PD-L1/PD-1 Expression in Non-Small Cell Lung Cancer: Clinicopathological Features and Treatment Strategies

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Non-small cell lung cancer (NSCLC) causes around 106310 deaths each year in the United States. Immune checkpoint inhibitors have had significant anti-cancer effects with lower toxicity than more traditional radio or chemotherapy interventions however, patient responses are variable. The level of expression of PD-L1 in the tumor is a key determinant of patient response and potentially influenced by several clinicopathological features. Patients that have NSCLC that are male, smoking and have epidermal growth factor receptor (EGFR) mutations are more likely to have tumors that have higher PD-L1 expression, suppressed immune responses and more aggressive disease. Now, six immune checkpoint inhibitors (ICIs) have been officially approved by the United States Food and Drug Administration (FDA), more are still in development. The efficacy of both monotherapies and their combination with chemoradiotherapy are summarized in this review, as are the contributing factors to patient responses. This report concludes that a number of clinicopathological features associated with ICI response and combination therapies may yield better outcomes while maintaining a moderate rate of adverse effects (AEs).

Introduction

Lung cancer is a severe disease that originates in the lung parenchyma or bronchi. Because of its high mortality and morbidity, it is the leading cause of cancer-related deaths worldwide[1]. According to an investigation about cancer incidence and morbidity, GLOBOCAN 2020, lung cancer has the highest number of deaths, which is estimated at 18.1% among the total number of new cancer cases that occurred in 2020 and were evaluated in this study[2]. There are two major types of lung cancer, small-cell lung carcinomas (SCLCs) and non-small cell lung carcinomas (NSCLCs)[3], in which NSCLCs account for 85% of all lung cancers[1]. It is classified into three main histological types, while lung adenocarcinoma (LUAD) is the most common subtype in NSCLC.

Programmed death 1(PD-1) is a protein expressed on the surface of activated T cells that acts as an inhibitory receptor. Programmed death-ligand 1(PD-L1) is its ligand expressed on antigen-presenting cells. These two critical immune checkpoint proteins bind together, forming a PD pathway which deactivate T cells, leading to T cell apoptosis and inhibitory tumor microenvironment[4]. About 24% to 60% of the population of NSCLC patients have prevalent PD-L1 expression[5]. Additionally, PD-1 and PD-L1 expressions have been observed to affect other species like cattle, cats, and dogs. For example, cats with high serum level PD-1 and PD-L1 tend to have certain types of mammary carcinoma, and PD-1 and/or PD-L1 expression on immune cells increases during chronic infections in cattle. In response to this high percentage and wide prevalence, immunotherapy was invented. Immune checkpoint inhibitors are prominent in immunotherapy because of their wide range of applicability. As of January 2024, 11 ICIs were approved by the FDA for cancer treatment. The American Association for Cancer Research reports that at least one of these drugs is used to treat over twenty types of cancer, including any solid tumors with specific shared molecular characteristics. Six of them can be applied to NSCLC treatment, and clinical trials have affirmed their effectiveness.

Methods

From a systematic search of “NSCLC”, “PD-1”, “PD-L1”, “anti-PD-1”, and “anti-PD-L1” on PubMed, this review aims to summarize the signaling pathway of PD-1/PD-L1; follow by the analysis of four factors that affect their expressions and the application of anti PD-1/ PD-L1, including monotherapies and combination with radiotherapy or chemotherapy; lastly identify side effects, limitations, and potential development for PD pathway as suggestions for future studies.

PD-1:

The most common immune cells are lymphocytes (T cells, B cells, and natural killer cells), neutrophils, monocytes, and macrophages. T cells, also called T-lymphocytes, are responsible for attacking pathogens and killing tumor cells, while B cells make antibodies to fight against pathogens. PD-1 is encoded in humans by the PDCD1 gene that lies on activated T cells,

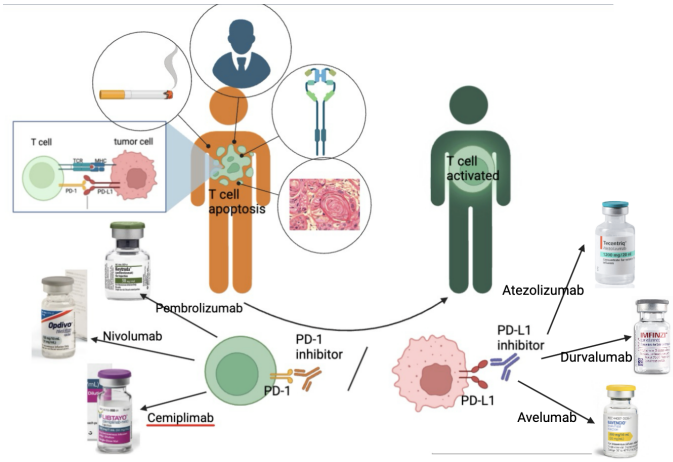

Figure 1. Graphical abstract for "PD-L1/PD-1 Expression in Non-Small Cell Lung Cancer: Clinicopathological Features and Treatment Strategies". The orange person represents non-small cell lung cancer patients, and the green figure on the right represents healthy people. The rectangular zoom-in bubble shows the interaction between PD-1 expressed in a T cell and its ligand PD-L1 expressed in a tumor cell. Four circular zoom-in bubbles indicate smoking, male, epidermal growth factor receptor mutation, and squamous cell carcinoma, which are clinicopathological features that affect PD-1/PD-L1 expressions. Six FDA-approved immune checkpoint inhibitors are listed at the bottom, The left three (pemb., nivo., cemi.) are anti-PD-1 ICIs, and the right three (atezo., durv., ave.) are anti-PD-L1 ICIs.

B cells and myeloid cells[6], but it is best characterized on T cells. PD-1 plays a role as an immune checkpoint and regulates the immune system's response through downregulating activated lymphocytes after prolonged inflammation. However, PD-1 can be a double-edged sword. On one hand, PD-1 can prevent autoimmune diseases by suppressing overactivated T cells' activity. Bcl-xL is a cell survival gene that functions against T cell apoptosis[7]. A study showed that Bcl-xL was downregulated following the binding of PD-1 and its ligands, suggesting that PD-1 engagement increases the risk of T cell exhaustion and apoptosis[8]. In this way, excessive immune response by T cells will be suppressed, and protect tissues from autoimmunity. When PD-1 expression is too low, it leads to active immune disease in Systemic lupus erythematosus (SLE) patients[9], since this autoimmune disease is caused by overactive immune response[10]. Herein, less PD-1 allows T cells to proliferate and target tissues mistakenly. On the other hand, overexpressing PD-1 may cause the rapid growth of malignant cells as it inhibits immune response. S-phase kinase associated protein 2 (SKP2) is a F-box protein (proteins used to regulate the activity and expression of oncogenes and tumor suppressor genes) that can lead to uncontrolled cell proliferation when overexpressed because it prevents the accumulation of cell cycle inhibitors which help cells change from G1 to S phase. PD1 suppresses SKP2 by reducing transcription, thereby blocking T cells' cell cycle progression and proliferation[11]. Two co-stimulations were examined in DO11.10 T cells, a type of T cells that endogenously express a substantial amount of PD-1, to investigate the efficacy of PD-1 inhibition. In this study, the presence of a strong CD28 (the receptor of CD80 and CD86 proteins that expressed on T cells and aim for naive T cells activation) co-stimulator, PD-1 still attenuated the inhibition of 80% of the T cells when compared with 97% inhibition with no CD28 co-stimulator. Besides, PD-1 effectively inhibits DO. DO11.10-CD28KO T cells from expressing IL-2, which can stimulate the proliferation of T cells[12]. The expression level of PD-1 serves as a pivotal biomarker in exploring the efficacy of PD-1 inhibitors and other novel treatment strategies, such as PD-1 inhibitors combined with chemoradiation therapy, which we will elaborate on in later passages. Also, by deepening the understanding of the mechanism of PD-1, different patients' treatments will be personalized according to their reaction to their PD-1 expression and response to different inhibitors

PD-L1:

Encoded by the CD274 gene, PD-L1 is one of the members of the B7 family (B7-H1) transmembrane protein[3], and is commonly expressed on antigen presenting cells (APCs). However, it also appears on the cell surface of various solid cancers, like endometrial cancer, kidney cancer, ovarian cancer, and melanoma[13]) '[14]. PD-L1 plays a vital role in immune escape in different mechanisms, for example, binding to its receptor PD-1 to anergize T cells, making CD 80 reverse signaling, and rendering tumor cells to resist T cells and Fas. Hence, no matter which protein PD-L1 affects, its eventual goal is to downregulate T cells[15]. Interferon-N (IFN), a cytokine released by activated T cells and natural killer (NK) cells

that triggers PD-L1, is the primary regulator of PD-L1. When PD-L1 and IFN-γ present together, the prognostic of locally advanced lung adenocarcinoma (LUAD) patients is apparently improved[16]. The appropriate amount of PD-L1 needs to be carefully controlled in human bodies, otherwise it may cause various other diseases. Marginally higher PD-L1 expression is better for immunotherapy since PD-L1 is more apparent to attract T cells to the cancer cells. But on the other hand, PD-L1 overexpression can increase immune escape rate. The reason for that is because these PD-L1 are usually expressed by tumor cells (TCs), and over-expression leads to more interaction between PD-L1 and PD-1 to reduce T cells' function.

PD pathway:

As figure 2.a illustrates that the binding T cell receptor (TCR) with the major histocompatibility complex (MHC) activates T cells and kills tumor cells. In figure 2.b, it shows that the interaction between PD-1 and PD-L1, commonly referred to as the "PD pathway,"is essential to the proliferation suppression and apoptosis of activated T cell[17]. According to the illustration, it has at least five interacting molecules: RGM-2, B7-1(CD-80), PD-L1, and PD-L2. Instead of systemic regulation of autoreactive T cell responses, the physiological role of the PD pathway is to control ongoing inflammatory reactions and to stop the spread of inflammation. IFNs are pleiotropic cytokines that are central coordinators of the immune response. By enabling the upregulation of neoantigen expression in cancer cells, IFNs allow the immune system to target cancer cells more easily. This effect can be applied for antiviral, antitumor, and immunomodulatory purposes. Among all IFNs, interferon-γ (IFN-γ) induces upregulation of PD-L1 and it is responsible for cancer-induced inflammation. Thus, IFN-γ ushers the apoptosis of CD8+ T cells through PD-1/PD-L1 interaction, contributing to tumor immune evasion[18]. Subsequently, chronic inflammation leads to proliferation and cell mutation, creating an environment for the unstoppable development for cancers. Another ligand of PD-1 that is similar to PD-L1 is called PD-L2. While both ligands bind to PD-1 to initiate inhibitory signaling pathways, PD-L2 is comparatively localized in the body. It can be expressed at a high level in tissues like the liver and pancreas, although it is preferentially expressed on the surface of activated macrophages and dendritic cell[19]. In comparison with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, a protein receptor that works similarly to PD-1), which is confined to T cells and only responsible for the primary phase of NSCLC, PD-1 functions at the effector phase and is expressed in nonlymphoid tissues, T cells, and nonhematopoietic cells[20]. Additionally, professional antigen-presenting cells (APCs), which are only found in lymph nodes or the spleen, express the B7 ligands for CTLA-4, but the ligands of PD-1, represented by PD-L1, are broadly expressed in peripheral tissues[21].

Clinicopathological Features that Affect PD-1/PD-L1 Expressions

According to the AME Lung Cancer Collaborative Group, they used a random-effects model to observe and analyze the association between PD-L1 expression and sex, smoking status, EGFR mutation, and histology type (table 1). From the table, we can conclude that male, smokers, EGFR mutation positive, and SCC patients generally express higher PD-L1.

Sex:

While NSCLC is not a sex-specific cancer, it exhibits a specific affinity towards males for many reasons, like carcinogen exposure, smoking status, sex hormone, diet, and inherent genetic differences[22]. Specifically, PD-1 expression is somewhat dependent on sex. Calculated from a study that men have about an 8% higher chance to have PD-L1 expression between 1-49% than women[23]. While another study shows that women have higher serum PD-1 levels than men[24]. Also, the efficacy of anti-PD-1/PD-L1 in both sexes was evaluated. When receiving chemotherapy as a control group, men who were treated with anti PD-1/PD-L1 had profoundly reduced death risk in comparison with females, whose progress was too little to have statistical significance[25]. Current studies on sex hormones are even fewe than the studies of general sex. Even though some results reveal that increasing testosterone can inhibit PD-1 expression on T cells and this effect may even overpower original sex barrier, other studies have found no relationship or inverse result between sex/sex hormones and PD-L1 expression in NSCLC, making it remain unclear[26,27].

Smoking status:

Smoking is the most prominent reason for lung cancer, which contributes to about 90% of lung cancers' causes[28]. The reasons for non-smokers getting NSCLC may include exposure to radon, secondhand smoke, and air pollution, which are different from patients who smoke. In smokers, PD-L1 expression is impacted through aryl hydrocarbon receptors (AhR)-related pathways or mTOR signaling. AhR is a transcription factor that can upregulate PD-L1 when exposed to tobacco carcinogens like Benzo[a]pyrene (BaP) and lead to T cell inactivation. The inhibition of the mammalian target of rapamycin (mTOR) kinase increases PD-L1 expression, however, results of current studies on mTOR pathway's effect on PD-L1 expression remain in conflict. In one study, PD-L1 expression in NSCLC patients was found to be 44% in current smokers, 20% in former smokers, and 13% in never smokers. This demonstrated that smokers are more likely to have PD-L1 expression at a higher intensity ($\geq$ 50%). In addition, long smoking pack years will increase the incidence of high PD-L1 expression[29]. According to a Japanese study, smokers have a shorter OS time than patients with low PD-L1 expression because they express more PD-L1. Therefore, PD-L1 ICI therapy prolonged OS and progression-free survival (PFS) in smokers but not in never smokers since PD-L1 positive

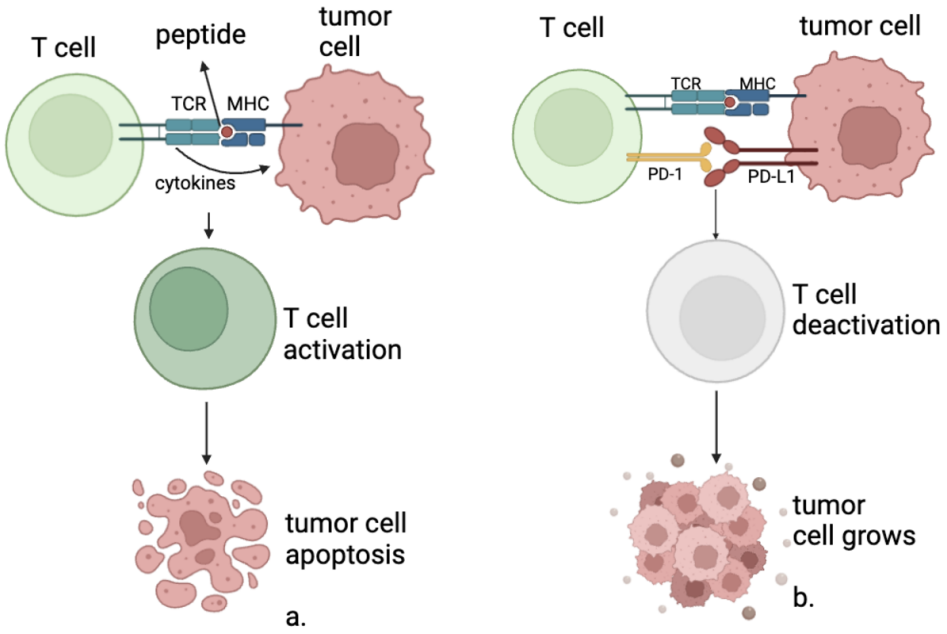

Figure 2. T cell kills a tumor cell VS. T cell deactivates through binding PD-1 with PD-L1; TCR, T cell receptor; MHC, major histocompatibility complex; Antigen, a peptide such as Ag.

Table 1. Summary of the association between PD-L1 expression and clinicopathological features in NSCLC. OS, overall survival; n, number of tested patients; EGFR, epidermal growth factor receptor; AC, adenocarcinoma; SCC, squamous cell carcinoma

Clinicopathological features	Main study	n	Location of study	Outcome	Note
sex (male vs female)	Gu et al. (2022)	Female: 46 (42.6%) Male: 62 (57.4%)	Guangzhou, China	No significant association between sex and PD-L1 expression however, non-significant trends suggest men have increased PD-L1 expression in the tumor.	N/A
Smoking status (never smokers vs smokers)	Azuma et al. (2014)	Never smoker: 95 (58.0%) Smoker: 69 (42.0%)	Kurume, Japan	Smokers have increased PD-L1 expression in the tumor.	Overall no uniform conclusion, but trend based on current studies indicate that smokers have relatively high PD-L1 expression level
Smoking status (never smokers vs smokers)	Calles et al. (2015)	Never smokers: 30 (26.3%) Smokers: 84 (73.7%)	Massachusetts, USA	Smokers have a higher PD-L1 expression level.	
EGFR mutation (EGFR + vs EGFR -)	Azuma et al. (2014)	EGFR +: 57 (34.8%) EGFR -: 107 (65.2%)	Kurume, Japan	Patients with EGFR mutation have a higher PD-L1 expression.	N/A
Histology (AC vs SCC)	Janzic et al. (2017)	AC: 29 (54%) SCC: 25 (46%)	Ventana, USA	There's no significant results that make the clear conclusion but trend based on current studies suggest SCC patients have higher PD-L1 expression	N/A

tumors responded more from ICI and the chance of improvement was greater in patients with high PD-L1 expression[30]. However, there is still a study claiming no direct relationship between smoking status/habits and PD-L1 expression, leaving researchers more space to explore[31].

EGFR mutation:

One somatic mutation in non-small cell lung cancer (NSCLC) is related to the epidermal growth factor receptor (EGFR), as abnormal EGFR activation induces PD-L1 production via the AKT-STAT3 pathway. Indeed, PD-L1 expression increases with increased activation of STAT3 and AKT according to some studies[32]. To investigate the favorable correlation between EGFR mutation and high PD-L1 expression, particularly in LUAD and squamous cell carcinoma (SCC), 164 patients with advanced NSCLC who underwent tumor resection surgery at Kumuri University between 2000 and 2010 were included in a retrospective analysis. The results indicated that the EGFR mutation-positive cell line had a greater PD-L1 mean fluorescence intensity (MFI) than the EGFR wild type cell line, and that only the EGFR mutation-positive cell line had downregulated PD-L1 expression following the use of EGFR-tyrosine kinase inhibitors (EGFR-TKIs)[32,33]. Specifically, flow cytometry analysis showed that PD-L1 protein decreased after receiving EGFR-TKI[33]. Patients using the first- and second-generation EGFR-TKI showed prolonged PFS when compared with traditional chemotherapy (median PFS for EGFR-TKI: 11.0 months vs chemotherapy: 5.6 months). Hence EGFR-TKIs combined with PD blockade is a potential treatment for EGFR wild type patients. However, another study suggests that patients receiving chemotherapy have a longer overall survival rate (OS) than those who use EGFR-TKI (12.8 months and 19.8 months, respectively)[34].

Histology type

Since two major types of NSCLC are lung adenocarcinoma (AC) and squamous cell carcinoma (SCC), some studies were conducted to show how they affect PD-L1 expression. SCC has a greater proportion of PD-L1 positive expression on TCs among all 25 samples when PD-L1 $\geq$ 5% and $\geq$ 10% are used as cut-off values (52% vs. 17% in SCC and AC, respectively for PD-L1 $\geq$ 5% and 14% in SCC and AC, respectively). This is true for 29 samples of AC as well. Furthermore, when the data cutoff was adjusted from $\geq$ 5% to $\geq$ 10%, the PD-L1 positive rate in both TCs and immune cells (ICs) in AC had reduced, while there had been little changes in SCC (AC: 72% at PD-L1 $\geq$ 5% vs 41% at PD-L1 $\geq$ 10%; SCC: 88% at PD-L1 $\geq$ 5% vs 76% at PD-L1 $\geq$ 10%). Overall, TCs with SCC had a greater percentage of PD-L1 positives, indicating that AC and SCC may induce distinct PD-L1 expression[35]. Yet another study claimed that there were no major histological differences between AC and SCC since PD-L1 expression level was not apparent enough to be considered as statistically significant (0.41 ± 0.03 in AC, 0.47 ± 0.03 in SCC). Instead, large cell carcinoma (LCC) had the highest PD-L1 expression (0.57 ± 0.12). Contrary to the findings of the first study mentioned above, the same study found no correlations between PD-L1 expression and tumor, node, metastasis features, the tumor stage, or survival rate in the SCC subtype[36]. But overall, SCC tends to express higher PD-L1 than AC after testing in other cancer types like cervical cancer and advanced gastroesophageal cancer[37,38].

Other factors

Genetic and epigenetic factors contribute to PD-1/PD-L1 expressions as well. Rearrangement of PD-L1 gene and chromosomal translocation can amplify its expression, leading to an increasing immune escape rate. Similar to PD-L1, KRAS—a gene that produces a protein that controls cell growth, maturation, and death— mutation, EML4-ALK—a fusion protein tyrosine kinase that results from a genetic abnormality on chromosome 2— chromosomal rearrangement etc. can alter PD-1 expression. Other than that, DNA phosphorylation, regulation of gene translation by miRNAs can also lead to different PD-1/PD-L1 expression as epigenetic factors[39].

As shown in figure 3, the goal of immunotherapy was to defeat TCs by activating T cells in the immune system to block the interaction between PD-1 and PD-L1 by applying PD-1/PD-L1 inhibitors[40]. Since 2015, anti-PD-1 and anti-PD-L1 immunotherapy has become the preferred option for first- or second-line treatment of IV-stage NSCLC because PD-L1 is the only biomarker for ICI therapy in NSCLC[41]. Unlike radiotherapy, chemotherapy, and surgery which directly target tumor cells, immunotherapy kills them by enhancing and normalizing immune cells' response. PD-L1 expression levels and clinical efficacy endpoints, including OS, PFS, and ORR, have been found to have good predictive relationships in the majority of clinical trials to date, which have demonstrated the greater efficacy of immunotherapy in advanced NSCLC[42]. Since 2006, the US Food and Drug Administration (FDA) has approved six Immune Checkpoint Inhibitors (ICIs) to improve immunotherapy. Among them, Nivolumab, pembrolizumab, and Cemiplimab are three anti-PD-1 drugs; Atezolizumab, Avelumab and Durvalumab are PD-L1 inhibitors[43].

Nivolumab

Nivolumab is a therapeutic antibody called IgG4 mAb developed by Bristol-Myers Squibb and works by selectively blocking and mediating PD pathways[44]. In previously treated patients with advanced or metastatic squamous cell NSCLC, Nivolumab had a one-year OS that far surpassed it achieved by docetaxel, according to the CheckMate-017 trial[45]. With only 20% severe or life-threatening treatment-related toxicities, single-agent nivolumab had a greater overall response rate (ORR) and better PFS in a cohort of patients receiving both erlotinib and nivolumab. Furthermore, compared to patients with PD-L1-negative tumors, those with PD-L1-positive tumors had numerically better ORR, median PFS, and 1-year OS in exploratory analyses. Furthermore, whereas only 17% of

Table 2. Summarization of the mechanisms, effectiveness, side effect rates of ICIs with overall treatment suggestions. PFS, progression-free survival; OS, overall survival; ORR, overall response rate; SAE, severe adverse event (grade $\geq$ 3); ICI, immune checkpoint inhibitor; TPS, tumor percentage score; TC, tumor cell.

ICI	Definition + Mechanism	Study	Efficacy	Grade 3-4 adverse events rate	Overall outcome
Nivolumab	A fully human IgG4 antibody that blocks the activity of PD-1, inhibiting T cell inactivation	CheckMate	They all show a longer PFS, ORR, and OS comparing to single-agent chemotherapy	Around 20%	Effective as a second or later line treatment with low side effect rate
Pembrolizumab	A humanized monoclonal IgG4 kappa antibody and works for PD-1 blockade	KEYNOTE		Around 20.7%	Preferred first-line treatment for high PD-L1 expression patients
Cemiplimab	A recombinant human IgG4 monoclonal antibody that binds with PD-1	EMPOWER		Around 28%	Preferred first-line treatment for patients whose TPS $\geq$50% but without EGFR mutation, ALK, and ROS1 rearrangement
Atezolizumab	A humanized IgG1 monoclonal antibody that functions as a PD-L1 inhibitor	IMpower		Around 16%	Suggested adjuvant treatment for patients with stage II to IIIA NSCLC whose PD-L1AA expression can be detected on $\geq$1% of tumor cells; may be viable first-line treatment
Durvalumab	A human IgG1 monoclonal antibody that is designed to bind to PD-L1	PACIFIC		Around 21%	When PD-L1 TC expression < 1%, it's better not to use Durvalumab
Avelumab	A human IgG1 monoclonal antibody that is used as PD-L1 inhibitor	JAVELIN		15-30%	Further studies are still in progress and there's no statistically significant results confirm that Ave. is better than chemo
ICI+Chemotherapy	Chemotherapy reduces the number of immunosuppression cells in the tumor microenvironment, and ICI blocks immune cell inhibitory signals. Together, they work to prevent immune cell inactivation	IMpower, KEYNOTE	The combination of chemo with ICIs increases PFS and median OS comparing to ICI single agent	Much higher than ICI monotherapy	Chemo-ICI combinations have a better overall efficacy than monotherapy but need to be further modified to reduce SAE.
ICI+Radiotherapy	Radiotherapy boosts antigen exposure and immune cell infiltration, letting more tumor cells be recognized and targeted. ICI blocks immune suppression.	PEMBRO-RT	The combination of radio and ICIs also increases PFS, OS, ORR in addition to monotherapy	SAE occurrence stays about the same percentage as ICI monotherapy	It's a reliable future treatment strategy for cancer treatment while more trials that combine other ICIs with radiotherapy need to be examined

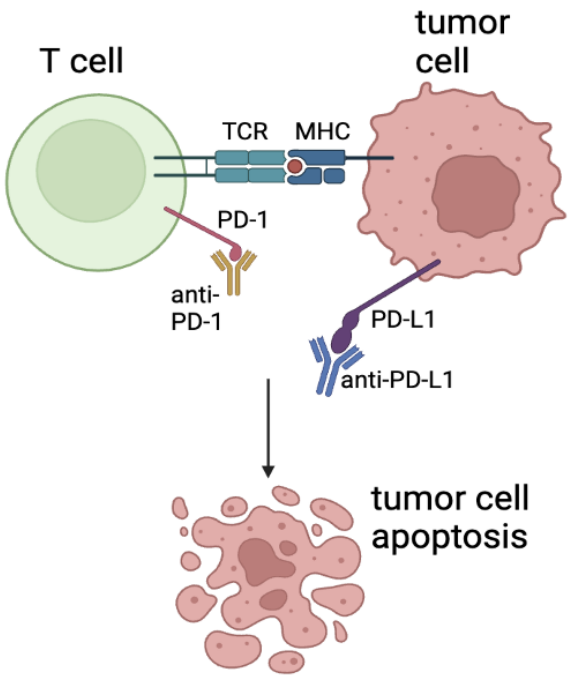

Figure 3. PD-1/PD-L1 inhibitors binding with PD-1/PD-L1 to regulate T cell activation and kill tumor cells.

participants in a multiple ascending dose study of patients with advanced stage solid tumors respond, more than half of those that respond see a reduction in tumor size in the first two months of treatment[46]. In another study whose patients used nivolumab as treatment, the incidence of both serious adverse events (SAEs) and fatal adverse events (FAEs) was lower than any grade of AEs of chemotherapy[47]. Above studies demonstrate Nivolumab is more effective than chemotherapy in treating NSCLC because of its longer OS, PFS, and less AEs, thus providing a better room for future development.

Pembrolizumab

Another mAb for target PD-1 is pembrolizumab, which is a highly selective humanized anti-PD-1 IgG4 kappa isotype mAb and was approved to be a treatment of metastatic NSCLC by FDA in 2016. A study named KEYNOTE-024 chose 305 patients with NSCLC from 16 different countries to compare the efficacy of Pembrolizumab with chemotherapy. When the data was cut off, pembrolizumab showed an average OS that was at least twice as long as chemotherapy (30.0 months for patients receiving pembrolizumab and 14.2 months for those receiving chemotherapy The benefits of pembrolizumab in regards to PFS were apparent in all subgroups that were examined, regardless of age, sex, smoking status, or histological type[48]. There are increases for PFS and OS with both single-agent pembrolizumab and its use in conjunction with chemotherapy. Pembrolizumab therapy has demonstrated the highest ORR, PFS, and OS advantage over chemotherapy in subgroups with varying levels of PD-L1 expression, indicating that pembrolizumab affects NSCLC in both adjuvant and single-treatment contexts[49]. In KEYNOTE-024, there were five treatment-related fatal AEs, and three of them were caused by chemotherapy[50]. Although pembrolizumab had a slightly greater frequency of serious treatment-related adverse events (AEs) compared to chemotherapy, with 20.7% in the pembrolizumab group versus 22.7% in the chemotherapy group, pembrolizumab had superior performance. The most common AEs for Pembrolizumab were diarrhea (16.2%) and fatigue (14.3%)[50]. Other than that, it can lead to chest pain, severe nausea or vomiting, and decreased appetite[51].

Cemiplimab

Sanofi/Regeneron developed cemiplimab, also known as Libtayo, which is a mAb that binds to the PD-1 receptor and prevents it from interacting with PD-L1[52]. When an adult patient with NSCLC has a tumor with high-level PD-L1 expression (tumor percentage score (TPS) $\geq$ 50%) without EGFR mutation, ALK, and ROS1 rearrangement, cemiplimab will be used as their first-line treatment. Seventy-one patients with NSCLC were randomly assigned to the cemiplimab and chemotherapy groups in the open-label EMPOWER-Lung 1 study. With nearly identical treatment-related mortality (2% in the cemiplimab group and 3% in the chemotherapy group), the average OS and PFS were

longer in the cemiplimab group compared to the chemotherapy group[53]. Additionally, Grade 3–4 treatment-emergent AEs occurred in 28% of cemiplimab-treated patients but in 39% of chemotherapy-treated patients[54]. Thus, a combination of them provides a potential solution. EMPOWER-Lung 3 examined the effectiveness of cemiplimab monotherapy and a combination of cemiplimab and chemotherapy. Compared to the control group, a combination of placebo and chemotherapy, the average OS of cemiplimab was 21.9 months while placebo was 13.0 months; the PFS of the two groups were 8.2 months and 5 months, respectively[55]. Cemiplimab in conjunction with platinum dual-agent chemotherapy is a viable first-line treatment choice for patients with advanced NSCLC, irrespective of PD-L1 expression levels, as demonstrated by the above data, which also shows the efficacy of cemiplimab in early-stage NSCLC.

Atezolizumab

On October 15, 2021, the FDA approved atezolizumab, a humanized IgG1 monoclonal antibody that functions as a PD-L1 inhibitor, as an adjuvant treatment for patients with stage II to IIIA NSCLC whose tumors express PD-L1 on $\geq$ 1% of TCs[56] ‘[57]. Fragment crystallizable (Fc) region refers to the tall part of an antibody which interacts with Fc receptors, especially FcRIII expressed on NK cells, to activate these cells and prompt them to kill cancer cells. By manipulating the Fc area of T cells, atezolizumab interrupts PD interaction, preventing T cell fatigue, decreasing Fc effector function, and minimizing antibody-dependent cell-mediated cytotoxicity (ADCC)[56]. In simple words, atezolizumab prevents PD-L1 from binding with T cells and uses modified Fc region to reduce the action of additional immune cells, thus protecting normal cells from being harmed by the side effects of the drug. To evaluate and compare the efficacy of atezolizumab and chemotherapy, a study tested 576 patients with EGFR/ALK wildtype NSCLC from 21 July 2015 to 20 February 2018, revealing atezolizumab works better than chemotherapy, and PD-L1 high expression patients benefit the most from atezolizumab. By using a stratified log-rank test, OS of both groups were estimated. The 12 months OS in atezolizumab group are 64.9% in high PD-L1 expression patients, 60.7% in high or medium PD-L1 expression patients, and 57.6% in any PD-L1 expression patients; in the chemotherapy group, it is 50.6%, 56.0%, 54.3%, respectively. Furthermore, compared to patients with high or intermediate PD-L1 expression (15.2 months, ranging from 0 to 35) and any PD-L1 expression (13.4 months, same range), patients with EGFR and ALK wild-type tumors with high PD-L1 expression had longer average follow-up duration for survival (15.7 months, same range). Additionally, among patients, the median OS with atezolizumab was much longer than that of chemotherapy (7.1 months)[58]. Compared to chemotherapy, patients experienced fewer grade 3–4 AEs (16% vs. 33%) and treatment-related fatalities (1% vs. 3%)[59]. As a result, atezolizumab monotherapy appears to be a viable first-line treatment option for patients with advanced NSCLC, particularly if they show high levels of PD-L1.

Durvalumab

Durvalumab, referred to as MEDI4736, is a human IgG1 monoclonal antibody that is specifically designed to block ADCC and binds to PD-L1 with high affinity[60]. By attaching itself to PD-L1 expressed on TCs, it prevents PD-L1 from interacting with PD-1 and CD80, which kills TCs by increasing T cell activation[61]. A phase III trial PACIFIC compared durvalumab with placebo to test the efficacy of durvalumab in extending patients' PFS and OS after concurrent chemoradiotherapy. In this trial, 476 patients were assigned to durvalumab treatments while 237 patients were assigned to placebo, and as the result suggested, the median OS for durvalumab was 47.5 months while it is 29.1 months for placebo (stratified HR, 0.72; 95% CI, 0.59 to 0.89). But there was a subgroup with a special OS result that cannot be ignored. When PD-L1 TC expression < 1%, OS favored patients with placebo more than durvalumab[62]. Additionally, patients with durvalumab, regardless of PD-L1 expressions, all showed higher AEs vs placebo group. Discontinuations led by ADs were more common in durvalumab except TC <1% (11.0% vs. 17.5% in durvalumab and placebo, respectively)[63]. These findings demonstrated durvalumab's effects in treating unresectable, stage III NSCLC, despite a small sample size favoring placebo.

Avelumab

Another fully human monoclonal antibody called Avelumab (MSB0010718C) binds selectively to PD-L1, blocking its interaction with PD-1 and B7/PD-L1[64]. However, its wild-type Fc region, which enables it to combine with Fc-γ receptors, sets it apart from Durvalumab and Atezolizumab. This aids in avelumab's use of both innate and adaptive immune systems to eradicate cancer cells[65]. Besides, it is currently mainly used for advanced Merkel cell carcinoma, a type of skin cancer. A study assessed PD-L1 expression every 6 weeks while treating patients with avelumab. According to the results, the ORR for PD-L1 positive patients was 14.4% compared to 10.0% for PD-L1 negative patients, and the median PFS for PD-L1 positive patients was 11.7 weeks compared to 5.9 weeks for PD-L1 negative patients[66]. Another study named JAVELIN Lung 100 found that compared to chemotherapy, avelumab had longer OS and PFS. However, the difference was not statistically significant, so its primary objective was not met since it failed to demonstrate the superiority of avelumab over chemotherapy[67]. As shown in above data, differences in two therapies were observed but cannot be solidly evident, hence the uses of avelumab to treat NSCLC patients are still in progress.

ICIs and chemotherapy combinations Despite that ICI monotherapy can improve cancer treatments, researchers expect its efficacy to be more significant. Therefore, more studies have turned to combining ICIs with chemotherapy. In a review containing 22 randomized controlled trials (RCTs), researchers

have analyzed several clinical trials to find the answer. 11 of 22 RCTs involved solely ICI intervention, including atezolizumab, pembrolizumab, avelumab, cemiplimab, durvalumab, and nivolumab; Other 11 trials had chemo-ICI combination intervention, including chemo-pembrolizumab, chemo-atezolizumab, chemo-tocilizumab, chemo-sintilimab, chemo-camrelizumab[68]. OS and PFS had improved largely with chemo-ICI regimen, suggested by the randomized clinical trial KEYNOTE-407, which focused on pembrolizumab plus carboplatin and paclitaxel or nab-paclitaxel, three chemotherapy drugs. In this study, they used Keplan-Meier to estimate the median OS, and for pembrolizumab plus chemotherapy was 17.1 months vs 11.6 months in placebo plus chemotherapy group[69]. However, when PD-L1 tumor proportion score(TPS) < 1%, combination therapies still did not show a great significance than chemotherapy monotherapy, indicating ICIs are more likely to target higher PD-L1 expression[70]. Camrelizumab (SHR-1210) as a humanized monoclonal antibody against PD-1 independently developed and approved by China, has a good development prospect in treating non-squamous NSCLC without EGFR and ALK changes. Through meta-analysis, a study provided evidence that camrelizumab combined with chemotherapy had a higher efficacy than camrelizumab monotherapy (PFS=11.3 months vs 8.3 months)[71]. In 22 countries, the randomized phase 3 trial IMpower-010 evaluated some patients who were treated with surgery and following chemotherapy. These patients were then divided into two groups, one receiving adjuvant atezolizumab after chemotherapy and the other one receiving best supportive care (BSC). In the study, when PD-L1 TC $\geq$ 1% atezolizumab was more advantaged in disease-free survival (DFS) than BSC, as of the deadline (January 21, 2021)[72]. In conclusion, chemo-ICI therapy exerts long-lasting effects for patients who have PD-L1 $\geq$ 1% than monotherapies by extending OS and PFS in all levels of PD-L1 expression[73].

ICI and radiotherapy combination

As the first line treatment for metastatic NSCLC, radiotherapy changes patients' tumor microenvironment (TME), triggers an adaptive immune response, and thus mediated tumor regression[74]. But high doses of ionizing radiation (IR) emitted in radiotherapy can cause tumor recurrence, which lead people to seek new paths by combining radiotherapy with ICIs[75]. PD-L1 enhances IR efficacy through eliminating myeloid-derived suppressor cell (MDSC), an immune cell that suppresses the immune responses in cancer[76,77]. Therefore, the combination therapies were tested. Through a randomized phase 2 study called PEMBRO-RT, researchers collected the median OS and PFS of patients who receive Pembrolizumab independently and Pembrolizumab after receiving Stereotactic body radiotherapy (SBRT). When treating with pembrolizumab alone, the median PFS was 4.4 months, while it was 9.0 months when pembrolizumab plus radiotherapy was used; median OS was 8.7 months when pembrolizumab was used applied alone while it was 19.2 months when used pembrolizumab plus radiotherapy[78]. This suggests an overall enhancement in the PD-1 blocking for NSCLC patients whose tumors have metastasized. Specifically, subgroups in a meta-analysis which contained 20 trials with 2027 patients, including PEMBRO-RT, showed that the RT-first group had a sequentially longer survival time than in the concurrent or sequential ICI-first group[79]. The effectiveness and safety of the ICI-radiation combination therapy remain debatable, as data reveals that RT increases the incidence of lymphopenia that is caused by immunotherapy[80]. Despite that, combination therapies can increase patients' OS, PFS, and ORR without increasing the occurrence of severe AE (grade $\geq$ 3) but only the rate of mild pneumonitis (grade 1–2)[78,79]), giving reliable evidence for the future application of this combination therapy.

Side effects and Toxicity

About 30% of NSCLC patients will have immune related adverse events (irAE) when treated with ICIs[81]. If only focus on drugs target PD-1 and PD-L1, the overall irAE incidence was 64% for PD-1 and 66% for PD-L1 inhibitors. Specifically, toxicity grade that is greater than 3 of PD-1 was 14% and PD-L1 inhibitors was 21Managed by toxicities specified to organs, AEs caused by anti PD-1/PD-L1 treatments have shown to be widely related to skin, lung, liver, pancreas, stomach, intestine, and so on[82]. Three often reported skin toxicities include rash, pruritus, and vitiligo. The rash in these conditions can be maculopapular, papulopustular, or mossy dermatitis, with maculopapular rash being the most prevalent type[83]. For the endocrine system, hypothyroidism is the most prevalent endocrine condition linked to nivolumab and pembrolizumab, but the endocrine system's prevalence of severe irAEs only accounts for $\leq$ 2% of all studied ICIs[84]. Immune-associated pneumonia typically happens at a high rate for patients with other existing lung diseases, such as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis[85,86]. Lung pneumonia is likely to endanger one's life rapidly, therefore immediate actions should be taken as soon as diagnosed. In addition to these, immune-related hepatitis, colitis, and myalgia are also examples of irAEs[82]. Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab, Avelumab monotherapies, and some of their combination therapies were tested in a study, showing that fatigue (19% in PD-1 inhibitor vs 21% in PD-L1 inhibitors) is the most common AE in both inhibitors, followed by diarrhea (9% in PD-1 inhibitors vs 12% in PD-L1 inhibitors) and rash (9% in PD-1 inhibitors vs 7% in PD-L1 inhibitors). In this study, the incidence of irAEs at any grade was greater with pembrolizumab than with nivolumab[87]. Another 36 head-to-head phase II and III randomized comparative trials evaluated the safety of more ICI therapies, coming to the result that

atezolizumab (66.4%), nivolumab (71.8%), and pembrolizumab (75.1%) ranked lowest to highest as regards of the risk of ICI drugs[88]. Other than mild AEs, SAEs can also be detected in NSCLC. For example, immune-associated encephalitis is a poorly understood fatal irAE[89]; Myasthenia Gravis, a serious neurotoxicity seems to appear more in patients receiving PD-1 mAb[90]; Acute interstitial nephritis (ATIN) who is necessary to add adjuvant medicine like Infliximab, mycophenolate to treat side effects; and myocarditis, who occurs the most in patients treating with Avelumab[91,92,93].

Discussion

Since there is a lack of studies on factors that affect PD-1/PD-L1 expression in NSCLC, it is essential to clearly figure out what and how clinicopathological features influence it, and how they lead to different effectiveness, suitability and toxicity of ICI drugs. According to this, investigators can have a more comprehensive understanding of PD as a whole, and thus improve PD-1/PD-L1 inhibitors. According to studies included in this review, patients typically have longer ORRs when their PD-L1 expression levels are greater than 50%[94]. This data highlights the tremendous benefit of ICIs towards patients who underwent higher PD-L1 expression. But for people whose PD-L1 expression is low or medium and who do not respond well to radiation and chemotherapy, their challenge to mediate tumor progression remains much greater than others. Besides PD-1/PD-L1 expressions decide which group of patients benefit more from ICI therapy, other biomarker testings are also essential to not only early diagnosis but also accurate prescription. Tumor mutational burden (TMB) is a typical factor that should be included in consideration since patients with high TMB rate exhibit increased PFS, undermining ICIs' efficacy. We can conclude that patients with high TMB do not usually favor the use of these drugs. A notable possibility to develop side effects is another problem we face. Even though anti-PD-1/PD-L1 mAbs are generally less toxic than anti-CTLA-4 mAbs and chemotherapy drugs like docetaxel, some ICIs, especially Pembrolizumab, have a higher chance to develop SAEs than chemotherapy, which gives researchers a direction and space for future exploration. For anti-PD-1 drugs, specifically, they can lead to general AEs associated with immune activation (rash, fatigue, diarrhea), organ-specific immune-related AEs, and AEs consistent with musculoskeletal problems[95]. Beyond NSCLC, melanoma, renal cell carcinoma, urothelial carcinoma, and other solid tumors are among the many solid malignancies for which blocking the PD pathway improves antitumor effects[96]. Based on the comparison above, even though different patients will take their personalized treatment, pembrolizumab is the relatively board approved drug for NSCLC because of its demonstrated efficacy and the availability in combination therapies.

Except six FDA-approved ICIs, other immunotherapy treatments are being investigated to treat NSCLC as well. For instance, CAR-T cell treatment is a method that uses genetically engineered chimeric T cells, containing new antigen receptors, to bind with cancer cells and kill them. Furthermore, bispecific antibodies (BsAbs) are designed to combine two different antibodies, allowing one arm of the BsAb binds to a T cell while the other arm binds to a target cell. This brings the T cell close to the target cell, which activates the T cell to release proteins that kill the cancer cell.

Review limitations

There are limitations on this review that may cause bias. Because multiple clinical trials were discussed in this review, there was no uniform drug dosage and control group. Therefore, this report may have omitted potential side effects and incomplete data, like the OS and ORR values for some trials. Also, most of the affecting factors have not yet been confirmed to make a definite conclusion on their impact on the expression of PD-1/PD-L1 in NSCLC. Therefore, a lack of studies on specific areas of NSCLC is an eminent problem, causing the conclusion to remain unclear or contradictory. Inadequate clinical trials, limited participants, confined geography, and insignificant outcomes all hinder further exploration of impacting factors and the development of checkpoint inhibitors or other more advanced therapies. Another reason for researchers to generalize findings from clinical trials to a broader population is because of the heterogeneity of NSCLC. It's a highly varied disease with unknown cancer cell origin, high frequency of non-synonymous mutations, complicated pathological features and so on, making it difficult to come to a united treatment. Higher volume clinical trials and additional side effects studies are needed to refine and deepen the current findings. First, a high-volume clinical trial with one universal control group and various experimental groups should be set up to achieve the goal of a unified constant, thus making data comparable and more straightforward for researchers to figure out new issues. Second, based on the current study and the few studies I selected in this review, AEs of ICIs should be further affirmed by applying more sophisticated equipment to detect existing complications at an early stage and studying whether there is any adverse reaction between the drug components and the relevant body cells and hence improve drug manufacturing, thereby minimizing side effects at any severity grade. Additionally, more accurate biomarkers to predict patients' response and ways to achieve higher reaction rate are yet to be studied. In conclusion, it has been demonstrated that the involvement of PD-1 and PD-L1 in NSCLC are crucial, and their inhibitors are durable for cancer immunology. We believe that through clarifying the association between clinicopathological features and PD-1/PD-L1 expression, its blockade will become the major first-line treatment in a few years with the combination of other therapies to reduce toxicity, pain, and duration of

treatment.

References

1 C. Zappa and S. Mousa, *Translational Lung Cancer Research*, 2016, **5**, 288–300.

2 H. Sung, J. Ferlay, R. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal *et al.*, *CA: A Cancer Journal for Clinicians*, 2021, **71**, 209–249.

3 N. Patsoukis, J. Brown, V. Petkova, F. Liu, L. Li and V. Boussiotis, *Science Signaling*, 2012, **5**, ra46.

4 C. Ghosh, G. Luong and Y. Sun, *Journal of Cancer*, 2021, **12**, 2735–2746.

5 H. Yu, T. Boyle, C. Zhou, D. Rimm and F. Hirsch, *Journal of Thoracic Oncology*, 2016, **11**, 964–975.

6 E. McCarthy, *Iowa Orthopaedic Journal*, 2006, **26**, 154–158.

7 E. Buchbinder and A. Desai, *American Journal of Clinical Oncology*, 2016, **39**, 98–106.

8 V. A. Boussiotis, *The New England Journal of Medicine*, 2016, **375**, 1767–1778.

9 Y. Tsujimoto, *Genes to Cells*, 1998, **3**, 697–709.

10 L. Francisco, P. Sage and A. Sharpe, *Immunological Reviews*, 2010, **236**, 219–242.

11 W. Qin, L. Hu, X. Zhang, S. Jiang, J. Li, Z. Zhang *et al.*, *Frontiers in Immunology*, 2019, **10**, 2298.

12 B. Wang, C. Chen, X. Liu, S. Zhou, T. Xu and M. Wu, *Frontiers in Immunology*, 2023, **14**, 1111005.

13 R. Mizuno, D. Sugiura, K. Shimizu, T. Maruhashi, M. Watada, I. Okazaki *et al.*, *Frontiers in Immunology*, 2019, **10**, 630.

14 L. Chen and X. Han, *The Journal of Clinical Investigation*, 2015, **125**, 3384–3391.

15 X. Wang, F. Teng, L. Kong and J. Yu, *OncoTargets and Therapy*, 2016, **9**, 5023–5039.

16 Z. Gatalica, C. Snyder, T. Maney, A. Ghazalpour, D. Holterman, N. Xiao *et al.*, *Cancer Epidemiology, Biomarkers Prevention*, 2014, **23**, 2965–2970.

17 P. Dong, Y. Xiong, J. Yue, S. Hanley and H. Watari, *Frontiers in Oncology*, 2018, **8**, 386.

18 Y. Gao, J. Yang, Y. Cai, S. Fu, N. Zhang, X. Fu *et al.*, *International Journal of Cancer*, 2018, **143**, 931–943.

19 M. Ji, Y. Liu, Q. Li, X. Li, W. Zhao, H. Zhang *et al.*, *Journal of Translational Medicine*, 2015, **13**, 5.

20 D. Kim, H. Kim, Y. Choi, S. Kim, J. Lee, K. Sung *et al.*, *Experimental & Molecular Medicine*, 2019, **51**, 1–13.

21 M. Wu, Q. Huang, Y. Xie, X. Wu, H. Ma, Y. Zhang *et al.*, *Journal of Hematology & Oncology*, 2022, **15**, 24.

22 B. Fife and J. Bluestone, *Immunological Reviews*, 2008, **224**, 166–182.

23 L. May, K. Shows, P. Nana-Sinkam, H. Li and J. Landry, *Cancers*, 2023, **15**, 1211.

24 F. Fu, C. Deng, W. Sun, Q. Zheng, Y. Jin, Y. Li *et al.*, *Respiratory Research*, 2022, **23**, 302.

25 Y. Gu, Y. Tang, J. Wan, J. Zou, C. Lu, H. Zhu *et al.*, *Frontiers in Immunology*, 2022, **13**, 1026214.

26 F. Conforti, L. Pala, E. Pagan, C. Corti, V. Bagnardi, P. Queirolo *et al.*, *ESMO Open*, 2021, **6**, 100251.

27 G. Lin, X. Fan, W. Zhu, C. Huang, W. Zhuang, H. Xu *et al.*, *Oncotarget*, 2017, **8**, 83986–83994.

28 H. Li, Y. Xu, B. Wan, Y. Song, P. Zhan, Y. Hu *et al.*, *Translational Lung Cancer Research*, 2019, **8**, 429–449.

29 A. Alberg and J. Samet, *Chest*, 2003, **123**, 21S–49S.

30 A. Calles, X. Liao, L. Sholl, S. Rodig, G. Freeman, M. Butaney *et al.*, *Journal of Thoracic Oncology*, 2015, **10**, 1726–1735.

31 L. Dai, B. Jin, T. Liu, J. Chen, G. Li and J. Dang, *EClinicalMedicine*, 2021, **38**, 100990.

32 F. Bie, H. Tian, N. Sun, R. Zang, M. Zhang, P. Song *et al.*, *Clinical Epigenetics*, 2021, **13**, 229.

33 S. Abdelhamed, K. Ogura, S. Yokoyama, I. Saiki and Y. Hayakawa, *Journal of Cancer*, 2016, **7**, 1579–1586.

34 E. Akbay, S. Koyama, J. Carretero, A. Altabef, J. Tchaicha, C. Christensen *et al.*, *Cancer Discovery*, 2013, **3**, 1355–1363.

35 C. Lee, L. Davies, Y. Wu, T. Mitsudomi, A. Inoue, R. Rosell *et al.*, *Journal of the National Cancer Institute*, 2017, **109**, 1–11.

36 U. Janzic, I. Kern, A. Janzic, L. Cavka and T. Cufer, *Radiology and Oncology*, 2017, **51**, 357–362.

37 K. Pawelczyk, A. Piotrowska, U. Ciesielska, K. Jablonska, N. Gletzel-Plucinska, J. Grzegrzolka *et al.*, *International Journal of Molecular Sciences*, 2019, **20**, 824.

38 A. Heeren, S. Punt, M. Bleeker, K. Gaarenstroom, J. van der Velden, G. Kenter *et al.*, *Modern Pathology*, 2016, **29**, 753–763.

39 H. Yoon, Z. Jin, O. Kour, L. Kankeu Fonkoua, K. Shitara, M. Gibson *et al.*, *JAMA Oncology*, 2022, **8**, 1456–1465.

40 M. Disis, *Seminars in Oncology*, 2014, **41**, S3–S13.

41 E. Dantoing, N. Piton, M. Salaün, L. Thiberville and F. Guisier, *International Journal of Molecular Sciences*, 2021, **22**, 12628.

42 Y. Xu, B. Wan, X. Chen, P. Zhan, Y. Zhao, T. Zhang *et al.*, *Translational Lung Cancer Research*, 2019, **8**, 413–428.

43 L. Meng, H. Wu, J. Wu, P. Ding, J. He, M. Sang *et al.*, *Cell Death & Disease*, 2024, **15**, 3.

44 H. Borghaei, L. Paz-Ares, L. Horn, D. Spigel, M. Steins, N. Ready *et al.*, *The New England Journal of Medicine*, 2015, **373**, 1627–1639.

45 J. Brahmer, K. Reckamp, P. Baas, L. Crinò, W. Eberhardt, E. Poddubskaya *et al.*, *The New England Journal of Medicine*, 2015, **373**, 123–135.

46 R. Sundar, B. Cho, J. Brahmer and R. Soo, *Therapeutic Advances in Medical Oncology*, 2015, **7**, 85–96.

47 B. Zhao, H. Zhao and J. Zhao, *Journal for Immunotherapy of Cancer*, 2018, **6**, 101.

48 J. Brahmer, D. Rodríguez-Abreu, A. Robinson, R. Hui, T. Csőszi, A. Fülöp *et al.*, *Journal of Thoracic Oncology*, 2017, **12**, S1793–S1794.

49 B. Yang, B. Wang, Y. Chen, N. Wan, F. Xie, N. Yang *et al.*, *Frontiers in Oncology*, 2023, **13**, 1044327.

50 M. Reck, D. Rodríguez-Abreu, A. Robinson, R. Hui, T. Csőszi, A. Fülöp *et al.*, *Journal of Clinical Oncology*, 2019, **37**, 537–546.

51 M. Reck, D. Rodríguez-Abreu, A. G. Robinson, R. Hui, T. Csőszi, A. Fülöp *et al.*, *The New England Journal of Medicine*, 2016, **375**, 1823–1833.

52 A. Markham and S. Duggan, *Drugs*, 2018, **78**, 1841–1846.

53 M. Özgüroğlu, S. Kilickap, A. Sezer, M. Gümüş, I. Bondarenko, M. Gogishvili *et al.*, *The Lancet Oncology*, 2023, **24**, 989–1001.

54 A. Sezer, S. Kilickap, M. Gümüş, I. Bondarenko, M. Özgüroğlu, M. Gogishvili *et al.*, *The Lancet*, 2021, **397**, 592–604.

55 M. Gogishvili, T. Melkadze, T. Makharadze, D. Giorgadze, M. Dvorkin, K. Penkov *et al.*, *Nature Medicine*, 2022, **28**, 2374–2380.

56 N. Shah, W. Kelly, S. Liu, K. Choquette and A. Spira, *Human Vaccines & Immunotherapeutics*, 2018, **14**, 269–276.

57 L. Mathieu, E. Larkins, A. Sinha, P. Mishra-Kalyani, S. Jafri, S. Kalavar *et al.*, *Clinical Cancer Research*, 2023, **29**, 2973–2978.

58 R. Herbst, G. Giaccone, F. de Marinis, N. Reinmuth, A. Vergnenegre, C. Barrios *et al.*, *The New England Journal of Medicine*, 2020, **383**, 1328–1339.

59 S. Lee, C. Schulz, K. Prabhash, D. Kowalski, A. Szczesna, B. Han *et al.*, *The Lancet*, 2023, **402**, 451–463.

60 R. Stewart, M. Morrow, S. A. Hammond, K. Mulgrew, D. Marcus, E. Poon *et al.*, *Cancer Immunology Research*, 2015, **3**, 1052–1062.

61 Y. Y. Syed, *Drugs*, 2017, **77**, 1369–1376.

62 D. R. Spigel, C. Faivre-Finn, J. E. Gray, D. Vicente, D. Planchard, L. Paz-Ares *et al.*, *Journal of Clinical Oncology*, 2022, **40**, 1301–1311.

63 L. Paz-Ares, A. Spira, D. Raben, D. Planchard, B. C. Cho, M. Özgüröğlu *et al.*, *Annals of Oncology*, 2020, **31**, 798–806.

64 A. Akinleye and Z. Rasool, *Journal of Hematology & Oncology*, 2019, **12**, 92.

65 H. Jin, V. D'Urso, B. Neuteboom, S. D. McKenna, R. Schweickhardt, A. W. Gross *et al.*, *Oncoimmunology*, 2021, **10**, 1958590.

66 J. L. Gulley, D. Spigel, K. Kelly, J. C. Chandler, A. Rajan, R. Hassan *et al.*, *Journal of Clinical Oncology*, 2015, **33**, 8034–8034.

67 M. Reck, F. Barlesi, J. C. H. Yang, V. Westeel, E. Felip, M. Özgüröğlu *et al.*, *Journal of Thoracic Oncology*, 2024, **19**, 297–313.

68 Y. Wang, H. Han, F. Zhang, T. Lv, P. Zhan, M. Ye *et al.*, *British Journal of Cancer*, 2022, **127**, 948–956.

69 L. Paz-Ares, D. Vicente, A. Tafreshi, A. Robinson, H. Soto Parra, J. Mazières *et al.*, *Journal of Thoracic Oncology*, 2020, **15**, 1657–1669.

70 L. Paz-Ares, A. Luft, D. Vicente, A. Tafreshi, M. Gümüş, J. Mazières *et al.*, *The New England Journal of Medicine*, 2018, **379**, 2040–2051.

71 C. Zhou, G. Chen, Y. Huang, J. Zhou, L. Lin, J. Feng *et al.*, *The Lancet Respiratory Medicine*, 2021, **9**, 305–314.

72 H. A. Wakelee, N. K. Altorki, C. Zhou, T. Csőszi, I. O. Vynnychenko, O. Goloborodko *et al.*, *Journal of Clinical Oncology*, 2021, **39**, 8500–8500.

73 M. Alifu, M. Tao, X. Chen, J. Chen, K. Tang and Y. Tang, *Frontiers in Oncology*, 2023, **13**, 1146905.

74 Y. Lee, S. L. Auh, Y. Wang, B. Burnette, Y. Wang, Y. Meng *et al.*, *Blood*, 2009, **114**, 589–595.

75 H. E. Barker, J. T. E. Paget, A. A. Khan and K. J. Harrington, *Nature Reviews Cancer*, 2015, **15**, 409–425.

76 F. Veglia, E. Sanseviero and D. I. Gabrilovich, *Nature Reviews Immunology*, 2021, **21**, 485–498.

77 L. Deng, H. Liang, B. Burnette, M. Beckett, T. Darga, R. R. Weichselbaum *et al.*, *Journal of Clinical Investigation*, 2014, **124**, 687–695.

78 W. S. M. E. Theelen, D. Chen, V. Verma, B. P. Hobbs, H. M. U. Peulen, J. G. J. V. Aerts *et al.*, *The Lancet Respiratory Medicine*, 2021, **9**, 467–475.

79 Y. Geng, Q. Zhang, S. Feng, C. Li, L. Wang, X. Zhao *et al.*, *Cancer Medicine*, 2021, **10**, 1222–1239.

80 Y. Cho, S. Park, H. K. Byun, C. G. Lee, J. Cho, M. H. Hong *et al.*, *International Journal of Radiation Oncology, Biology, Physics*, 2019, **105**, 1065–1073.

81 D. H. Owen, L. Wei, E. M. Bertino, T. Edd, M. A. Villalona-Calero, K. He *et al.*, *Clinical Lung Cancer*, 2018, **19**, e893–e900.

82 C. Su, H. Wang, Y. Liu, Q. Guo, L. Zhang, J. Li *et al.*, *Frontiers in Oncology*, 2020, **10**, 554313.

83 J. Naidoo, D. B. Page, B. T. Li, L. C. Connell, K. Schindler, M. E. Lacouture *et al.*, *Annals of Oncology*, 2015, **26**, 2375–2391.

84 P. F. Wang, Y. Chen, S. Y. Song, T. J. Wang, W. J. Ji, S. W. Li *et al.*, *Frontiers in Pharmacology*, 2017, **8**, 730.

85 N. M. Mark, J. Kargl, S. E. Busch, G. H. Y. Yang, H. E. Metz, H. Zhang *et al.*, *American Journal of Respiratory and Critical Care Medicine*, 2018, **197**, 325–336.

86 M. J. Bott, S. C. Yang, B. J. Park, P. S. Adusumilli, V. W. Rusch, J. M. Isbell *et al.*, *Journal of Thoracic and Cardiovascular Surgery*, 2019, **158**, 269–276.

87 R. N. Pillai, M. Behera, T. K. Owonikoko, A. O. Kamphorst, S. Pakkala, C. P. Belani *et al.*, *Cancer*, 2018, **124**, 271–277.

88 C. Xu, Y. P. Chen, X. J. Du, J. Q. Liu, C. L. Huang, L. Chen *et al.*, *The British Medical Journal*, 2018, **363**, k4226.

89 A. Vogrig, S. Muñiz-Castrillo, B. Joubert, G. Picard, V. Rogemond, C. Marchal *et al.*, *Journal of Neurology, Neurosurgery & Psychiatry*, 2020, **91**, 772–778.

90 H. Safa, D. H. Johnson, V. A. Trinh, T. E. Rodgers, H. Lin, M. E. Suarez-Almazor *et al.*, *Journal for ImmunoTherapy of Cancer*, 2019, **7**, 319.

91 T. Makunts, I. M. Saunders, I. V. Cohen, M. Li, T. Moumedjian, M. A. Issa *et al.*, *Scientific Reports*, 2021, **11**, 17324.

92 Q. Fan, Y. Hu, C. Yang and B. Zhao, *Frontiers in Pharmacology*, 2019, **10**, 1207.

93 R. Koda, H. Watanabe, M. Tsuchida, N. Iino, K. Suzuki, G. Hasegawa *et al.*, *BMC Nephrology*, 2018, **19**, 48.

94 E. J. Aguilar, B. Ricciuti, J. F. Gainor, K. L. Kehl, S. Kravets, S. Dahlberg *et al.*, *Annals of Oncology*, 2019, **30**, 1653–1659.

95 S. Baxi, A. Yang, R. L. Gennarelli, N. Khan, Z. Wang, L. Boyce *et al.*, *The British Medical Journal*, 2018, **360**, k793.

96 Q. Lei, D. Wang, K. Sun, L. Wang and Y. Zhang, *Frontiers in Cell and Developmental Biology*, 2020, **8**, 672.