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LUNG CANCER PRACTICE, IMPLEMENTING EVIDENCE FROM AROUND THE WORLD
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Immunotherapy for lung cancer

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ABSTRACT

Treatment of lung cancer remains a challenge, and lung cancer is still the leading cause of cancer-related mortality. Immunotherapy has previously failed in lung cancer but has recently emerged as a very effective new therapy, and there is now growing worldwide enthusiasm in cancer immunotherapy. We summarize why immune checkpoint blockade therapies have generated efficacious and durable responses in clinical trials and why this has reignited interest in this field. Cancer vaccines have also been explored in the past with marginal success. Identification of optimal candidate neoantigens may improve cancer vaccine efficacy and may pave the way to personalized immunotherapy, alone or in combination with other immunotherapy such as immune checkpoint blockade. Understanding the steps in immune recognition and eradication of cancer cells is vital to understanding why previous immunotherapies failed and how current therapies can be used optimally. We hold an optimistic view for the future prospect in lung cancer immunotherapy.

Key words: antigen, antineoplastic agents, immunotherapy, lung Cancer, vaccine.

Abbreviations: APCs, antigen-presenting cells; AEs, adverse events; ASCO, American Society of Clinical Oncology; BORR, best overall response rate; CI, confidence interval; CTLA-4, cytotoxic T-lymphocyte antigen-4; DC, dendritic cell; DCB, durable clinical benefit; dLN, draining lymph node; HR, hazard ratio; IC3, immune cells 3; irBORR, immune-related best overall response rate; irPFS, immune-related progression-free survival; KIR, killer-cell immunoglobulin-like receptors; MAGE-A3, melanoma associated antigen-A3; MHC, major histocompatibility complex; MVA, modified virus of Ankara; NDB, no durable benefit; NK, natural killer; NSCLC, non-small cell lung cancer; ODN, oligodeoxynucleotides; ORR, objective response rate; OS, overall survival; PAMPs, pathogen-associated molecular patterns; PD-L1, programmed death ligand-1; pDCs, plasmacytoid dendritic cells; PFS, progression-free survival; PR, partial response; PRR, pattern recognition receptor; RCC, renal cell carcinoma; SCLC, small cell lung cancer; TCR, T-cell receptor; TC3, tumour cells 3; TH, transversion high; TL, transversion low; TrPAL, triple-positive activated lymphocytes; TLR, toll-like receptor; WHO, World Health Organization.

INTRODUCTION

Worldwide, lung cancer is one of the most deadly of the solid cancers.[1] Around 95% of lung cancers are classified as either non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC). With advancing stage, survival decreases progressively down to four months in stage IV disease,[2] so early intervention is important. Systemic cytotoxic chemotherapy has been the mainstay of treatment for advanced stage NSCLC, but the benefit of chemotherapy has reached a plateau. And new forms of treatment are required. Although there is now a better understanding of the role of driver mutations in NSCLC and how to target these mutations in treatment of NSCLC, for example, epidermal growth factor receptor (*EGFR*) mutation and anaplastic lymphoma kinase (*ALK*) fusion oncogene,[3–5] success remains limited. Without a doubt, the most powerful new therapy for lung cancer is immunotherapy.

HISTORICAL BACKGROUND OF LUNG CANCER IMMUNOTHERAPY

Cancer growth and spread are not only dependent on tumour cell characteristics but are also affected by the interaction with the immune system.[6,7] The use of immunotherapy for treatment of malignancy was described more than a century ago by Dr William B. Coley. He used what was later known as 'Coley's toxin', a streptococcal vaccine, which incorporated *Serratia marcescens*, to treat a variety of malignant diseases. He later observed that the best response was achieved in patients with inoperable soft tissue sarcomas, where long-term (more than 5 years) disease-free survival was achieved for approximately 50% of these patients.[8] Despite the success of several immunotherapies in some solid cancers, immunotherapy in lung cancer has not, until recently, shown significant survival benefit.[9] Lessons were learnt however, and recently, there has been a renewed interest in lung cancer immunotherapy following positive results using immune checkpoint inhibitors, which work by modulating the

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interactions of T cells and either antigen-presenting cells (APCs) or tumour cells to help unleash suppressed immune responses.

WHAT IS REQUIRED FOR EFFECTIVE IMMUNE ATTACK ON TUMOURS?

Immune destruction of tumours by tumour-specific cluster of differentiation 8 T (CD8 T) cells is a seven-step process (Figure 1). Each of these steps is required, and each can be regulated to strengthen or reduce the response and to avoid 'collateral damage' to normal tissues. Tumour neoantigens must be present in a tumour (Step 1), and these antigens must reach/load 'professional' APCs, such as dendritic cells (DC) where they can present the antigen to immune cells in the draining lymph node (dLN, Step 2). Tumour-specific T cells must then 'see' these antigens via T-cell receptor (TCR) recognition of major histocompatibility (MHC)::peptide complex, and receive the right co-stimulatory signals and any additional 'help' (e.g. CD40 ligation) before they become activated and proliferate (Step 3). Activated tumour-specific T cells then exit the dLN and circulate through the periphery before entering the tumour (Step 4). Once inside the tumour, these T cells encounter a number of local immune-suppressive mechanisms, which they need to overcome (Step 5), and probably also require re-stimulation by the APCs within the tumour (Step 6). Finally, once these activated T cells have passed these six steps, they need to attack the tumour (Step 7), a process that involves recognition of antigen expressed by the tumour and the release of potent molecules such as perforin and granzymes that ultimately kill the tumour cell. Memory cells can also be generated from this process, which may be important in control of emerging micrometastases.

Importantly, each of the steps can be modulated, for example, suppressed by regulatory CD4 T cells (Treg), which normally control autoimmunity but can restrict anti-tumour responses or boosted by 'helper' CD4 T cells.

WHY HAVE PREVIOUS IMMUNOTHERAPIES FAILED?

Lung cancer immunotherapy has failed in the past at any one or several of these steps. It is difficult to be certain exactly where the failures have occurred, but from what we now know about these seven difficult steps, it is not surprising that immunotherapy has failed so often. Reasons for failure include a lack of a sufficient load of mutated tumour antigens (Step 1), consistent with the recent observation that neoantigenic load parallels the success of immunotherapy,[10] a restriction in cross presentation (Step 2) due to suppressed APC traffic from the tumour[11] or lack of the necessary signals such as CD40, largely delivered by CD4 T cells.[12] Active specific immunotherapy of lung cancer has probably also failed here in that the lysed tumour cells used in the vaccines

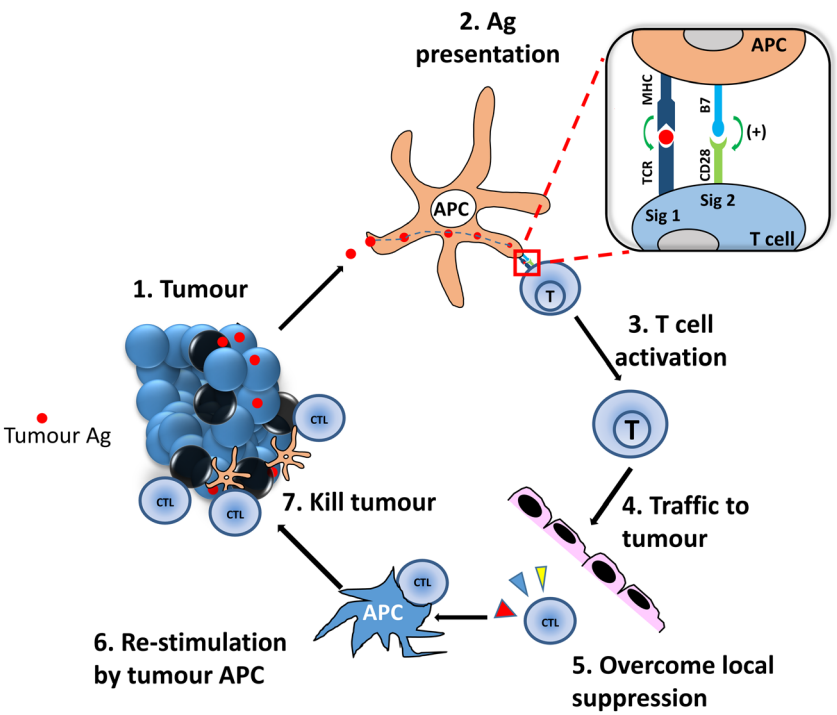

Figure 1 Tumour antigen cross-presentation and induction of anti-tumour immunity. (1) Tumour antigen release: Tumour-specific antigens (red dots) are picked up by professional antigen-presenting cells (APCs), such as dendritic cells, and migrate from the tumour site to the draining lymph node. (2) Antigen presentation: As the APCs migrate, they mature during which time the tumour antigen is processed into small peptides, which are then presented on the surface of the APC in the context of a MHC-peptide complex to lymphocytes. (3) T-cell activation: Recognition of the MHC peptide complex by cognate T-cell receptor constitutes signal 1. However, a second co-stimulatory signal (CD28::B7.1/B7.2) is required before a naïve T cell can become fully activated cytotoxic CD8 T lymphocytes (CTL). T cell activation can be enhanced by CD4 helper T cells. (4) T-cell trafficking: Activated T cells leave the dLN and traffic through the peripheral blood vessels back to the tumour site. (5) Overcoming local suppression: Activated CTLs need to be able to overcome tumour immuno-suppressive mechanisms in the tumour microenvironment. (6) APC re-stimulation: Tumour resident APC restimulates antigen-specific CTLs. (7) Tumour killing: Antigen-specific CTLs recognize and kill antigen bearing tumour cells.

would not have been effectively presented.[13] There may also be a limitation in the number of responding CD8 T cells (Step 3), which is one of the reasons why adoptive immunotherapy using T cells obtained from the tumours was first used in lung cancer.[14] A failure of T-cell traffic to tumours (Step 4) and the presence of local intratumoural suppressive influences (Step 5) are both well described.[7,15] One way the balance between inflammation and suppression occurs is by altering the local cytokine milieu. Cytokine therapy in lung cancer probably failed because the concentration of cytokines that could be achieved within the tumour following systemic administration was never high enough to achieve this change in balance. A limitation in re-stimulation by APCs within tumours (Step 6) is a more recent notion.[16] Finally, tumours can escape by immunological selection by losing antigen or the antigen-presenting molecule MHC class I, something noticed recently when lung squamous carcinomas were first sequenced.[17] As each of the steps can be modulated, the status of those modulatory influences may also explain immunotherapy failures, for example, an excess of regulatory CD4 T cells or a lack of helper CD4 T cells. The development of assays to analyse each of these seven steps in animal models has been a key to understanding how surgery, chemotherapy and immunotherapy interact.

Our discussion of current immunotherapies in lung cancer, described later, can be understood by reflecting on these seven steps.

IMMUNE CHECKPOINT BLOCKADE

To prevent any unwanted damage caused by activated T cells to surrounding tissues, the immune system has evolved a variety of inbuilt mechanisms, or 'checkpoints' that are used to modulate the duration and amplitude of the immune response. This dampens the immune response and protects against damage because of inflammation and autoimmunity. This is achieved primarily by upregulation of 'co-inhibitory' receptors that act to 'turn off' activated T cells. Ultimately, it is the balance between co-stimulatory and co-inhibitory signals that dictates the fate of activated T cells (Figure 2).

The receptor/ligand nature of T cell activation/inhibition means that these interactions can be manipulated by the use of agonistic or antagonistic antibodies. Indeed, over the last decade there has been significant advancement in the use of antibody-mediated immune checkpoint blockade as a cancer immunotherapy.

CTLA-4 inhibition

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) protein is expressed on the surface of T cells and competes with CD28 for B7 binding in an inhibitory fashion, therefore acting as suppressor of T-cell activation. CTLA-4 inhibitors have been well studied and are among the earliest immune checkpoint inhibitors in clinical development.[18–20] Antibodies to CTLA-4 in effect block the inhibition of CD28/B7 T-cell activation and in turn prolong anti-tumour activity (Figure 2).

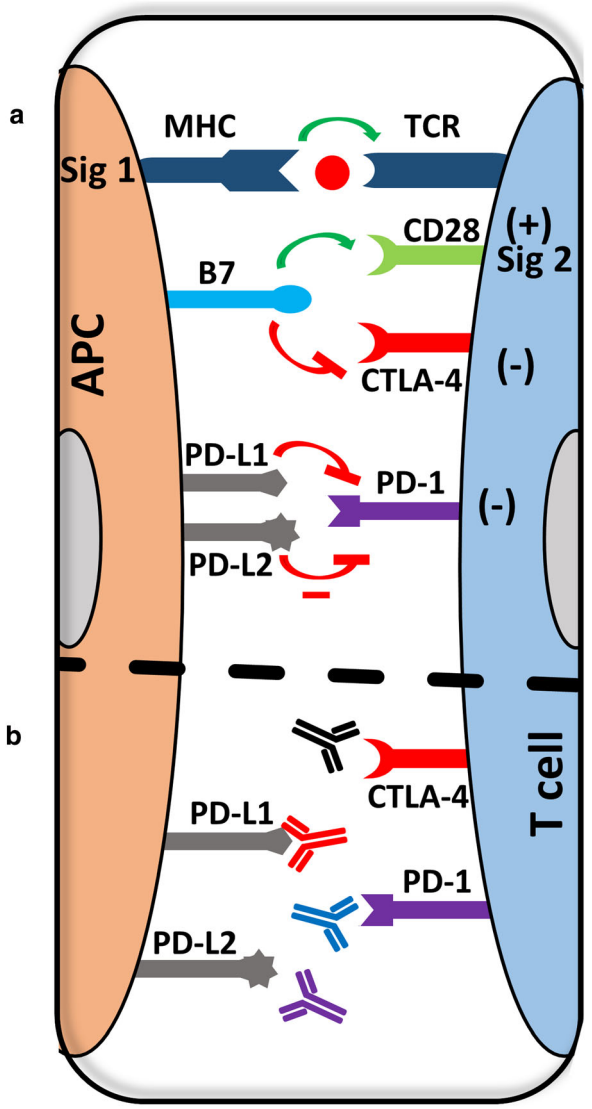

Figure 2 Mechanism of immune checkpoint blockade. T-cell activation is ultimately dictated by the balance of co-stimulatory versus co-inhibitory signals. (a) Upon T-cell activation (signal 1 + signal 2), co-inhibitory receptors such as CTLA-4 and PD-1 are upregulated. CTLA-4 binds with greater avidity to the B7 molecules CD80/CD86 and outcompetes binding of CD28 leading to inhibition of T-cell activation. (b) Monoclonal antibodies directed against inhibitory receptors block their ability to bind to their respective ligand, favouring the co-stimulatory signal and thus prolonging T cell activation and the anti-tumour immune response.

Ipilimumab

Ipilimumab is a fully humanized monoclonal antibody that binds to CTLA-4 and prevents it from binding to its ligand. In a phase II study of chemotherapy-naïve NSCLC patients ($n=204$), phased ipilimumab (two doses of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin), but not concurrent ipilimumab (four doses of ipilimumab plus paclitaxel and carboplatin followed by two doses of placebo plus paclitaxel and carboplatin), improved immune-related progression-free survival (irPFS) versus the control group

(chemotherapy alone) (median 5.7 vs 4.6 months, HR (hazard ratio) 0.72, $P = 0.05$) and progression-free survival (PFS) versus the control group (median 5.1 vs 4.2 months, HR 0.69, $P = 0.02$). Overall survival (OS) in the phased group appeared to be higher than the control group (median 12.2 vs 8.3 months, log rank $P = 0.23$). Immune-related best overall response rate (irBORR) and best overall response rate (BORR) versus the control group were 32% versus 18% and 32% versus 14%. In a post hoc analysis based on histology, both PFS and OS were improved in the phased ipilimumab group for patients with squamous histology (HR for PFS 0.40 (95% CI: 0.18–0.87), HR for OS 0.48 (95% CI: 0.22–1.03)). Overall incidence of treatment-related grade 3 and 4 adverse events (AEs) was similar across groups (control, 37%; concurrent, 41%; phased, 39%). Serious immune-mediated events included rash (4%), colitis (10%) and hypophysitis (one case).[21]

There is currently an ongoing phase III trial comparing chemotherapy alone to chemotherapy with phased ipilimumab in patients with squamous histology NSCLC (NCT01285609).

PD-1 checkpoint blockade

One of the mechanisms for immune resistance in NSCLC is the expression of inhibitory molecules in the tumour microenvironment. There are three classes of these molecules, namely, cytokines, membrane ligands and metabolites.[22] PD-L1 (B7-H1) is the most studied membrane inhibitory ligands in NSCLC although there are others, which are found to be up-regulated in lung cancer. PD-L1 is expressed on tumour cells in approximately half of NSCLC, and its expression could contribute to poor prognosis by suppressing T-cell function and promoting tumour cell immune escape.[23] Both PD-L1 and PD-L2 (B7-DC) bind to PD-1, and they are induced by different inflammatory cytokines. PD-L2 is largely expressed on DCs and macrophages, whereas PD-L1 can be expressed on diverse epithelial and haematopoietic cell types, although as mentioned previously, PD-L1 expression is predominantly confined to the tumour microenvironment.[22,24–27] PD-L2 expression has been found on Th2 helper cells[28] and thus may also have a role in cancer immunotherapy.[29]

Programmed death-1 checkpoint blockade uses antibodies directed against either the receptor, PD-1 or its ligand PD-L1. In contrast to CTLA-4 inhibitors, which only seem to work in lung cancer when used in combination with chemotherapy, PD-1 checkpoint blockade has shown activity as a single agent treatment in NSCLC.

Nivolumab

Nivolumab is a human IgG4 monoclonal antibody to PD-1. In a phase I trial with a large expansion cohorts of patients with NSCLC, melanoma and renal cell carcinoma (RCC), 129 patients with NSCLC received nivolumab (1, 3 or 10 mg/kg IV every 2 weeks). Fifty-four per cent of these patients had received greater that or equal to three previous systemic therapies. Twenty-two out of 122 (17%) patients with NSCLC had a partial response (PR) based on response evaluation criteria in solid tumors (RECIST) 1.0 criteria, and 10% had stable disease at ≥24 weeks. Anti-tumour effect was particularly observed at the 3 mg/kg dosing level so this dose was selected for further study. At this dose, the response rate was 28% (5/18) for non-squamous and 27% (4/15) for squamous histology. The most common side effects in this cohort were fatigue (24%), decreased appetite (12%) and diarrhoea (10%). Fourteen per cent of patients experienced grade 3/4 treatment-related adverse events. The most concerning toxicity observed was pneumonitis (8 patients or 6%), which resulted in two deaths.[30] In patients who had significant response and disease stability with nivolumab, durability of responses was common with a median duration of 74 weeks. The most recent results from the ASCO Meeting 2014[31] reported prolonged survival with a median of 9.2–14.9 months across the cohorts. The median OS for the 3 mg/kg cohort was 14.9 months, 1-year OS of 56% and 2-year OS of 45%. In a randomized phase III study of nivolumab versus docetaxel in advanced squamous NSCLC involving 272 patients, the median OS was 9.2 months (95% CI: 7.3–13.3) with nivolumab versus 6.0 months (95% CI: 5.1–7.3) with docetaxel while the median PFS was 3.5 months with nivolumab versus 2.8 months with docetaxel (HR for death or disease progression, 0.62; 95% CI, 0.47-0.81; $P < 0.001$). At 1 year, the overall survival rate was 42% (95% CI: 34–50) with nivolumab versus 24% (95% CI: 17–31) with docetaxel. The response rate was 20% with nivolumab versus 9% with docetaxel ($P = 0.008$). Importantly, treatment-related adverse events and treatment-related serious adverse events occurred less frequently with nivolumab than with docetaxel. 7% of patients in the nivolumab group had grade 3 or 4 adverse events in comparison with 55% of those in the docetaxel group. In this group, the expression of PD-L1 was neither prognostic nor predictive of benefit from anti-PD-1 therapy.[32] In general terms, although the expression of PD-L1 by immunohistochemistry was associated with response to therapy, patients who were PD-L1 negative could respond to nivolumab but at lower rates.[33] The FDA has recently approved the use of nivolumab in advanced squamous and non-squamous NSCLC with progression on or after platinum-based chemotherapy.

There are other agents utilizing PD-1 checkpoint blockade, and they seem to have similar efficacy in pretreated advanced stage NSCLC. Selected ongoing clinical trials of immune checkpoint blockade in thoracic malignancy are summarized in Table 1.

Pembrolizumab

Pembrolizumab, another PD-1 inhibitor is also approved as second-line treatment for NSCLC after chemotherapy. In a phase I study of pembrolizumab in advanced NSCLC, the objective response rate in all patients was 19.4% (95% CI: 16.0–23.2), with median duration of response of 12.5 months (range, 1.0–23.3). Median PFS was 3.7 months (95% CI: 2.9–4.1) for all patients, 3.0 months (95% CI: 2.2–4.0) for previously treated patients and 6.0 months (95% CI: 4.1–8.6) for previously untreated patients. Median OS was 12.0 months (95% CI: 9.3–14.7) for all patients,

Table 1 Current trials of checkpoint blockade in thoracic malignancy

Target	Drug name	Malignancy	Phase	Comparison treatment	Company	Clinical trial ID	Status	Ref
PD-1	nivolumab	NSCLC	II	neoadjuvant in resectable NSCLC	BMS	NCT02259621	recruiting	†
	nivolumab	advanced stage NSCLC	II	—	BMS	NCT02350764	recruiting	†
	nivolumab	non-squamous NSCLC	III	vs docetaxel	BMS	NCT01673867	ongoing, but not recruiting	34‡
	nivolumab	squamous NSCLC	III	vs docetaxel	BMS	NCT01642004	ongoing, but not recruiting	32
	nivolumab	Stage IV or recurrent NSCLC	IV	—	BMS	NCT02041533	recruiting	†
	pembrolizumab	carcinoma, NSCLC, melanoma	I	—	MSD	NCT01295827	ongoing, but not recruiting	35
	pembrolizumab	NSCLC	I	—	MSD	NCT02007070	ongoing, but not recruiting	†
	pembrolizumab	malignant mesothelioma	II	—	MSD	NCT02399371	recruiting	†
	pembrolizumab	NSCLC	II–III	vs docetaxel	MSD	NCT01905657	ongoing, but not recruiting	†
	pembrolizumab	NSCLC	III	vs Paclitaxel, carboplatin, pemetrexed, cisplatin, gemcitabine	MSD	NCT02142738	recruiting	†
	pembrolizumab	NSCLC	III	vs carboplatin + paclitaxel or pemetrexed	MSD	NCT02220894	recruiting	†
PD-L1	avelumab	solid tumours	I	—	Merck KGaA and Pfizer	NCT01772004	recruiting	36‡ 37‡
	avelumab	NSCLC	III	vs docetaxel	Merck KGaA and Pfizer	NCT02395172	recruiting	†
	MEDI4736	NSCLC	II	—	AstraZeneca	NCT02087423	recruiting	†
	MEDI4736	NSCLC	III	completely resected NSCLC	AstraZeneca	NCT02273375	recruiting	†
	MEDI4736	NSCLC	III	—	AstraZeneca	NCT02125461	recruiting	†
	MPDL3280A	solid cancers	I	—	Genentech	NCT01375842	recruiting	38‡
	MPDL3280A	NSCLC	II	vs docetaxel	Genentech	NCT01903993	ongoing, but not recruiting	39‡
	MPDL3280A	NSCLC	II	—	Genentech	NCT01846416	ongoing, but not recruiting	40‡
	MPDL3280A	NSCLC	II	—	Genentech	NCT02031458	ongoing, but not recruiting	†
	MPDL3280A	NSCLC	III	vs docetaxel	Genentech	NCT02008227	recruiting	†
	MPDL3280A	non-squamous NSCLC	III	vs Gemcitabine + cisplatin or carboplatin	Genentech	NCT02409355	recruiting	†
CTLA-4	ipilimumab	SCLC	II	—	BMS	NCT02046733	recruiting	†
	tremelimumab	malignant mesothelioma	II	—	MedImmune LLC	NCT01655888	unknown	41 42‡
	tremelimumab	malignant mesothelioma	II	—	MedImmune LLC	NCT01649024	unknown	42‡
	tremelimumab	unresectable pleural or peritoneal malignant mesothelioma	II	—	MedImmune LLC	NCT01843374	ongoing, but not recruiting	†

†Study details available at clinicaltrials.gov.
‡Conference abstract at American Society of Clinical Oncology (ASCO) 2015.
NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; PD-1, programmed death-1; PD-L1, programmed death ligand-1; CTLA, cytotoxic T-lymphocyte antigen.

9.3 months (95% CI: 8.4–12.4) for previously treated patients and 16.2 months (95% CI: 16.2–not reached). In patients with a PD-L1 proportion score of 50%, response rate was 45.2%, with median PFS of 6.3 months (95% CI: 2.9–12.5) and median OS was not reached. Common treatment-related adverse events were fatigue, pruritus and decreased appetite. 9.5% had grade 3 or higher adverse events. Immune-mediated adverse events include infusion reactions (3%), hypothyroidism (6.9%) and pneumonitis (3.6%). Patients developing hypothyroidism were successfully treated with medical therapy while one patient died with pneumonitis.[35]

Anti PD-L1 antibody

MPDL3280A is a human Immunoglobulin G1 (IgG1) monoclonal antibody to PD-L1. A phase I study that was conducted in advanced solid cancers revealed activity in NSCLC, melanoma, renal cell carcinoma, colorectal cancer, gastric cancer and head and neck squamous cell carcinoma. PD-L1 expression and prevalence were assessed by immunohistochemistry and were graded 0 to 3.[43] The result in NSCLC was updated in ASCO 2015, showing that adverse events occurred in 73% of patients and 11% had grade 3–4 adverse events, mostly dyspnoea, hypoxia, fatigue and hyponatraemia. Objective response rate (ORR) in all patients was 21% (95% CI: 13–30). Patients with PD-L1 expression of TC3 (PD-L1 positive tumour cells 3) or IC3 (tumour-infiltrating immune cells 3) had an ORR of 45% (95% CI: 23–68) versus 14% (95% CI: 6–25) for patients with PD-L1 expression of TC 0/1/2 and IC 0/1/2. One-year OS in all patients was 82% (95% CI: 72–91).[38]

MEDI4736 is a human IgG1 antibody, which binds specifically to PD-L1 preventing binding to PD-1 and CD80. In the Society for Immunotherapy of Cancer 2014 Meeting in, Brahmer *et al.* reported the early update of a phase I MEDI4736 study of a NSCLC cohort that included 155 patients, with 29% experiencing treatment-related adverse events, none experiencing treatment-related colitis of any grade and none experiencing grade 3/4 pneumonitis. Of the 58 patients who had ≥12-week follow-up, 16% had partial response, with the duration of response ranging 5–54+ weeks, disease control rate 35%.[44]

Programmed death-1 checkpoint blockade seems to be conferring a durable response and a favourable side effect profile. It remains to be seen, however, if the responders will continue to benefit over many years. If the data on melanoma are also replicated in patients with NSCLC, then the outlook is very promising indeed.[45]

Biomarkers in PD-1 checkpoint blockade

There is a need for a biomarker to predict the response of PD-1-directed therapies, and association between PD-L1 expression in NSCLC and treatment response has been investigated in multiple studies. In non-squamous NSCLC treated with nivolumab, positive PD-L1 expression across all cut-off points was associated with better clinical response. The ORR and median OS are 31% and 17.7 months in PD-L1 positive (⩾1%) patients while only 9% and 10.5 months in PD-L1 negative (<1%) patients.[46] In squamous NSCLC, however, PD-L1 expression was not associated with clinical response.[32] A meta-analysis exploring the predictive role of PD-L1 expression in advanced melanoma, NSCLC and genitourinary cancer, which included 20 trials (1475 patients), found a significant difference in the ORR of patients with PD-L1 positive versus PD-L1 negative patients (34.1% vs 19.9%, $P < 0.0001$).[47] It is worth noting however that some PD-L1 negative patients respond to anti-PD-1/PD-L1 antibody.

The role of PD-L1 expression in predicting response to PD-1 checkpoint blockade is controversial. Some issues include reliability of detection methods, differences in cut-off values in determining positivity, heterogeneity of PD-L1 expression and site of PD-L1 expression (tumour cells and/or immune cells). Additionally, PD-L1 expression has a dynamic nature, and it can vary with tumour microenvironment; therefore, PD-L1 expression at a single time point may not be the most useful as a predictive biomarker.[48,49] The complex landscape associated with using PD-1/L1 expression as a predictive biomarkers has been recently reviewed,[50] and further studies are needed to clarify the suitability of PD-L1 expression as a predictive biomarker.

More recently, there was evidence that treatment response to PD-1 checkpoint blockade is associated with higher nonsynonymous mutation burden, molecular smoking signature, higher neoantigen burden and DNA repair pathway mutations.[51] In this paper, Rizvi *et al.* hypothesized that the mutational landscape of NSCLC would influence the response to PD-1 blockade. They sequenced the exomes of two independent groups of patients treated with pembrolizumab and their matched normal DNA and found that higher somatic nonsynonymous mutation burden was associated with the clinical response to pembrolizumab. In the discovery cohort, patients with durable clinical benefit (DCB) (partial or stable response lasting >6 months) had a median number of nonsynonymous mutations of 302 versus 148 in those with no durable benefit (NDB) ($P = 0.02$). There was a high concordance between nonsynonymous mutation burden and DCB, and by applying a cut-off point of nonsynonymous mutation burden ≥178, which combined maximal sensitivity with best specificity in the discovery cohort, to the validation cohort, the rate of DCB in patients with tumours with ≥178 mutations was 75% versus 14% in those with <178 (sensitivity 86% and specificity 75%). The authors then applied a previously validated binary classifier to identify the molecular signature of smoking[52] to differentiate transversion-high (TH, smoking signature) from transversion-low (TL, never smoking signature) tumours. They found that efficacy was greatest in patients with tumours harbouring the smoking signature. TH tumours were associated with ORR of 56% versus 17% in TL tumours ($P = 0.03$) and DCB of 77% versus 22% ($P = 0.004$). The observation that higher nonsynonymous mutation burden is associated with PD-1 blockade efficacy is consistent with the hypothesis that recognition of neoantigens is crucial for the activity of PD-1 blockade. Predicted candidate neoantigens,

which have binding affinity for patient-specific class I human leukocyte antigen (HLA) alleles, were identified. Not surprisingly, they found that tumours from patients with DCB had significantly higher candidate neoantigen burden compared with those with NDB and that high candidate neoantigen burden was associated with improved median PFS (14.5 vs 3.5 months). It is tempting to speculate that patients who have tumours with high nonsynonymous mutation burden and consequently high candidate neoantigen burden could be ideal candidates for personalized vaccination protocol.

OTHER NON-SPECIFIC IMMUNOTHERAPY

Toll-like receptors are a family of pattern recognition receptors (PRR), which recognize pathogen-associated molecular patterns (PAMPs) to induce antigen-specific innate immunity. TLR agonists have been investigated for their ability to enhance anti-tumour immune responses. TLR9 is expressed by human B cells and plasmacytoid dendritic cells (pDCs). Synthetic unmethylated 5'—C—phosphate—G—3' (CpG) oligodeoxynucleotides (CpG ODN) can activate TLR9 to reduce immune tolerance and promote anti-tumour response. Two international phase III trials evaluating TLR9 agonist PF-3512676 in combination with first line paclitaxel/carboplatin and gemcitabine/cisplatin, respectively, in advanced NSCLC were terminated after the first interim analysis because of lack of efficacy and increased toxicity.[53,54] Other TLR9 agonists, such as IMO-2055 and MGN1703, are still in early development.[55,56] We and others have demonstrated anti-tumour potential for TLR7 agonists in pre-clinical studies.[57–59] In a murine mesothelioma model, a combination of imiquimod (a TLR7 agonist) and anti-CD40 produced a systemic anti-tumour response in a CD8 T cell-dependent manner.[57,58] TLR7 also appears to have a dual role in lung cancer microenvironment. TLR7 ligands promote anti-tumour immunity via activation of pDCs,[60,61] whereas TLR7 expression on lung cancer cells and its stimulation by TLR7 agonists promote tumour progression and resistance to chemotherapy.[62,63] Similarly, TLR3 agonists can enhance tumour-specific T-cell responses and are currently used as adjuvants for cancer vaccines (reviewed in[64]). Talactoferrin alfa, a recombinant human lactoferrin, is an orally active dendritic cell-mediated immunotherapy. It is thought to interact with DCs in the gut wall and stimulate their migration and maturation as tumour antigen-presenting DCs.[65–67] Despite demonstrating anti-tumour activity in a variety of different pre-clinical models,[65,68,69] these findings were not validated in the FORTIS-M study, an international randomized trial of talactoferrin alfa versus placebo in patients with advanced NSCLC who had failed two or more prior regimens.[70]

CANCER VACCINES

Vaccine therapy results are not always encouraging (see review by Freeman-Keller *et al.*[71]). Despite this fact, the trials have played a significant role in shedding light on what challenges need to be overcome to successfully employ tumour vaccine therapy. There are multiple lung cancer vaccine therapies that have been trialled clinically, with most being utilized for NSCLC and a much smaller proportion being developed for SCLC. Antigen-specific vaccines aim to induce specific anti-tumour immunity directed against specific tumour associated antigens (TAAs).[72] Conversely, tumour/whole cell vaccines composed of either autologous or allogeneic tumour cells expose the immune system to a variety of often unknown tumour antigens.[9,72]

Non-small cell lung cancer patients whose tumours express the TAA melanoma associated antigen-A3 (MAGE-A3) are often associated with poorer prognosis. The MAGE-A3 vaccine is a recombinant MAGE-A3 protein combined with the immunostimulant AS02B that was assessed in a large, double-blind, randomized phase III trial (MAGRIT) in patients with stage IB, II or IIIA MAGE-A3 positive NSCLC. Unfortunately, this was a negative study as there was no increase in DFS in the vaccine group compared with placebo in either the overall population or in the patients who did not receive chemotherapy.[73]

TG4010 is a vaccine targeted against Mucin1 (MUC1) antigen composed of a recombinant vaccinia virus (modified virus of Ankara or MVA) that encodes human MUC1 and IL-2 (MVA-MUC1-IL-2). In a phase IIb of TG4010 and first line chemotherapy for advanced NSCLC (TIME), TG4010 group had a median PFS of 5.9 months versus 5.1 months in the placebo group (HR 0.74 (95% CI: 0.55–0.98), $P = 0.019$). When taking into account of the baseline value of CD16, CD56 and CD69 triple-positive activated lymphocytes (TrPAL value) $\leq$ Q3 (the third quartile of the TrPAL distribution), PFS was significantly improved by addition of TG4010 to chemotherapy, whereas there was no benefit in patients with TrPAL value $>$ Q3. The highest benefit was noted in patients with non-squamous histology and TrPAL value $\leq$ Q3.[74]

Belagenpumatucel-L is an allogeneic tumour cell vaccine containing four NSCLC cell lines and an antisense plasmid of TGF-β. The vaccine aims to increase the immune response to NSCLC through downregulation of TGF-β expression. In a randomized phase II trial in patients with stage II-IV NSCLC, no significant adverse events were observed with the vaccine, and a dose related survival difference was observed in those who received $\geq 2.5 \times 10^7$ cells/injection ($P = 0.0069$). In clinical responders, increased cytokine production and elevated antibody-mediated response to vaccine MHC ($P = 0.014$) were observed. A phase III study of belagenpumatucel-L (STOP trial) did not meet its predefined endpoint, but a non-statistically significant increase in OS was seen in several subsets of patients who started belagenpumatucel-L within 12 weeks of the completion of frontline chemotherapy.[75]

With the exception of the current TG4010 phase III trial mentioned earlier, vaccines have not shown significant activity in lung cancer. Like many other solid tumours, lung cancer may utilize a variety of mechanisms to subvert host immune response including downregulation of HLA/MHC molecules, loss or

Table 2 Current and planned trials of combination therapy involving checkpoint blockade in thoracic malignancy

Target	Drug name	Cancer	Phase	Combination therapy	Company	Clinical trial ID	Status	Ref
PD-1	nivolumab	NSCLC, pancreatic and breast	I	nab-Paclitaxel, gemcitabine, carboplatin	BMS	NCT02309177	recruiting	†
	nivolumab	adult solid neoplasm, anal carcinoma, HIV infection, Kaposi sarcoma, lung carcinoma	I	ipilimumab	BMS	NCT02408861	not yet recruiting	†
	nivolumab	NSCLC	I	cisplatin + gemcitabine/pemetrexed, carboplatin + paclitaxel, bevacizumab, erlotinib, ipilimumab	BMS	NCT01454102	recruiting	84‡
	nivolumab	NSCLC, melanoma, head and neck ca, ovarian ca, colon ca	I and II	varlilumab (anti-CD27)	BMS	NCT02335918	recruiting	†
	nivolumab	NSCLC, hepatocellular carcinoma, glioma	I and II	galunisertib (LY2157299)	BMS	NCT02423343	not yet recruiting	†
	pembrolizumab	lung cancer, melanoma	I and II	stereotactic body radiation therapy (SBRT)	MSD	NCT02407171	not yet recruiting	†
	pembrolizumab	NSCLC	I and II	carboplatin + Nab-paclitaxel	MSD	NCT02382406	not yet recruiting	†
	pembrolizumab	NSCLC, melanoma	Ib/II	entinostat	MSD	NCT02437136	not yet recruiting	†
	pembrolizumab	NSCLC	I and II	gemcitabine	MSD	NCT02422381	recruiting	†
	pembrolizumab	NSCLC	I and II	paclitaxel, carboplatin, bevacizumab, pemetrexed, ipilimumab, erlotinib, gefitinib	MSD	NCT02039674	recruiting	85‡86‡
	pembrolizumab	NSCLC, gastric adenocarcinoma, gastro-oesophageal junction adenocarcinoma, TCC	I	ramucirumab	MSD	NCT02443324	not yet recruiting	†
PD-L1	MEDI4736	NSCLC	I	gefitinib	AstraZeneca	NCT02088112	recruiting	†
	MEDI4736	NSCLC	I and II	tremelimumab	AstraZeneca	NCT02000947	recruiting	87‡88‡
	MEDI4736	NSCLC	III	vinorelbine, gemcitabine, erlotinib, tremelimumab (anti CTLA-4)	AstraZeneca	NCT02352948	recruiting	89‡
	MPDL3280A	Recurrent NSCLC, stage IV NSCLC	I	stereotactic body radiation therapy	Genentech	NCT02400814	not yet recruiting	†
	MPDL3280A	non-squamous NSCLC	III	carboplatin + paclitaxel with or without bevacizumab	Genentech	NCT02366143	recruiting	†
	MPDL3280A	NSCLC	III	carboplatin + paclitaxel or Nab-paclitaxel vs carboplatin + Nab-paclitaxel	Genentech	NCT02367794	recruiting	†
	MPDL3280A	NSCLC	III	carboplatin and Nab-paclitaxel	Genentech	NCT02367781	recruiting	†
	MPDL3280A	non-squamous NSCLC	III	cisplatin or carboplatin + pemetrexed	Genentech	NCT02409342	recruiting	†
CTLA-4	ipilimumab	melanoma, head and neck cancer, NSCLC	I	MGA271	BMS	NCT02381314	recruiting	†
	ipilimumab	extensive stage SCLC	II	carboplatin + etoposide	BMS	NCT01331525		†

(*Continues*)

Table 2. (Continued)

Target	Drug name	Cancer	Phase	Combination therapy	Company	Clinical trial ID	Status	Ref
							ongoing, but not recruiting	
	ipilimumab	NSCLC	II	radiotherapy	BMS	NCT02221739	recruiting	†
	ipilimumab	NSCLC	III	ipilimumab and carboplatin + paclitaxel vs carboplatin + paclitaxel alone	BMS	NCT01285609	ongoing, but not recruiting	†

†Study details available at clinicaltrials.gov.
‡Conference abstract at American Society of Clinical Oncology (ASCO) 2015.
NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; PD-1, programmed death-1; PD-L1, programmed death ligand-1; CTLA-4, cytotoxic T-lymphocyte antigen-4.

modulation of tumour antigen expression or secretion of immunosuppressive cytokines; all of which have been proposed as reasons why active immunotherapy and vaccines have not worked in lung cancer in an excellent recent review.[76] An interesting and potentially very attractive new approach is to take a personalized approach to immunotherapy by harnessing the power of gene sequencing. Lung cancers (especially those caused by tobacco smoking) carry high numbers of nonsynonymous mutations, with molecular smoking signature,[77] which may generate tumour-specific MHC class I-restricted epitopes. Every patient's tumour is therefore highly specific and carries a unique antigenic profile. This so-called tumour mutanome can be revealed by deep sequencing and the immunogenicity of mutated peptides predicted *in silico*. These peptides then can be used to track tumour-specific T-cell responses and be incorporated into an individualized vaccine.[78,79] The peptide neoantigen vaccine may not be enough in inducing an effective tumour-specific immune response, and a combination therapy approach may be needed. For example, combination of checkpoint blockade and vaccine is a logical next step in cancer immunotherapy.[22,71,80] Despite the advances with checkpoint blockade, not all patients respond, and the addition of vaccine therapy may further enhance T-cell proliferation. In a murine melanoma model (B16 melanoma), which was thought to be highly tumourigenic but poorly immunogenic, combination of GM-CSF-expressing tumour cell vaccine and CTLA-4 blockade induced tumour eradication in 80% of the cases while each treatment by itself produced little or no effect.[81]

IMMUNOTHERAPY FOR LUNG CANCER: LIMITATIONS

Immunotherapy in lung cancer has become a reality, and it is now used in clinical practice. However, there are a number of aspects that have the potential to limit the acceptance of immunotherapy for lung cancer. For instance, it is still uncertain how best to predict response from PD-1 blockade. Using PD-L1 expression in tissues as a biomarker is promising although the dynamic expression of PD-L1 and the fact that some patients who did not exhibit PD-L1 responded to PD-1 blockade make it somewhat less reliable as a predictive biomarker.

Like any cancer drug, there are adverse effects that clinicians have to deal with when they do arise. For CTLA-4 inhibition, one of the more serious side effects is colitis, whereas PD-1 checkpoint blockade can result in pneumonitis, a rare event but nevertheless potentially fatal. Grading of immune-related side effects has been used in clinical trials, and treatment with cessation of immunotherapy and if necessary corticosteroid administration has been applied. Pneumonitis is a particular concern in lung cancer population as patients with lung cancer not infrequently have poor lung reserve because of current or past smoking history. Thus, pneumonitis can seriously impair their already poor lung reserve and in

some cases may well be fatal. Despite this concern, pneumonitis is a rare event.

RECIST VERSUS IRRECIST: HOW DO WE BEST ASSESS THE RESPONSE TO IMMUNOTHERAPY?

As early as the ipilimumab trial on melanoma, it was suggested that conventional response assessment criteria such as RECIST and WHO criteria are insufficient in fully characterising patterns of tumour response to immunotherapy. In 2009, irRECIST criteria were proposed based on the discussion by 200 oncologists, immunotherapists and regulatory experts. The immune-related Response Criteria (irRC) were evaluated in large multinational studies, involving 487 patients with advanced melanoma treated with ipilimumab.[82] It is possible that irRC will replace RECIST or WHO criteria in future clinical studies in immunotherapy.

FUTURE PROSPECTS OF LUNG CANCER IMMUNOTHERAPY

The path to successful immunotherapy in lung cancer has not been straightforward, but valuable lessons have been learned from both animal and human studies. Armed with better understanding of lung cancer immunoescape and immunosubversion, along with cancer immunosurveillance, immunoediting and ways to reactivate cancer immunity, we can now embark on a future filled with possibilities in utilizing immunotherapy as reliable lung cancer therapy.

The logical combination of nivolumab with ipilimumab in melanoma and NSCLC is showing promise although with substantial side effects.[45,83] There are multiple studies investigating the effect of combining nivolumab (anti PD-1 antibodies) with various anticancer agents including chemotherapy, EGFR TKI, bevacizumab and lirilumab (antibody targeting killer-cell immunoglobulin-like receptors (KIR) on NK cells) and other combination immunotherapy involving checkpoint blockade (Table 2). Epigenetic therapy could also be utilized to prime the tumour to be more responsive to immunotherapy.[22] The long-term efficacy of immune checkpoint blockade remains unknown, and clinicians are faced with the problems of how best to identify patients for treatment (i.e. biomarker issues) and how best to manage the toxicities that occur and the costs of these therapies.

We predict that future NSCLC therapies will include neoantigen vaccines (personalized therapy) combined with chemotherapy (which 'uses the tumour as its own vaccine') and with multiple checkpoint inhibitors.

CONCLUSION

It is an exciting time for lung cancer immunotherapy as our understanding of immune response to control, and eliminate tumour cells have greatly expanded in the last few years. This area is expanding rapidly, and we expect to see further development in it in the next few years.

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