$) than the other groups. These results allude that *STK11/LKB1* mutations are the main driving factors for primary resistance to PD-1 inhibitors in *KRAS*-mutant lung adenocarcinoma. Certain studies[78,79] have also found that the somatic *JAK1/2* mutations in TCs were responsible for the inability to respond to IFN-γ as well as the absence of reactive PD-L1 expression.

### Gut microbiome

The gut microbiome has been proposed to have an impact on the development of cancer and systemic immunity.[80,81] Even though the specific mechanism is not yet verified, increasing evidence shows that the microbiome could be a potential biomarker for the therapeutic outcome. Gopalakrishnan *et al*[82] deduced that the diversity of gut microbiome was positively correlated with the response to immunotherapy in melanoma patients, and this finding was also verified in a mouse model. Mice raised in a germ-free environment did not respond well to anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4 therapy.[83] Even though no evidence was shown in immunotherapy-based combination in NSCLC, recently, a study of renal cell carcinoma (RCC) observed that either in nivolumab monotherapy (77%) or nivolumab plus ipilimumab (23%), greater microbial diversity was associated with clinical benefit.[84] In general, the abundance of microbiome could be a potential indicator for response to ICI-based therapy.

There are currently no effective markers for combination therapy prediction to choose which patients are more suitable for immunotherapy-based combination. Therefore, the choice of combination regimen is based on predictive indicators of immune monotherapy. The latest perspective is related to the concept of the landmark "Landscape," which covers many aspects such as PD-L1, TMB, tumor neoantigen, genetic mutation, host germline genome, TME, and gut microbiome.[85] In conclusion, a single biomarker is insufficient to differentiate the responders from the non-responders. Emerging biomarkers are needed to refine the selection process. Furthermore, the development of comprehensive predictive models involving a combination of different components is the need of the hour.

## *Treatment of special populations*

### *EGFR/ALK* alterations

Most prospective clinical studies have excluded patients with *EGFR* mutations or *ALK* rearrangements, and only limited trials have included such patients. Among them, IMpower150[33,86] exposed that patients with non-squamous advanced NSCLC, including subgroups with *EGFR/ALK* mutations, could benefit from ABCP. The study asserted the potential value of anti-angiogenetic agents in this setting. KEYNOTE-789 is an ongoing clinical trial

investigating a combination of pembrolizumab plus chemotherapy after the progression of first-line TKIs in NSCLC patients with *EGFR* mutations. However, the initial results have been disappointing.

Therefore, current evidence mostly suggests that ICIs are not suitable for NSCLC patients with *EGFR/ALK* alterations. Even though the IMpower150 trial found that the combination of anti-angiogenetic agents and ICIs might exhibit synergistic anti-tumor activity in this population, it could also result in fatal adverse reactions. Therefore, for these patients, TKI monotherapy should be the first-line treatment. ICIs and combinations of ICIs and TKIs are not recommended.[39] If the TKI treatment fails or if the patient is not able to tolerate its adverse effects, it is imperative to detect the PD-L1 expression, TMB levels, and tumor immune microenvironment. ICI monotherapy or combination of ICIs with chemotherapy and anti-angiogenic drugs could be considered for NSCLC patients with high PD-L1 expression.[87]

### Elderly patients

The clinical benefit of immunotherapy in elderly patients is still controversial.[88] In the subgroup analysis of IMpower132 trial, the PFS was comparable between the older (≥65 years) and younger (<65 years) NSCLC patients receiving immunotherapy plus chemotherapy (HR: 0.55 *vs*. 0.63); however, the OS was higher in the older patients than that in the younger patients (HR: 0.71 *vs*. 0.89).[89] A meta-analysis involving 5265 patients explored the differential activity of immunotherapy in four histological settings. When 65 to 70 years was used as the cut-off value, the older and younger groups were found to receive comparable benefits.[90] A previous study reported that immunosenescence (a measure of immunological age), rather than chronological age is more closely associated with poor response of ICIs.[91]

In summary, the use of combination therapy in elderly patients is still controversial. However, the treatment could offer certain benefits for elderly patients with a good general status, and age should not be the only factor in deciding whether immunotherapy should be administered.

### Liver metastasis

Advanced NSCLC with liver metastasis is associated with unfavorable prognosis[92] and poor response in ICI monotherapy as well as in combinations of ICIs and chemotherapy.[93] This result could be attributed to the fact that the liver is the primary site of T cell activation and that incomplete activation could lead to abortive activation, exhaustion, and early death of T cells.[94] The presence of liver metastasis is also correlated with reduced marginal infiltrating CD8+ T cells, which sheds light on the potential causes for these disappointing outcomes.[95] However, the combination of bevacizumab, atezolizumab, and chemotherapy in the IMpower150 trial improved the survival in this population, suggesting the key role of anti-angiogenics in patients with liver metastases. Such patients do not constitute the dominant group for immunotherapy. When compared with chemotherapy, immunotherapy combined with anti-angiogenics is a better choice for this population.

## Treatment-related adverse events

With the widespread application of ICIs, irAEs have gradually attracted the attention of the researchers. Numerous large-scale clinical trials have reported that the incidence of irAEs is 60% to 80%, and it is similar in combination strategies.[56,96] In the clinical settings, the irAEs depend on the toxicity profiles of the ICIs and originate from the non-specific activation of the immune system.[97,98] The adverse reactions can occur in any organ or tissue and chiefly involve the skin, gastrointestinal tract, endocrine glands, liver, and lungs.[99,100] Even though irAEs are generally manageable (grade 1–2), some serious adverse reactions such as immune-related pneumonia, immune-interstitial nephritis, and immune-related myocarditis could be life-threatening.

Checkpoint inhibitors-associated pneumonitis (CIP), a kind of lung injury caused by ICIs. CIP has differing clinical, imaging, and pathological features and is the main irAE of grade ≥3. A meta-analysis of ICI treatment combined with chemotherapy showed that the relative risk of CIP for the combined treatment was 2.37 (95% CI: 1.27–4.32, $P$ = 0.007).[101] When compared with ICI monotherapy, the combined treatment with PD-1 and CTLA-4 inhibitors was linked to an increased total incidence of CIP (6.6% *vs*. 1.6%, $P < 0.001$) and severe CIP (1.5% *vs*. 0.2%, $P = 0.001$).[102] The combined strategy of immunotherapy and targeted therapy can also exacerbate the incidence of CIP. In a phase Ib trial of durvalumab and osimertinib for NSCLC treatment, up to 38% of the patients developed CIP, of which the incidence of severe CIP was 15%.[103]

Although the NSCLC patients benefit from immunotherapy combinations, the upregulated incidence of CIP requires the attention of clinicians. In general, with regard to irAEs reported in the literature, the identification, monitoring, and follow-up of adverse reactions should be carried out throughout the process.

## Selection of the optimal combination strategy

Several combination algorithms have been successful in all pathological types of metastatic NSCLC, and most of them have been approved by the FDA. However, in different settings, ICI agents and/or chemotherapy regimens are not exactly the same. The selection criteria for combined therapy are also not completely clear.

First, the existing combination strategies are simply a combination of several agents, leading to just modest ORR in some trials. The optimal kind of drug, dose, and sequence should be chosen to identify the optimized model of the combinations in every setting. Second, it is necessary to consider issues such as adverse reactions. Although irAEs are mostly tolerable, severe irAEs are possible and they can be life-threatening. Even though there is little evidence to suggest that combination strategies are associated with increased mortality or severity of toxicity, clinical benefit and treatment-related toxicity should be

balanced. Third, most of NSCLC patients enrolled in the trials were supported by the company, and hence, the expenses of the combination strategies are not taken into account. The current strategies may be suitable only for patients who are economically sound. Therefore, the cost effectiveness factor needs to be considered for routine use of the treatment in the future.

The current clinical trials have focused on the development of novel agents or combination algorithms for immunotherapy. However, the most suitable drugs and dosage, the sequence and the best application time of immunotherapy remain unestablished. In the later stages, more head-to-head phase III trials with large sample sizes are needed to identify the most effective combination strategy. If the toxicity is acceptable, the optimal combination strategy can enhance innate as well as adaptive immunity.

## Future challenges

Immunotherapy has truly revolutionized the treatment approach to NSCLC. As per the existing research evidence, immune monotherapy cannot completely satisfy the needs of NSCLC patients. However, immunotherapy combinations are expected to become the trend in the future.

In recent years, treatment algorithms such as immunotherapy combined with chemotherapy, anti-angiogenesis therapy, and targeted therapy have witnessed rapid development. In addition, immunotherapy combined with other treatments such as interventional therapy, ablational therapy, and vaccination is also likely to become the key approaches in tumor management.

Herein, we have proposed four main challenges for the development of immune combined therapy. The first one addresses how to improve the efficiency of immunotherapy. Currently, the optimal combined algorithms, such as the best combined immunotherapy regimen, the dosage, and the best application time, require further optimization. It is well known that the prognosis is significantly better for patients showing efficacy during the early period of the application of the immune combination therapy, such that it has become necessary to promote early efficacy manifestation. Meanwhile, the development and research of new immunomodulatory agents, including CAR-T-cell/CAR-natural killer-cell therapies, should be accelerated to break through the bottleneck of immune combination therapy. In this aspect, more numbers of pre-clinical and clinical trials are needed in the future to promote the efficacy of immunotherapy for lung cancer.

The second one addresses how to improve the preciseness of immunotherapy. Although combined algorithms appear to be the future trend, the existence of ITH poses a considerable challenge in the implementation of precision oncology. The PD-L1 expression levels and TMB have been routinely assessed in clinical trials, which should actually be the foundation of the biomarker exploration. It is, therefore, necessary to introduce the concept of "Landscape," which covers multiple aspects such as PD-L1, TMB, tumor neoantigen, genetic feature, TME, and imaging in order to explore the individualized markers of immune efficacy.

The third one addresses measures to prolong the benefit of immunotherapy. The endogenous and exogenous mechanisms through which the immune combination can regulate multiple aspects to overcome drug resistance remain unclear. Ascertaining the primary drug-resistant population is also difficult. On the one hand, a pre-clinical study should explore the in-depth understanding of the mechanisms of drug resistance during immune combination therapy. On the other hand, clinical practice needs to delay or overcome drug resistance by bringing immunotherapy forward to the first-line in a combined fashion, by turning cold tumors into hot ones.

Finally, we addressed the mechanism to make immunotherapy more secure. When compared with the use of chemotherapy and targeted therapies, the specific damage caused by immunotherapy remains unclear. It may affect the multiple systems throughout the body (eg, the heart, thyroid, small intestine, and pituitary) and some irAEs of grade $\geq$3 can progress rapidly within a short period of time. Therefore, irAEs require close attention and effective clinical management methods are urgently needed for irAEs. In the future, avoiding the occurrence of serious irAEs, adopting an individualized approach to manage irAEs in a better way, and timing the "re-challenge" after the control of irAEs will be the hotspots of clinical concern.

The future of NSCLC treatment is thus immune combination. However, it is undeniable that the exploration of immunotherapy for NSCLC is still in its infancy. This industry needs innovation, integration, and transformation to promote the continuous advancement of immune combination. We are confident that once the mystery shrouding immunotherapy is unveiled, the adoption of this strategy will mark the beginning of a new era.

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## Conflicts of interest

None.

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