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REVIEW ARTICLE

Immune Checkpoint Inhibitors for Non-Small Cell Lung Cancer: Review

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Abstract

The aim of this review was to evaluate the efficacy of immune checkpoint inhibitors (ICIs) for non-small cell lung cancer (NSCLC). NSCLC is the most common lung malignancy (85% of all lung cancers) and has a poor prognosis. Though chemotherapy is an effective therapeutic option, factors such as drug resistance, drug toxicity, and adverse side-effects cause poor response rate and ~10-20% survival within 2 years in metastatic NSCLC patients. The efficacy of ICIs in NSCLC has been widely studied as an alternative treatment option. This review discusses the role of ICIs in tumorigenesis, recent clinical trials, clinically available ICIs, including their efficacy, toxicity, and application as a single agent and in combination with other agents. Currently, ICIs such as Nivolumab and Pembrolizumab have been approved as effective therapeutic options for NSCLC. Durable response rate, improved overall survival, and tolerable safety profiles have been recorded in clinical trials for these ICIs. Combination treatments with ICIs such as Nivolumab + Ipilimumab and Durvalumab + Tremelimumab have been shown in clinical trials to provide better safety and efficacy profiles than single ICI treatment. However, further research is required to study different ICI combination therapies in treating metastatic NSCLC and variations in efficacy among smokers and non-smokers. It is recommended that future research should consider combination therapy with other conventional therapies including chemotherapy, radiotherapy, and personalized targeted therapy; the efficacy of ICIs for early-stage cancer; and identification of predictive biomarkers.

Keywords: Immune checkpoint inhibitors, efficacy, non-small cell lung cancer, programmed cell death 1, programmed cell death ligand 1, cytotoxic T-lymphocyte–associated protein 4

Introduction

Lung cancer is the most common cancer, accounting for ~1.6 million deaths worldwide in 2012 and 36.4 million disability-adjusted life-years in 2015[1,2]. The World Health Organisation 2012 data shows that lung cancer affects more men (~1.1 million deaths) than women (~491 thousand deaths), and the highest mortality rates are recorded in Europe and North America (Figure 1)[1]. Around 80% of diagnosed cases of lung cancer are due to cigarette smoking or chronic exposure to passive tobacco smoke[3]. Tobacco smoke damages the lung tissue and leads to gene mutations. Risk factors such as ageing, unhealthy diet, obesity, consumption of arsenic-contaminated water, and chronic exposure to air pollution and carcinogenic chemicals can also predispose one to lung cancer[3,4].

Lung cancer is divided into four stages (Figure 2) and are classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC)[5]. NSCLC consists of non-squamous cell carcinomas, squamous cell carcinomas and large cell carcinomas which have been grouped together due to their similar behaviour and response to treatment[5]. Non-squamous cell carcinoma is the most common type of NSCLC[5]. SCLC is an aggressive type of lung cancer with two main types (small cell and combined small cell carcinomas), which are usually caused by smoking. The different types of NSCLC account for ~85% (Figure 3) of all lung cancer[5,6]. Patients demonstrate poor response to chemotherapy in metastatic NSCLC, with a median overall survival of 8–10 months after diagnosis and a 2–year survival of about 10-20%[7]. Patients with poorly managed metastatic NSCLC have median overall survival of about 4–5 months, with 10% survival in the first year of diagnosis[8,9].

Both NSCLC and SCLC have similar symptoms upon disease progression which include persistent coughing, shortness of breath, wheezing, chest pain when breathing or coughing, coughing up blood, loss of appetite, and unexplained tiredness and weight loss[10].
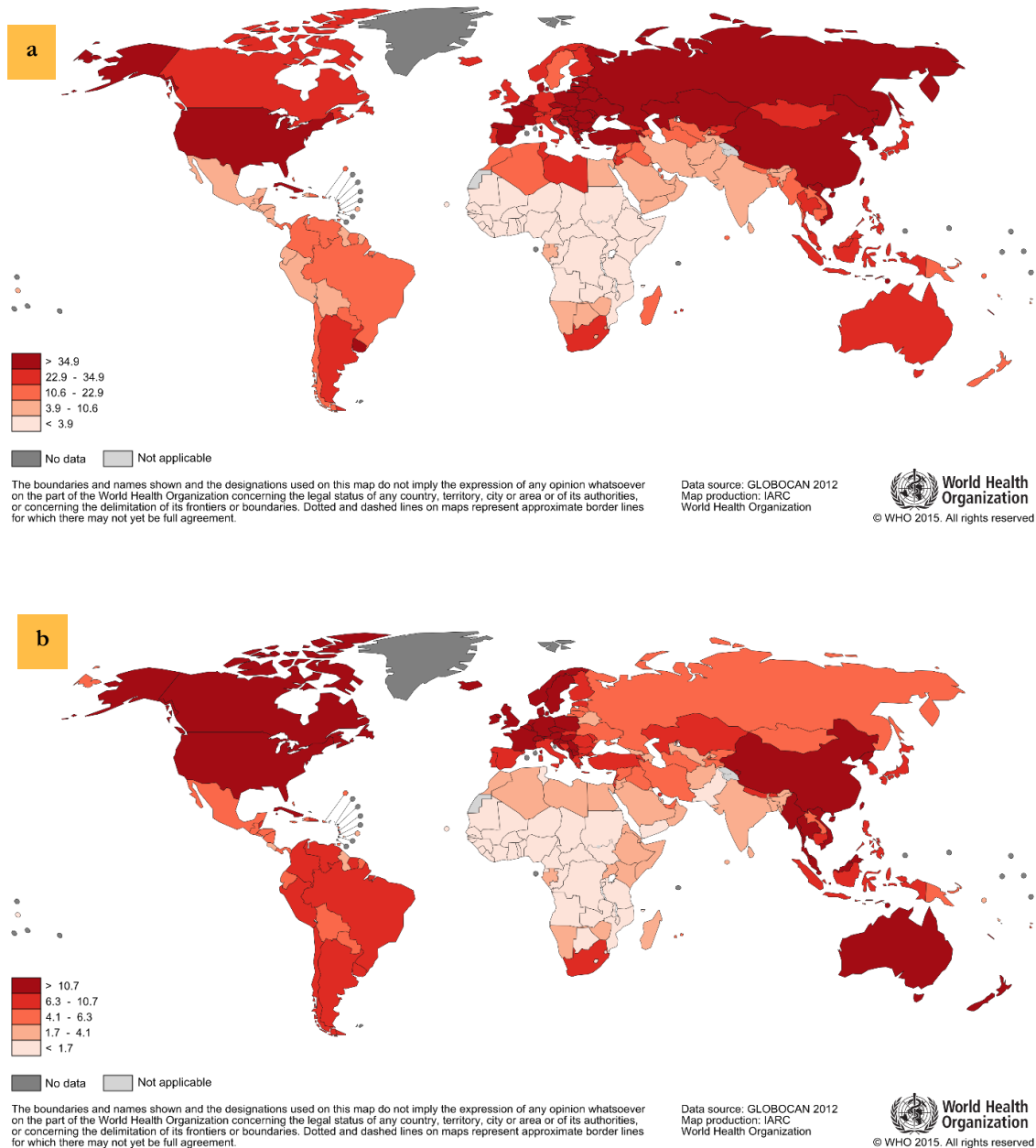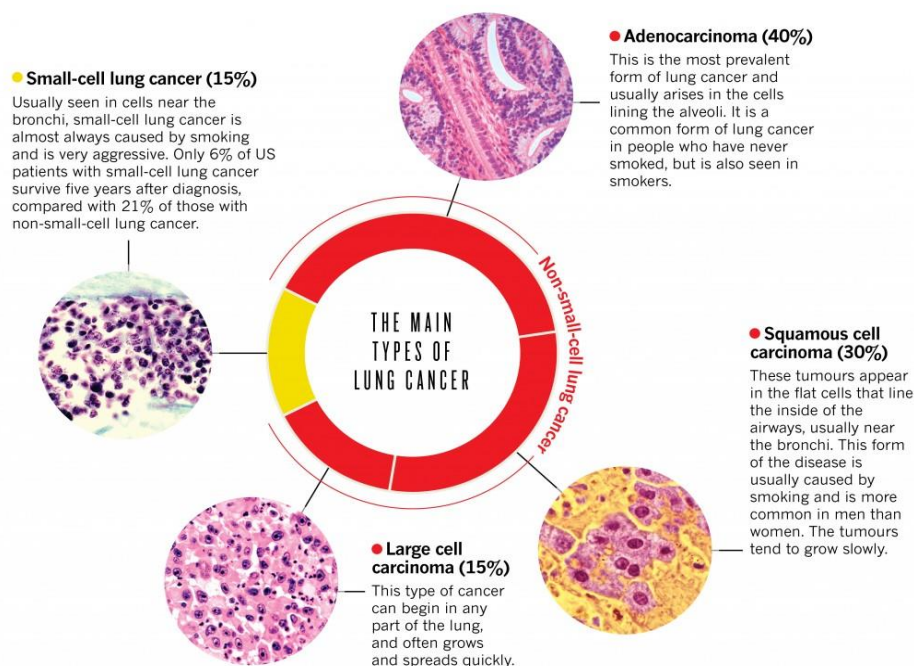

Figure 1 - *Lung Cancer mortality in (a) men and (b) women worldwide in 2012 (World Health Organization[1]).*
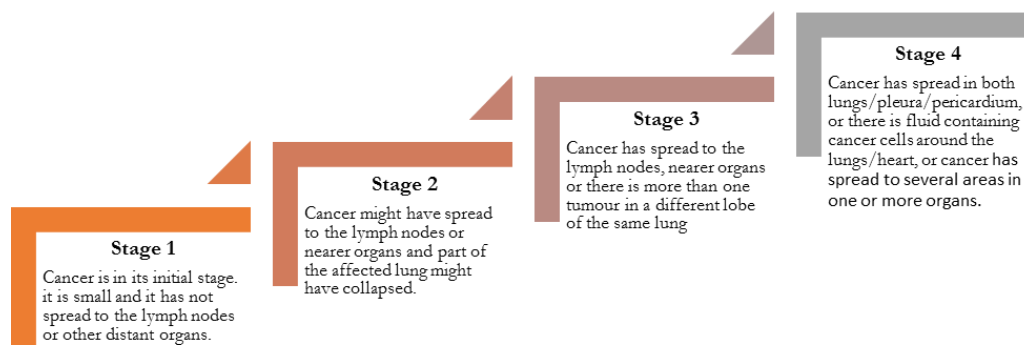
Figure 2 - *Stages of lung cancer[8]*

Small-cell lung cancer (15%)
Usually seen in cells near the bronchi, small-cell lung cancer is almost always caused by smoking and is very aggressive. Only 6% of US patients with small-cell lung cancer survive five years after diagnosis, compared with 21% of those with non-small-cell lung cancer.

Adenocarcinoma (40%)
This is the most prevalent form of lung cancer and usually arises in the cells lining the alveoli. It is a common form of lung cancer in people who have never smoked, but is also seen in smokers.

Squamous cell carcinoma (30%)
These tumours appear in the flat cells that line the inside of the airways, usually near the bronchi. This form of the disease is usually caused by smoking and is more common in men than women. The tumours tend to grow slowly.

Large cell carcinoma (15%)
This type of cancer can begin in any part of the lung, and often grows and spreads quickly.

THE MAIN TYPES OF LUNG CANCER

Figure 3 - *Lung Cancer types*[6].

Treatment options for lung cancer include surgery, radiotherapy, chemotherapy, or targeted therapy. Surgery with stereotactic body radiotherapy was reported to be relevant in patients with stage 1 NSCLC[9]. Post-surgery adjuvant therapy such as chemotherapy, radiotherapy, targeted therapy is usually given to lower the risks of cancer recurrence. Chemotherapy is the most widely used treatment for lung cancers, involving single or multiple standard chemotherapeutic drugs to induce cancer apoptosis[9,11]. Though effective, conventional treatment options result in minimal survival benefits largely due to drug resistance, adverse side-effects, and toxicity. Modern treatment options for lung cancer have focused on immunotherapy which enhances the immune systems' innate capacity to fight cancer[12].

This review focuses on the efficacy of immunotherapy, specifically immune checkpoint inhibitors (ICIs) in NSCLC. The role of ICIs in tumorigenesis, recent clinical trials, clinically available ICIs, including their efficacy, toxicity, and application as a single agent and combination with other agents are discussed in this review.

Immunotherapy

Immunotherapy prolongs overall survival and provides durable remissions to advanced stage chemoresistant cancer patients. Although the immune system is programmed to eliminate tumour cells, they are limited by the immunosuppression activities of tumour cells including secretion of cytokines, loss of tumour antigens, and expressions of immune inhibitory ligands such as programmed cell death-protein 1 and 2 ligands (PD-L1 and PD-L2)[13]. To overcome these challenges, immunotherapeutic approaches such as checkpoint inhibitors, therapeutic vaccines, adoptive cellular therapies, oncolytic viral therapies, and monoclonal antibodies have been used to stimulate an immune response against tumour progression[14–18].

One of the most promising immunotherapies for NSCLC treatment is the ICIs. This technique works by blocking the checkpoints on the immune system, thus allowing it to attack the tumour cells more effectively. Different types of ICIs have been used to target and block different checkpoints on immune/cancer cells.

Immune Checkpoint Inhibitors

The immune system can distinguish between foreign cells and normal cells in the body. This process helps the immune system to fight invaders thus keeping the normal cells protected. Immune checkpoints are proteins that are hardwired in the immune system and they are involved in many inhibitory pathways which are vital for the immune cells to maintain self-tolerance. They also modulate the duration and amplitude of physiological immune responses in peripheral tissues, minimizing collateral tissue damage[19]. Cancer cells utilise these checkpoints to escape immune surveillance. Drugs targeting these immune checkpoints can be effective cure for various types of malignancies including NSCLC. Immune checkpoint inhibitors are drugs that bind to checkpoint proteins found on both immune and tumour cells to prevent cancer evasion[13]. Different types of ICIs have been used to target and block different checkpoints on immune or cancer cells (Table 1). This paper focusses on the PD-1/PDL1 and Cytotoxic-T-lymphocytes antigen-4 (CTLA-4) pathways and ICIs that target these checkpoints.

Table 1 - *Immune checkpoint proteins and their inhibitors*[20].

| Checkpoint Protein	Binding Partner	Inhibitor in Clinical Trial
PD-1 (programmed death-1)	PD-L1, PD-L2	Nivolumab, Pembrolizumab, Pidilizumab, AMP-224
PD-L1 (programmed death-ligand 1)	PD-1	Atezolizumab, Durvalumab, Avelumab, BMS-936559, MPDL33280A
CTLA-4 (cytotoxic T-lymphocyte antigen-4)	CD80, CD86	Ipilimumab and Tremelimumab
LAG-3 (lymphocyte-activation gene-3)	MHC-II	IMP321
KIR (killer cell immunoglobulin-like receptor)	MHC I-II	Lirilumab
TIM3 (T-cell immunoglobulin domain and mucin domain 3)	Galectin-9	-
BTLA (B and T lymphocyte attenuator)	Herpes virus entry mediator (HVEM)	-
A2aR (adenosine receptor)	Adenosine	-

PD-1/PD-L1 Pathway

The PD-1/PD-L1 inhibitory pathway is an effective therapeutic target due to its immunosuppressive activity. PD-1 is a 55-kDa IgG1 transmembrane protein found on activated T-cells[21]. PD-L1 and PD-L2 are the two type 1 transmembrane ligands expressed on cancer cells for a PD-1 receptor, with PD-L1 being the main immunosuppressive mediator[13,22,23]. The PD-L1-PD-1 complex down regulates T-cell activation, suppressing type1–based antitumor immunity which promotes tumour immune escape[24]. The PD-L1-PD-1 interaction forms a microcluster that initiates the activity of Src-homology 2 domain containing protein tyrosine phosphatase (SHP-2) which activates various signalling molecules via dephosphorylation on the cell membrane. These act downstream to reduce the cytotoxicity of T-cells towards cancer cells by significantly inhibiting both cytokine production and T-cell proliferation[24].

In tumour microenvironment, tumour cells overexpress PD-L1 and PD-L2 to hinder the immune surveillance. The binding of the ligands to PD-1 receptors inhibits T-cell activation and T-cell attack. This leads to the formation of a suitable tumour microenvironment for tumour cells proliferation[25]. Immune suppression regulated by PD-1/PD-L1 pathway includes multiple mechanisms. These are:

(i) Activated T cells death: PD-1 induces apoptotic pathway of T cells impacting apoptotic genes. The survival of T cell is sustained by the ligation of CD28 by driving the expression of Bcl-xL, an anti-apoptotic gene. PD-1 prevents the expression of the Bcl-xL by inhibiting P13K activation, which is crucial for the Bcl-xL upregulation. Several studies have demonstrated that PD-L1 positive expressions on human and murine tumour cells cause apoptosis of activated T-cells. ICIs blockade of PD-L1 can down-regulate T-cell apoptosis and mediate anti-tumour immunity[26,27].

(ii) Loss of T-cell immune response and exhaustion: There is an association between the occurrence of tumour and chronic infection[28]. During chronic infection, PD-1 on T-cells are overexpressed and this leads to the upregulated tumour immune escape. Inhibition of the PD-1/PD-L1 pathway can restore T cell proliferation, secretion and cytotoxicity[29].

(iii) Increased function of regulatory T-cells: PD-L1 assists in the generation of induced regulatory T-cells by down-regulating the AKT, mTOR, S6 and the phosphorylation of ERK2 and increasing PTEN, hence controlling the effector T-cell activity[30]. Blockade of a PD-1/PD-L1 pathway inhibits the regulatory T-cells function and upregulates the effector CD8 T-cell function, increasing the anti-tumour response.

(iv) Inhibition of T-cell proliferation: PD-1 ligation inhibits PKCθ phosphorylation which is essential for interleukin-2 production[31], and induces T-cells arrest in the G1 phase, hindering proliferation facilitated by the activation of Smad3, a factor that arrest cell cycle[32].

(v) Down-regulation of T-cell activation and interleukin-2 production: PD-1/PD-L1 blocks the downstream signalling events resulting in impaired T-cell activation and interleukin-2 production. It also reduces long-term immune memory[33] and promotes tumour progression.

High levels of PD-L1 has been observed in stage 1 to 4 NSCLC patients with poor survival, showing that PD-1/PD-L1 is significant in epithelial-originated malignancy prognoses[34]. In a study by Zhang et al.,[35] epithelial-cancer patients with positive PD-L1 expressions in tumours were associated with significantly poorer overall survival than negative PD-L1 patients. Another study by Sorensen et al.[34] of NSCLC-patients in cancer stages from 1 to 4 concluded that increased PD-L1 expression is associated with worst survival. High positive PD-L1 expression in infiltrating lymphocytes is also associated with short overall survival of patients. Thus, expressions of PD-1/PD-L1 may be a clinically important prognostic in epithelial-originated malignancies. The well-studied ICIs for the PD-1/PD-L1 pathway are Nivolumab, Pembrolizumab, Atezolizumab, and Durvalumab. Blockade of PD-1/PD-L1 checkpoints with monoclonal antibodies (Figure 4) has shown a great promise in clinical activity by significantly inhibiting the tumour growth in NSCLC.
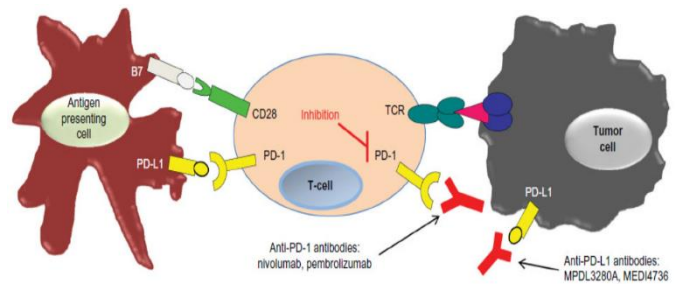
Figure 4 - *The PD-L1/PD-1 pathway. Examples of anti-PD-1 antibodies such as Nivolumab, Pembrolizumab and anti-PD-L1 antibodies such as Atezolizumab (MPDL3280A) and Durvalumab (MED14736) have been used to illustrate the inhibition of both PD-1 and PD-L1 in tumour microenvironment*[36].

Nivolumab

In 2015, the first ICI, Nivolumab (Opdivo®), an IgG4 PD-1 human monoclonal antibody (hmAb), was approved by the United States Food and Drugs Administration (FDA) and European Commission for the treatment of metastatic squamous NSCLC[37,38]. Nivolumab targets and blocks the interaction of PD-1 and PD-L1[38]. The approval was based on an open-label (CheckMate 057) phase 3 trial[38] where 272 patients with metastatic squamous NSCLC were randomized (1:1) to receive Nivolumab (n=135) at a dose of 3 mg/kg intravenously every-2-weeks or a chemotherapeutic agent Docetaxel (n=137) at 75 mg/m² intravenously every-3-weeks. Nivolumab was found to extend the overall survival by 3.2 months, with 41% reduction in risk of death than Docetaxel (Hazard ratio (HR) = 0.59; p<0.001). Fatigue, dyspnea and lymphopenia were among the most common and frequent adverse effects observed in at least 30% of Nivolumab treated patients.

Nivolumab was also approved for metastatic non-squamous NSCLC based on another phase 3 trial in 2015[39]. A total of 582 patients with metastatic non-squamous NSCLC were studied using the Checkmate 057 protocol. Nivolumab resulted in an improved median overall survival (12.2 months) than Docetaxel (9.4 months) (HR = 0.73; p=0.0015) and the median duration of response (DOR) in both drugs were 17 months and 6 months, respectively. The HR for patients with PD-L1 expression levels of ≥1% (0.59) was lower than those with <1% (0.90). A similar trend was observed for PD-L1 expression levels of ≥5% (0.43) against those with <5% (1.01). This clearly showed that patients with PD-L1-

positive tumours have longer survival when treated with Nivolumab than Docetaxel. The number of patients that had grade 3/4 treatment-related adverse effects (TRAEs) were 9 out of 131 and 71 out of 129 in the Nivolumab and Docetaxel-treated groups, respectively. The safety analysis found decreased appetite, coughing, pruritis and constipation as the most common adverse effects in ≥10% of the Nivolumab-treated patients. The most frequent adverse effects were musculoskeletal pain and fatigue.

In summary, both studies demonstrated improved overall survival in Nivolumab-treated patients. The TRAEs and the PD-L1-status in the second trial show that Nivolumab is favourable.

Pembrolizumab

In 2016, US FDA approved Pembrolizumab (Keytruda®), an IgG4-kappa isotype hmAb, for the treatment of metastatic non-squamous NSCLC with PD-L1 expression[40,41]. Pembrolizumab binds to PD-1, blocking the interactions with PD-L1/L2[41].

A phase 1 trial[41] administered Pembrolizumab to 495 patients at a dose of either 2 mg or 10 mg/kg every-3-weeks or 10 mg/kg every-2-weeks. The patients were assigned into a training group (n=182) and a validation group (n=313). The overall median objective response rate (ORR) was 19.4%, overall survival was 12 months and progression-free-survival (PFS) was 3.7 months. The median DOR was 12.5 months and there was no disease progression in 84.4% of patients with a response. Patients with PD-L1 expression in ≥50% of tumour cells had a response rate of 45.2% in the validation group. The overall median PFS for patients with a PD-L1 score of ≥50% was 6.3%.

In another open-label, randomized, phase 2/3 study[42], previously treated PD-L1-positive NSCLC patients were assigned (1:1:1) to receive Pembrolizumab 2 mg/kg (n=345; Group 1), 10 mg/kg (n=346; Group 2) or Docetaxel 75 mg/m² (n=343; Group 3) every-3-weeks. The median overall survival of Groups 1 (10.4 months) and 2 (12.7 months) was significantly longer than Group 3 (8.5 months) (HR=0.71, p=0.0008; and HR=0.61, p<0.0001, respectively). A similar trend was observed in patients with a PD-L1 score of ≥50%. The median PFS for Groups 1, 2 and 3 were 3.9, 4.0, and 4.0 months, respectively. Pembrolizumab-treated patients had less common grade 3/4 treatment-related serious adverse reactions (13% and 16%, respectively) compared with Docetaxel-treated patients (35%).

In both studies, patients treated with 10 mg/kg Pembrolizumab every-2/3-weeks or 2 mg/kg Pembrolizumab every-3-weeks demonstrated durable response, improved overall survival, and acceptable amount of TRAEs profile than Docetaxel-treated patients. There was no significant difference between the efficacy and safety profile of Groups 1 and 2. This finding is consistent with the results of melanoma cohorts (KEYNOTE-001)[43–45]. Usually, the lowest dose is recommended when the efficacy is similar to the high dose. The efficacy of 2 mg dose of Pembrolizumab is currently being evaluated in KEYNOTE-001 and phase 2-3 KEYNOTE-010 cohorts (ClinicalTrials.gov number, NCT01905657).

Atezolizumab

Atezolizumab, an IgG1 hmAb, targets PD-L1 on tumour cells and tumour-infiltrating immune cells by binding to PD-1 and B7.1[46]. This prevents binding of PD-L1 thereby activating T-cells[46]. In 2015, Atezolizumab received "Breakthrough Therapy" by the US

FDA because it is active against chemotherapy-refractory NSCLC, especially tumours expressing PD-L1, tumour-infiltrating immune cells, or both[46,47]. In an open-label phase 2 trial (POPLAR)[46], chemotherapy-naïve (patients who had never received chemotherapy) NSCLC patients were randomly assigned into two groups (1:1) according to previous line-of-therapy, PD-L1 tumour-infiltrating immune cell status, and histology. Patients received 1200 mg Atezolizumab (n=144) or 75 mg/m² Docetaxel (n=143) intravenously once every-3-weeks. The overall survival for Atezolizumab (12.6 months) was higher than Docetaxel (9.7 months) (HR = 0.73; p=0.04), with higher PD-L1 expressions. The treatment was discontinued for 8% Atezolizumab- and 22% Docetaxel-treated patients due to TRAEs. Moreover, 11% Atezolizumab- and 39% Docetaxel-treated patients had grade 3-4 TRAEs, and 1 (<1%) patient and 3 (2%) patients respectively died from TRAEs.

A recent ongoing phase 2, open-label, multicenter study[48] evaluated the efficacy of Atezolizumab in 659 PD-L1-positive metastatic NSCLC patients assigned to 3 cohorts (first (n=139), second (n=268) and third (n=252) line cohorts). Participants received the same dose of Atezolizumab as the POPLAR study. The overall median ORR was 18% - 22%. The median overall survival for the first line cohort (23.5 months) was higher than the second (15.5 months) and third (13.2 months) after ≥20 month follow up. Although most responses are still understudy, the trial found enhanced overall survival in patients with increased PD-L1 expressions. This indicates that positive response to Atezolizumab could be predicted from the PD-L1 status of patients.

Durvalumab

Durvalumab, an IgG1 anti-PD-L1 hmAb, binds to PD-L1 and blocks its interaction with PD-1 and CD80[49]. An ongoing phase 1/2, open-label, multicenter study[50,51] in metastatic NSCLC patients has shown durable anti-tumour activity along with an acceptable tolerability profile (TRAEs in 8% of patients). Durvalumab treatment with 10 mg/kg every-2-weeks resulted in higher ORR in patients with PD-L1-positve tumours (27%) than patients with PD-L1-negative tumours (<25%). Combination therapy with anti-CTLA-4 Tremelimumab has been suggested to counter the limited response in patients with PD-L1-negative tumours[52]. Another ongoing phase 3 trial[53] is evaluating the efficacy of Durvalumab in unresectable NSCLC patients following a definitive chemoradiation.

CTLA-4 Pathway

Cytotoxic-T-lymphocytes antigen-4 (CTLA-4) is a CTLs and CD28 homodimeric glycoprotein receptor for B7-ligands[54]. T-cell activation upregulates CTLA-4 expression, which inhibits T-cell activation through competitive binding to CD80 (B7.1) and CD86 (B7.2)[18,54,55]. This maintains the balance between immune activation and inhibition.

In the tumour microenvironment, tumour cells secrete a suppressive cytokine TGF-beta that mediates the upregulation of CTLA-4 molecules on T-cells[55]. This suppresses T cell-mediated immune response. The expression of CTLA-4 is significantly higher in precancerous lesions and primary lung cancer sample than in normal lung tissues: 75% in NSCLC, 52.8% in metastatic non-squamous NSCLC, and 35% in metastatic squamous NSCLC[55].

Anti-CTLA-4 mAb such as Ipilimumab and Tremelimumab have been used to suppress the inhibitory signals (Figure 5) resulting in the generation of antitumour T-cell responses.
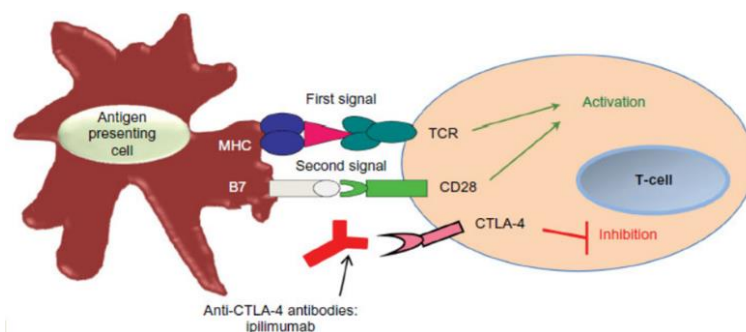
Figure 5 - *The CTLA-4 pathway. Ipilimumab, an example of anti-CTLA-4 antibody, has been used to illustrate the inhibition of CTLA-4 pathway in tumour microenvironment*[36]

Ipilimumab

Ipilimumab (Yervoy®), an anti-CTLA-4 IgG1 hmAb, blocks the CTLA-4 and B7-ligands' interaction[56] (Figure 5). In 2011, US FDA approved Ipilimumab for metastatic melanoma[57] and it is currently being tested in clinical trials for solid tumours including NSCLC.

Ipilimumab in combination with carboplatin or paclitaxel (Ipi+Car/Pac) has been used in a randomized phase 2 clinical trial[56] for both advanced SCLC (n=130) and NSCLC (n=204). Patients were randomized into 3 groups (1:1:1): placebo/chemotherapy alone, Ipilimumab plus chemotherapy and phased Ipilimumab. In the phased cohort, the HR for immune-related-PFS were 0.55 and 0.82 in patients with squamous and non-squamous histology, respectively. Another separate case report[58] of a recurrent NSCLC patient treated with palliative concurrent radiotherapy and Ipilimumab showed an increase in tumour regression, tumour-infiltrating immune cells, and normalization of tumour markers. PET/CT imaging showed no evidence of the disease after 1 year.

Lynch *et al.*[59] also assessed the efficacy of Ipi+Car+Pac in 204 randomly assigned (1:1:1) chemotherapy-naïve NSCLC patients. Car+Pac (175 mg/m²) with either Ipilimumab or placebo (concurrent Ipilimumab, and phased Ipilimumab) was administered intravenously every-3-weeks for ≤4¼ months. Both treatments were administered continuously to eligible patients every-12-weeks as maintenance therapy. Phased Ipilimumab, concurrent Ipilimumab and control treatments resulted in: median PFS of 5.1, 4.1, and 4.2 months; median immune-related-PFS of 5.7, 5.5, and 4.6 months; best immune-related-ORR of 32%, 21%, and 18%; best ORR of 32%, 21%, and 14%; and median overall survival of 12.2, 9.7, and 8.3 months, respectively. Treatment-related grade 3/4 were 15%, 20%, and 6%, respectively. Two patients (1 concurrent; 1 control) died due to TRAEs. The phased Ipi+Car+Pac is currently under further studies in a phase 3 trial.

Ipilimumab is actively being investigated for NSCLC through ongoing phase 3 studies. The Phased Ipilimumab plus paclitaxel and carboplatin improved immune-related-PFS and PFS in the phase 2 trial, which supports additional investigation of Ipilimumab in NSCLC.

Tremelimumab

Tremelimumab, another anti-CTLA-4 IgG2 hmAb, is being studied in an ongoing, open-label, multicenter, randomized phase 2 clinical trial[60]. The initial study was conducted in 87 metastatic NSCLC patients who responded after ≥4 cycles of first-line-platinum-based chemotherapy. Patients were randomized between 15 mg/kg Tremelimumab and best supportive care until disease progression. The resulting PFS was 3 months for 9 (20.9%) and 6 (14.3%) patients, respectively. The observed TRAEs was higher in the Tremelimumab-treated patients (61.4%) than the best supportive care patients (7%). Nine patients (20.5%) receiving Tremelimumab reported grade 3/4 adverse effects relative to patients receiving best supportive care (0%). Colitis and diarrhoea (9.1%) were the most common grade 3/4 adverse effects. Tremelimumab was tolerable with consistent safety in patients with metastatic NSCLC. An ORR of 4.8% was observed in the Tremelimumab group which may support future combination studies.

Combination ICIs Approaches

Clinical trials of ICI combination therapy have shown effective outcomes in patients with different cancer types including NSCLC. Blocking multiple checkpoints simultaneously allows continued T-cell survival, proliferation, enhanced infiltration, and tumour rejection[61]. Certain combination therapies including Nivolumab + Ipilimumab (Niv + Ipi) and Durvalumab + Tremelimumab (Dur + Tre) have been recommended due to their efficacy and safety profile.

Hellmann *et al.*[18] assessed the potency of Niv+Ipi in 77 randomly assigned (1:1:1) recurrent stage IIIB/IV and chemotherapy-naïve NSCLC patients. The phase 1, open-label, multicohort study (CheckMate 012) administered 1 mg/kg Nivolumab every-2-weeks plus 1 mg/kg Ipilimumab every-6-weeks (cohort 1), 3 mg/kg Nivolumab every-2-weeks plus 1 mg/kg Ipilimumab every-12-weeks (cohort 2), or 3 mg/kg Nivolumab every-2-weeks plus 1 mg/kg Ipilimumab every-6-weeks (cohort 3). The latter 2 regimens were considered potentially suitable for further clinical development and its' outcome (Table 2) have been reported in the study. The ORR was 47% (18) for cohort 2 and 38% (15) for cohort 3. Median DOR was not reached in either cohorts with median follow-up times of 12.8 months and 11.8 months in the second and third cohorts, respectively. Patients expressing PD-L1 ≥1% achieved confirmed ORR of 57% (12 out of 21) and 57% (13 out of 23) in the second and third cohorts, respectively. PFS at 24 weeks were 68% and 47% in the second cohort and third cohort, respectively. Median PFS was longer (80%) in cohort 2 than cohort 3 (65%). Overall survival at 1 year was 69% for the third cohort.

The overall survival for the second cohort and median overall survival for all cohorts have not been reported. The combination treatments with its significant clinical benefits was enhanced with the increased PD-L1 expressions.

Table 2 - Efficacy of the combination treatment Nivolumab and Ipilimumab.

Endpoints	Treatment Cohort 2	Treatment Cohort 3
Objective response rate	47%	38%
Confirmed objective response upon PD-L1 ≥1% expression	57%	57%
Median DOR	Was not reached with median follow-up times of 12.8 months	Was not reached with median follow-up times of 11.8 months
PFS at 24 weeks	68%	47%
Median PFS	80%	65%
Overall survival at 1 year	Have not been reported	69%
Grade ¾ TRAEs	37%	33%
The most common grade ¾ TRAEs	Increased lipase (8%), pneumonitis (5%), adrenal insufficiency (3%), and colitis (3%)	Increased lipase (no patients), pneumonitis (3%), adrenal insufficiency (5%), and colitis (5%)
Treatment-related serious adverse effects	32%	28%
Treatment-related deaths	Not reported	Not reported
Treatment discontinuation	76%	82%
Total number of deaths	29%	38%

Grade 3/4 TRAEs occurred in 14 (37%) and 13 (33%) patients from cohort 2 and cohort 3, respectively. The most common grade 3/4 TRAEs were increased lipase (8% and no patients), pneumonitis (5% and 3%), adrenal insufficiency (3% and 5%) and colitis (3% and 5%) in cohort 2 and cohort 3, respectively. Treatment-related serious adverse effects in the second and third cohorts were 32% (12) and 28% (11), respectively. There was no report on treatment-related deaths. Sixty-one (79%) patients (29 (76%) from cohort 2 and 32 (82%) from cohort 3) discontinued the treatment due to disease progression. Eleven (29%) and fifteen (38%) patients from cohort 2 and cohort 3, respectively, died from disease progression.

The CheckMate 012 trial represents the first evidence of improved benefits in the first-line treatment of recurrent NSCLC. This combination showed potential clinical activity and tolerable safety profile. Currently, several phase-3 ongoing trials are investigating the efficacy of dual checkpoint inhibitor blockade or immunotherapy plus chemotherapy.

Another immunotherapy combination study focused on the potency of Dur + Tre in 102 immunotherapy-naïve patients with metastatic squamous and non-squamous NSCLC. In the phase 1b, open-label, multicenter, and non-randomized study[62], patients received Durvalumab in doses of 3 mg/kg, 10 mg/kg, 15 mg/kg, or 20 mg/kg every-4-weeks, or 10 mg/kg every-2-weeks, and Tremelimumab in doses of 1 mg/kg, 3 mg/kg, or 10 mg/kg every-4-weeks for 6 doses then every-12-weeks for 3 doses. The trial found an objective response in 23% (n=6) of the combined tremelimumab 1 mg/kg cohort (n=26). Of this group, two were PD-L1 positive whilst four were PD-L1 negative. The study showed that patients responded to the treatment within the allocated period (Table 3; Table 4). However, to establish the treatment-related effects on survival, a longer and a more adequate follow-up period has been suggested. Twenty-nine out of 102 patients (28%) discontinued the treatment due to TRAEs. Treatment-related serious adverse effects occurred in 37 out of 102

patients (36%). Eighty-two (80%) patients had ≥1 TRAEs and the most common adverse effects were diarrhoea (32%), fatigue (24%), and pruritus (21%). The most common grade 3/4 TRAEs were diarrhoea (11%), colitis (9%), and increased lipase (8%). A total of 22 deaths were reported, of which 3 were treatment related. The causes of treatment-related deaths were the complications from pericardial effusion (20 mg/kg durvalumab every-4-weeks + 1 mg/kg tremelimumab), myasthenia gravis (10 mg/kg durvalumab every-4-weeks + 1 mg/kg tremelimumab), and neuromuscular disorder (20 mg/kg durvalumab every-4-weeks + 3 mg/kg tremelimumab). Clinical activity was observed in both PD-L1-positive and PD-L1-negative patients.

Table 3 – Combination of Durvalumab and Tremelimumab.

	Durvalumab	Tremelimumab
Administered doses	3 mg/kg, 10 mg/kg, 15 mg/kg, or 20 mg/kg every-4-weeks, or 10 mg/kg every-2-weeks	1 mg/kg, 3 mg/kg, or 10 mg/kg every-4-weeks for 6 doses then every-12-weeks for 3 doses
Adverse effects related treatment discontinuation	28%	
Treatment-related serious adverse effects	36%	
The most common adverse effects	Diarrhoea (32%), fatigue (24%), and pruritus (21%)	
The most common grade ¾ TRAEs	Diarrhoea (11%), colitis (9%), and increased lipase (8%)	
Total number of deaths	22 (3 of which were treatment-related deaths)	

Table 4 - Efficacy of Durvalumab and Tremelimumab with PD-L1 status[63]. Data are number of patients/total number of patients (% [95% CI]).

	10 – 20 mg/kg Durvalumab every-2-weeks or 4-weeks plus 1 mg/kg Tremelimumab	10 – 20 mg/kg Durvalumab every-2-weeks or 4-weeks plus 3 mg/kg Tremelimumab	15 mg/kg Durvalumab every-4-weeks plus 10 mg/kg Tremelimumab
All evaluable patients with ≥24 weeks of follow-up			
Objective response	6/26 (23% [9-44])	5/25 (20% [7-41])	0/9 (0% [0-34])
Disease control	9/26 (35% [17-56])	8/25 (32% [15-54])	1/9 (11% [0-48])
PD-L1 positive (≥25%)			
Objective response	2/9 (22% [3-60])	2/5 (40% [5-85])	0/4 (0% [0-60])
Disease control	3/9 (33% [8-70])	2/5 (40% [5-85])	1/4 (25% [1-81])
PD-L1 negative (<25%)			
Objective response	4/14 (29% [8-58])	2/17 (12% [2-36])	0/4 (0% [0-60])
Disease control	6/14 (43% [18-71])	5/17 (29% [10-56])	0/4 (0% [0-60])
PD-L1 negative (0%)			
Objective response	4/10 (40% [12-74])	1/10 (10% [0-45])	0/3 (0% [0-71])
Disease control	5/10 (50% [19-81])	3/10 (30% [7-65])	0/3 (0% [0-71])
PD-L1 status unknown			
Objective response	0/3 (0% [0-71])	1/3 (33% [1-91])	0/1 (0% [0-98])
Disease control	0/3 (0% [0-71])	1/3 (33% [1-91])	0/1 (0% [0-98])

The combination of 20 mg/kg durvalumab treated every-4-weeks plus 1 mg/kg tremelimumab showed efficient antitumour activity regardless of PD-L1 status (even in patients with no PD-L1 staining in tumour cell membrane). It also had better tolerability profile and was selected as the dose for the ongoing phase 3 trials[52].

Conclusion

Immune checkpoint inhibitors have established a new generation of treatment for metastatic NSCLC with durable responses and tolerable safety profiles. The ICI modulators, Nivolumab and Pembrolizumab have shown promising clinical activities in NSCLC. Combination therapy using Niv + Ipi and Dur + Tre have been shown to be more effective than single-agent ICIs to manage cancers. It is recommended that future research should investigate the efficacy of combination therapy with other ICIs, chemotherapy, radiotherapy, and personalized targeted therapy using randomised controlled trials. Other important research areas include the efficacy of ICIs for early stage cancer and identification of predictive biomarkers.

The review of relevant literature in this paper highlighted the following limitations or research gaps in clinical studies that could improve the understanding of the efficacy of ICIs and immunotherapy research:

1. The effect of p16 status on the efficacy of ICIs in NSCLC[64]: Ferris *et al.*[65] found that patients with a tumour PD-L1 expression level of 1% or more and/or with p16-positive tumours in head and neck squamous cell carcinoma had greater effect from Nivolumab treatment compared to those with a PD-L1 level less than 1% and/or p16-negative tumours. The expression levels of p16 might also be a prognostic factor for NSCLC.[66]

2. The difference in ICI response rate between smokers and non-smokers: Garon *et al.*[41] demonstrated that the response rate of Pembrolizumab in NSCLC is higher in smokers (22.5%) than non-smokers (10.3%). This observation is attributed to higher mutational burdens of smokers. Further research is required to elucidate the biochemical mechanisms underlying this observed effect.

3. The difference in ICI response rate between previously treated and untreated patients[41]: Previously untreated patients had showed higher drug response profile compared to previously treated patients. Further studies are required to provide more insight into this observation. An understanding of the biochemical basis for this difference may help in the design of effective combination therapy for NSCLC.

Conflict of interest
None declared

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