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Immunotherapy in the treatment of non-small cell lung cancer

Raghav Sundar^a, Richie Soong^{b,c}, Byoung-Chul Cho^d, Julie R. Brahmer^e, and Ross A. Soo^{a,b,f,*}

^aDepartment of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore

^bCancer Science Institute of Singapore, National University of Singapore, Singapore

^cDepartment of Pathology, National University Health System, Singapore

^dDivision of Medical Oncology, Yonsei Cancer Center, Seoul, South Korea

^eDepartment of Oncology, The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins, Baltimore, MD, United States

^fDepartment of Surgery, University of Western Australia, Australia

Abstract

Advances in the understanding of the role of the immune system in tumor immunosurveillance have resulted in the recognition that tumors can evade immune destruction via the dysregulation of co-inhibitory or checkpoint signals. This has led to the development of a generation immunotherapeutic agents targeting the immune checkpoint pathway. Recent early phase studies of immune checkpoint modulators, such as CTLA-4, PD-1 and PD-L1 inhibitors in NSCLC have reported promising results with prolonged clinical responses and tolerable toxicity. This article provides an overview of co-stimulatory and inhibitory molecules that regulate the immune response to tumors, recent therapies that have been developed to exploit these interactions and the role of predictive biomarkers in treatment selection.

Keywords

Immunotherapy; Non-small cell lung cancer; PD-L1; CTLA-4; Ipilimumab; Nivolumab

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*Corresponding author at: Department of Haematology-Oncology, National University Cancer Institute, National University Health System, 1E Kent Ridge Road, NUHS Tower Block Level 7, Singapore 119228, Singapore. Tel.: +65 67795555. ross_soo@nuhs.edu.sg (R.A. Soo).

Conflict of interest statement

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1. Background

Lung cancer is one of the most common malignancies in the world, with 1.6 million new cases diagnosed annually and is also the leading cause of cancer deaths worldwide, causing 1.4 million deaths annually [1]. Whilst the identification of activating sensitizing EGFR mutations and other driver oncogenes [2,3] has led to improved treatment outcomes in selected subgroups of patients with advanced stage NSCLC, survival remains dismal and novel therapeutic approaches are needed [4].

Tumorigenesis is not only dependent on the properties of cancer cells but also by interaction with the immune system [5]. Testament to this is the numerous immunotherapies in clinical use in cancer, such as Sipuleucel-T (metastatic castrate resistant prostate cancer), ipilimumab and interleukin (IL)-2 (advanced melanoma) and IL-2 (renal cell carcinoma). Nonetheless, NSCLC has traditionally been thought to be a non-immunogenic tumor due to the inactivity of similar agents such as Bacillus Calmette-Guerin (BCG) [6], IL-2 [4] and interferon [7] have been unsuccessful in NSCLC. However the development new generation of cancer vaccines and immune modulators has renewed interest in immunotherapy in NSCLC. With respect to vaccines, although initial studies were promising, several phase III trials have been disappointing. Studies with tecemotide (START study) [8], and belagenpumatucel-L (STOP study) [9] in the locally advanced setting, and melanoma associated antigen A3 (MAGE-A3) (MAGRIT study) in the adjuvant setting [10] have been negative. The role of cancer vaccines has been reviewed elsewhere and will not be discussed [11].

The expression of antigens in cancer cells differs from host cells due to genetic and epigenetic variations. In order for the immune system to eliminate cancer cells, tumor recognition must first occur. This is followed by tumor antigen presentation to T cells, which leads to T cell activation and finally cell kill can occur. Co-stimulatory and inhibitory signals regulate T cell mediated immune response. T cell mediated immune response is modulated by stimulatory and inhibitory signals. Immune co-stimulatory molecules include CD28, CD137, glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR), and OX-40. Negative regulatory molecules immune checkpoint molecules prevent overstimulation of immune responses. Checkpoint molecules (co-inhibitory molecules) include cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), T-cell immunoglobulin- and mucin domain-3-containing molecule 3 (TIM3), Lymphocyte-activation gene 3 (LAG3) and killer cell immunoglobulin-like receptor (KIR). (Table 1) These immune checkpoints exist in a normal physiological state to protect against autoimmunity and inflammation. In a neoplastic state, dysfunction of these immune checkpoint proteins can lead to tumor tolerance and eventually allow for tumor “escape” from the immune system.

Targeting the molecules that regulate the immune response using antibodies has been the subject of much research and has yielded some promising results. This article provides an overview of co-stimulatory and inhibitory molecules that regulate the immune response to tumors, and recent therapies that have been developed to exploit these interactions.

2. Co-inhibitory interactions

2.1. CTLA4

The presentation of an antigen to T cell receptor (TCR) by a major histocompatibility complex on an antigen presenting cell (APC) as well as the binding of B7 molecules on APCs with the CD28 receptors on the T cells leads to the activation of CD4 and CD8 T cells. B7 has two subtypes of ligands, B7.1 or CD80 and B7.2 or CD86. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is also binds to B7, and acts competitively with CD28, but in an inhibitory fashion. The CTLA4 binds to B7, it releases signals that revert an activated T cell into an inhibited T cell. CTLA4 is up regulated only following T cell activation, and is only barely detectable on naïve T cells [31]. CTLA4 also reduces IL2 production; IL2 receptor expression and can directly inhibit TCR signals [32]. As a result of CTLA4 activation, peripheral tolerance of antigen specific T cells occurs. Mice with CTLA4-knockout have been shown to have phenomenal and lethal lymphoproliferation, indicating the importance of the role CTLA4 plays in inhibiting T cell activation [33]. CTLA4 expression is constitutive on regulatory T cells (Tregs) and is induced in CD4 and CD8 T cells.

CTLA-4 expression is not only confined to T lymphocytes but it is also expressed in NSCLC tumors in 51–87% of cases (Table 2). In one study CTLA-4 was associated with non-squamous histology but was not prognostic for overall survival [34] whilst in a second study CTLA-4 tumor expression was associated with older age and poorer tumor differentiation [35].

The anti-tumor effect of CTLA4 inhibition is via the reduction in the inhibition of CD28/B7 T cell activation. This also results in reduced Tregs, ultimately leading to an accelerated immune response to tumor associated antigens. The CTLA-4 inhibitor, ipilimumab has been shown to be effective in advanced stage melanoma and is currently being studied in NSCLC.

Ipilimumab is a humanized monoclonal antibody that binds to CTLA4, thereby preventing it from binding to its ligand and reducing the inhibition of CD28/B7 T cell activation by CTLA4. CTLA4 inhibition also reduces Tregs, ultimately leading to an accelerated immune response to tumor associated antigens.

In a randomized phase II trial in patients with advanced NSCLC [36], patients were randomized to receive one of the three regimens. All treatment arms received up to six cycles of paclitaxel and carboplatin. The first arm (early) received ipilimumab on D1 of cycle 1–4, and placebo for the remaining 2 cycles. The second arm received placebo for the first 2 cycles and ipilimumab on D1 of cycle 3–6 (delayed). The third arm (control) received only placebo. Maintenance ipilimumab was given in patients in the first two treatment groups once every 12 weeks until progression. Immune-related progression free survival (irPFS), which takes into account the immune related response criteria (described later in the article), was the study's primary endpoint. In the delayed arm, the irPFS was 5.7 vs 4.6 months (HR = 0.72; $P = 0.05$), whilst in the early arm, no improvement in irPFS was seen (5.5 vs 4.6 months HR = 0.81; $P = 0.13$). In the delayed group, a non-statistical improvement in OS was also seen (12.2 vs 8.3 months HR = 0.87; $P = 0.23$). Although not

statistically significant, patients with squamous histology had longer OS (HR = 0.55, 95% CI, 0.27–1.12).

The side effects reported were rash, pruritus and diarrhea. Grade 3/4 irAE was 20% for the early phase, 15% for the delayed phase and 6% for the control group. One death from toxic epidermal necrolysis was attributed to ipilimumab.

A larger phase III trial is being conducted, aiming specifically at the squamous subtype NSCLC (NCT01285609). Ipilimumab is also being studied in combination with EGFR and ALK tyrosine kinase inhibitors (NCT01998126). The role of ipilimumab is also being investigated in small cell lung cancer (NCT01331525, NCT01450761, NCT02046733).

Tremelimumab, a monoclonal antibody similar to ipilimumab has been studied in a phase II study of pre-treated patients with advanced stage NSCLC [37]. Patients were randomized into two arms-tremelimumab or best supportive care after 4 cycles of a platinum doublet chemotherapy regimen of investigators choice. The ORR was 5% and there was no difference in PFS.

2.2. PD1

PD-1 receptor is expressed on CD4 and CD8 lymphocytes, Tregs, B lymphocytes and NK cells [13]. Known ligands of PD-1 include PD-L1 (or CD274, B7-H1) and PD-L2 (CD 273, B7-DC). The binding of PD-1 with PD-L1 or PD-L2 leads to decreased cytokine production, reduced proliferation and cell lysis. In many tumors, PD-1 is up regulated in tumor infiltrating lymphocytes (TILs), while many tumors have increased PD-L1 expression [38]. It is proposed that through this mechanism, tumors can induce T cell anergy and avoid the processing tumor antigens by APCs that lead to recognition. PD-1 antagonists include PD-L1 antibodies such as nivolumab (BMS936558), lambrolizumab (MK-3475), and pidilizumab (CT-011) and the fusion protein AMP-224.

Nivolumab (BMS-936558, MDX-1106, ONO-4538) is a fully human IgG4 monoclonal antibody without detectable antibody-dependent cellular cytotoxicity (ADCC). In a phase I study of patients with advanced stage solid tumors [39], escalating doses of nivolumab biweekly were given for up to 12 cycles (2 years). In the NSCLC cohort ($n = 129$) the majority of patients were heavily pretreated, with 55% receiving at least 3 prior lines of therapy. The ORR was 17% with a median duration of response of 74 weeks (range, 6.1–133.9 weeks). The median survival was 9.9 months with one and two year survival rates of 42 and 24%, respectively. The median PFS was only 2.3 months. Nivolumab was generally well tolerated with skin toxicities (20%), gastrointestinal (15%) and pulmonary (9%) being the most commonly observed adverse events (AEs). A lower frequency of gastrointestinal toxicities was seen: 2% (grade 3/4) as compared to 20% with ipilimumab. Pneumonitis was reported in 6% (8/129) of patients with two deaths [40]. Biomarker analysis for PD-L1 expression was performed in 49% (63/129) patients. PD-L1 positive cases, defined as expression in at least 5% of tumor cells on immunohistochemistry (IHC), were seen in 49% (31/63) of patients. The ORR in patients with PD-L1 positive and PD-L1 negative tumors was 16% and 13%, respectively [41], suggesting that in a pretreated group, archival tumor tissue may not be ideal for assessing PD-L1 status.

Phase III trials of nivolumab versus docetaxel in patients with either squamous NSCLC (NCT01642004) or non-squamous NSCLC (NCT01673867) have completed accrual and results are eagerly awaited (Table 3).

Lambrolizumab (MK-3475) is a monoclonal antibody targeting PD-1 with significant antitumor activity in melanoma [42]. Preliminary results from a NSCLC phase 1 expansion cohort, a median survival of 51 weeks and a partial response of 25% as assessed by immune related response criteria [43]. Common AEs were fatigue, rash and pruritis, whilst grade 2 pneumonitis ($n = 1$) and grade 3 pulmonary edema ($n = 1$) were reported. In the tumor biomarker studies, fresh pre-treatment tumor biopsies were obtained. Tumor PD-L1 expression by IHC was a predictor of response with the ORR of 67% (6/9) and 4% (1/24) in PD-L1 positive and negative tumors, respectively. Based on these results, a randomized phase II/III trial of lambrolizumab vs docetaxel in patients with PDL1 positive advanced NSCLC is being conducted (NCT01905657) (Table 3).

2.3. PDL1

Programmed death receptor ligand 1 (PD-L1, B7-H1), the ligand for PD-1, is a member of the B7 superfamily, is involved in the negative regulation of immune response [44]. PD-L1 is expressed in T and B cells, macrophages and dendritic cells and is up regulated in a range of solid tumors including NSCLC. Upon induction by cytokines such as interleukin-4 (IL-4), IL-10, interferon- α , -B or - γ , PD-L1 activates PD-1 on T cells, and down-regulates T cell effector function and is one mechanism by which cancer cells evade host immune surveillance. PD-L1 is expressed in 27–57.5% of NSCLC (Table 2) and is localized in the cell membrane $\pm$ cytoplasm [45–51]. In sarcomatoid NSCLC, PD-L1 is expressed in 69% of cases compared with 27% in NSCLC [52]. The prognostic role of PD-L1 is currently unclear with studies reporting no association [45,49,51], a worse overall survival [48,50] or improved survival [46]. The conflicting results could be attributed to different methodologies to detect and score PD-L1 expression. PD-L1 expression has been reported to be associated with vascular invasion and higher-grade differentiation but improved relapse free survival [45]. PD-L1 expression is associated with increased macrophages [47], increased dendritic cells [50] and increased inflammatory infiltrate [46]. In contrast, one study found PD-L1 expression was inversely related to TILs [51]. With regards to histology, PD-L1 expression was associated with squamous cell carcinoma [46] whilst in contrast, with adenocarcinoma in a separate study [50]. One study found no association between PD-L1 expression and EGFR/KRAS mutations or ALK rearrangement [45] (Table 2). Given the key role PD-L1 has in lung cancer, the inhibition of PD-L1 is an attractive therapeutic approach. PD-L1 inhibitors undergoing clinical development include the monoclonal antibodies MPDL3280A, BMS-936559, MEDI4736 and MSB0010718C (Table 3).

In the expanded phase I study of BMS-936559 (MDX-1105) in solid tumors, the ORR in NSCLC was 10% and 18% had stable disease (SD) of at least 24 weeks [53]. The development of BMS-936559, however, has apparently been halted.

MPDL3280A is an engineered IgG anti-PD-L1 antibody with modified Fc domain that prevents antibody-dependent cell-mediated cytotoxicity (ADCC) in other immune cells expressing PD-L1. In a phase I study of MPDL3280A in pre-treated patients with advanced

NSCLC, the ORR was 24% and the 24-week PFS was 46%. The ORR in patients with PD-L1 positive and negative tumors was 100% (4/4) and 15% (4/26), respectively. Interestingly, the ORR in former/current smokers was 25% (8/31) versus 16% (1/6) in the never smokers [54].

MEDI4736, similar to MPDL3280A, is engineered with a triple mutation in the Fc domain to avoid Fc-mediated ADCC. Preliminary results from the phase I study of patients with solid tumors reported clinical activity and durable disease stabilization in different tumor types including NSCLC with no dose limiting toxicities or grade 3–4 treatment-related adverse events [55].

2.4. TIM3

Unlike other immune checkpoint molecules, TIM3 is not up regulated on all T cells post-activation but only in CD4+ T helper 1 (Th1) and CD8+ T cytotoxic cells and is involved in co-inhibition [56]). After activation by its ligand galectin-9, TIM3 inhibits effector Th1 cells and induces peripheral tolerance [57,58]. TIM3 plays a key role in the T cell exhaustion in tumors [59,60]. It can be co-expressed with PD-1 and in patients with melanoma; this co-expression represents the most exhausted population of CD8+ T cells in tumors [60]. In patients with NSCLC, TIM-3 in TiLs is up regulated in CD4+ and CD8+ TiLs but not expressed in peripheral blood T cells. The expression of TIM-3 in CD4+ T cells was associated with nodal metastasis and advanced cancer stage [61]. As well as being present in T cells, TIM3 has been reported in NSCLC in 86.7% of NSCLC tumor cells and is associated with T stage and histology and is an independent prognostic factor in NSCLC (relative risk of 4.481; 95% CI, 1.790–11.22) [62]. In preclinical models, the combined inhibition of TIM3 and PD-1 has been reported to be more effective than inhibiting either pathway alone in a range of solid tumors [59,63,64]. Taken together, the data supports the development of TIM3 inhibitors in humans.

2.5. LAG3

Lymphocyte-activation gene 3 (LAG3; CD223) is a co-inhibitory receptor expressed in activated T cells, Treg, Dendritic cells and NK cells. LAG3 is a CD4-related protein that binds to major histocompatibility complex (MHC) class II and reduces T cell proliferation resulting in tumor evasion [65,66]. LAG3 gene expression is up regulated in a silica-mediated lung inflammation murine model [67]. A phase I study of BMS-986016, a LAG3 monoclonal antibody with or without nivolumab in advanced solid tumors is currently ongoing (NCT01968109) (Table 3).

2.6. KIR

KIR is a family of natural killer (NK) cell regulators which presents a novel class of immunotherapy targets that are beginning to garner interest in various tumor types. KIR is one of the negative regulators of NK cell effector function. The inhibition of KIR results in NK cell mediated anti-tumor activity [68]. Lirilumab is an antibody that binds to KIR2DL1, –2 and –3 receptors, causing NK cell mediated cell kill [69]. Increased co-expression of KIR2DL1 in NSCLC patients has been described, which could potentially lead to decreased natural killer cell function [70]. Two phase I studies of lirilumab in combination with

ipilimumab and nivolumab in patients with NSCLC are currently ongoing (NCT01714739 and NCT01750580) (Table 3).

2.7. BTLA and A2AR

Co-inhibitory receptor B and T lymphocyte attenuator (BTLA) has been studied in the role of autoimmunity, and along with its ligand herpes virus entry mediator (HVEM) has been associated with lymphomagenesis [71]. A2A adenosine receptor (A2AR) is a G protein-coupled receptor, which binds to adenosine and may potentially regulate the MAPKinase pathway [20]. Currently no studies are being conducted on these molecules in lung cancer.

3. Co-stimulatory interactions

3.1. OX40

OX-40 (CD134, TNFRSF4 tumor necrosis factor receptor superfamily, member 4) is a co-stimulatory molecule present in activated T cells at sites of inflammation [72] and regulates antigen-specific T-cell expansion, survival and cytokine IL-2, IL-4, IL-5, IFN- γ production [73,74]. The immunological effects of OX40 signaling make it an attractive target for improving tumor immunotherapy [75]. Pre-clinical studies have shown OX40 agonists have anti-tumor activity in melanoma, glioma, breast and colon carcinoma, sarcoma, renal carcinoma, and prostate cancer [76–79]. In a phase I study, patients with solid tumors were treated with one cycle of a murine agonistic anti-human OX40 Ab at three dose levels. Toxicities were acceptable and included fatigue, fever/chills, transient lymphopenia, and mild rash. Tumor reduction was seen in 12/30 patients and both humoral and cellular immunity were enhanced [80]. High levels of human anti-mouse antibodies were detected, thus future studies of humanized OX40 agonists are required for further development of this antibody [81]. Studies of OX40 agonist combined with radiotherapy are ongoing in breast and prostate cancer (NCT01862900, NCT01303705).

3.2. CD137

CD137 (4-1BB) is an inducible T-cell surface molecule of the tumor necrosis factor (TNF) receptor superfamily. Binding to its ligand CD137L co-stimulates CD4 and CD8 cells [82]. CD137 is also expressed on Treg, activated NK cells. Urelumab (BMS 663513) a CD137 agonist [83] has been tested in a phase I/II study with promising activity although marked hepatic toxicities has been reported [83,84]. A study of urelumab in NSCLC has been terminated (NCT00461110).

3.3. GITR

Glucocorticoid-induced TNF receptor (GITR) is a member of the TNF receptor superfamily. GITR acts as a co-stimulatory molecule to both CD4⁺ and CD8⁺ naïve T cells, leading to T cell proliferation and effector function [85]. GITR is found on Treg, effector T cells, B cells, NK cells, and activated dendritic cells [86]. TRX518, a monoclonal antibody GITR agonist, is being investigated in a phase I setting (NCT01239134).

3.4. CD40, CD28, CD27 and ICOS

CD40 is a member of the tumor necrosis factor superfamily and is involved in cellular differentiation, survival and apoptosis [87]. Preclinical studies have shown a potential use of anti-CD40 antibodies may suppress tumor growth and metastases [88]. CD27 is another stimulatory receptor of T cells and an antibody against it is currently being tested in a phase I study (NCT01460134). Inducible T-cell costimulator (ICOS) is a T-cell co-stimulator related to CD28 [89]. TGN1412, an antibody against CD28 was found to be extremely toxic and has hence no further trials have been conducted with this class of drugs [90].

4. Combination therapy

The concept of using multiple drugs to target the various pathways of tumorigenesis has been a common approach utilized in cancer therapy. This includes the use of combination cytotoxic chemotherapeutic agents with different mechanisms of action and toxicity profiles to maximize cell kill. Indeed, the standard of care for first line chemotherapy regimen for NSCLC is a platinum based doublet [91]. With the emergence of molecular-targeted therapy in NSCLC, it has become increasingly apparent that combinations require careful investigation in their consideration. While some combinations of targeted therapy and chemotherapy have been found to be of benefit, other combinations may be detrimental [92]. While, each agent has proven to be efficacious individually, the combination of these agents has not shown to improve overall survival. Similarly, especially with its distinctive toxicity profile, the combination of immunotherapeutic agents with one another and with chemotherapy regimens to affect the multiple aspects of the immunomodulation of the neoplastic process requires further study.

CTLA4 inhibitors in combination with chemotherapy have already been discussed previously. Currently there is a phase III study looking at the role of ipilimumab with paclitaxel and carboplatin in squamous NSCLC (NCT01285609). Nivolumab and Ipilimumab in combination with KIR inhibitors (NCT01714739, NCT01750580), and nivolumab in combination with various chemotherapeutic and targeted therapies (NCT01454102) has also been described above (Table 3).

Of interest is the combination of immunotherapeutic agents with each other, and currently several are being tested. CTLA4 blockade after vaccination with irradiated, autologous tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor (GM-CSF) showed antitumor immunity without major toxicities in patients with advanced melanoma [93]. Multiple immune checkpoint blockades with combination PD1 and CTLA4 Ab can allow for increased T cell responsiveness and decreased T cell anergy, in preclinical models [94].

The timing of immunotherapy in the schema of anti-neoplastic therapy requires further study. In the phase II study of ipilimumab and chemotherapy described earlier, the arm which introduced ipilimumab in a delayed manner compared to the upfront introduction of ipilimumab appeared to have a greater benefit, and the biology of this is not clearly understood yet.

5. Predictive biomarkers

The need for predictive biomarkers is important as immune modulators can result in durable response but only in a very select group of patients. Several biomarkers for CTLA4 have been described that may translate into clinical application [95]. A rise in absolute lymphocyte counts has been shown to be associated with clinical benefit [96]. Studies have shown a positive correlation between ipilimumab treatment and frequency of CD4⁺ cells expressing high levels of ICOS [97]. HLA-DR has been associated with increased expression on CD4 cells after treatment with ipilimumab [98]. Similar results have also been shown with CD45RO [99]. Tumor infiltrating lymphocytes have been found to correlate with clinical benefit from treatment with ipilimumab [100]. Forkhead box P3 (FOXP3)-positive and indoleamine 2,3-dioxygenase (IDO)-positive patients benefited from CTLA4 blockage as well [100].

It is known that PD-L1 expression varies with the tumor microenvironment and may not be a static expression at a single time point. PD-L1 expression has been associated with the presence of IFN- γ in the tumor microenvironment [101]. This may be the explanation for PD-L1 expression not being a good predictive marker for nivolumab as described earlier [41]. Predictive biomarker research in this area is at an early stage of development and multiple studies are ongoing examining issues such as the following: Is a predictive biomarker/companion diagnostic necessary? Is tissue micro-localization of biomarker expression important (TILS versus tumor)? What is the optimal detection method? Does biomarker expression alter over time (time at diagnosis versus time at relapse) and space (primary versus metastasis)? What is the impact of chemotherapy, molecular targeted therapy and radiotherapy on biomarker expression?

6. Evaluation of tumor response to immunotherapeutic agents

One accepted standardized method to evaluate tumor response to cancer treatment is the use of RECIST criteria [102]. However, according to RECIST, an increase in the size of a tumor of 30% or more, even early in treatment, is considered as disease progression. However treatment responses with immunotherapy may occur even after progression of disease as defined by RECIST criteria. Patients appear to have clinical benefit even though the tumor appears to progress radiologically and sustained SD may represent sustained response. Based on this, an immune related Response Criteria has been created [103]. Immune related progression free survival (irPFS) accounts for the apparent increase in tumor size followed by sustained tumor response which has been documented with these agents in the past [104]. This phenomenon of “pseudo-progression” may be due to peritumoral lymphocyte infiltration or delayed immune activity.

7. Conclusion

An improvement in the understanding of the immune system in tumor immunosurveillance has resulted in the development of a new generation of immunotherapeutic agents. In particular, it is now recognized tumors can evade immune destruction via the dysregulation of co-inhibitory or checkpoint signals. Results from early phase studies of immune checkpoint modulators such as CTLA-4, PD-1 and PD-L1 inhibitors in a range of solid

tumors including NSCLC are highly promising and may provide new therapeutic options in the treatment of NSCLC. Future challenges include incorporating immunotherapeutic agents either together or in combination with cytotoxic chemotherapy, molecular targeted therapy and radiotherapy, its administration in early stage disease and the identification of predictive biomarkers.

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Table 1

Co-inhibitory and co-stimulatory molecules and their binding partners.

Receptor		Binding partner			References
Name	Expression in immune cells	Therapeutic agent	Name	Expression in normal cells	
Co-inhibitory molecules					
CTLA-4	Activated T cells, Tregs	Ipilimumab, Tremelimumab	CD80, CD86	Activated B cells, monocytes	[12]
PD-1	Activated CD4, CD8, Tregs, Activated B cells and NK cells	Nivolumab, Lambrolizumab, pidilizumab (CT-011), AMP-224	PD-L1(B7-H1), PD-L2	Pancreas, thymus, liver, heart, endothelium, small intestine	[13,14]
TIM3	Monocytes, NK cells, CD4TH1 cells		Galectin-9	Small intestine	[15,16]
LAG3 (CD223)	CD4, CD8, $\gamma\delta$ T cells, Treg, B cells, NK cells and plasmacytoid DC	IMP321	MHC-II	Antigen presenting cells, B lymphocytes	[17]
BTLA	TH1		HVEM	Peripheral T and B cells	[18,19]
A2aR	Ubiquitous		Adenosine B7-H3,B7-H4	Ubiquitous Haematopoetic cells	[20] [21]
KIR	NK cells	Lirilumab	MHC-II or MHC-I	Antigen presenting cells, B lymphocytes	[22]
Co-stimulatory molecules					
OX-40(CD134)	Activated T cells, Treg	MEDI6469	OX40-L	Peripheral B lymphocytes	[23]
CD137(4-1BB)	Activated T cells, dendritic cells, NK cells	BMS-666513, PF-05082566	CD137L	Most leukocytes	[24]
CD40	Dendritic cells, B lymphocytes, macrophages, endothelial cells, smooth muscle cells, fibroblasts, monocytes	Dacetuzumab, Lucatumumab, CP-870893	CD40L(CD154)	CD4, CD8, skin mast cells and blood basophils	[25,26]
CD27	Resting T lymphocytes	CDX1127	CD70	Activated T and B lymphocytes	[27]
GITR	Treg, CD4, CD8,	TRX518	GITR-L	Antigen presenting cells	[28]
ICOS	Activated T cells	MEDI570, AMG557	B7RP1	B cells, macrophages	[29]
CD28	T cells, eosinophils	TGN1412	CD80, CD86	Activated B cells, monocytes	[30]

A2aR: A2A adenosine receptor; BTLA: B and T lymphocyte attenuator; DC: dendritic cells; GITR: glucocorticoid-induced tumor necrosis factor receptor; HVEM: herpes virus entry mediator; ICOS: Inducible T-cell co-stimulator; KIR: killer cell immunoglobulin-like receptor; LAG3: lymphocyte-activation gene 3; PD-1: programmed death-1; NK: natural killer; PD-L1: programmed death receptor ligand 1; TIM3: T-cell immunoglobulin- and mucin domain-3-containing molecule 3; Tregs: T regulatory cells.

Table 2

Tumor expression of CTLA-4, PD-L1 in resected NSCLC.

Author	N	Histologic subtype	Pathologic stage	Detection method/Ab clone	Cellular localization	% PD-L1 + ve	Clinpathological association	Association with immune cells/TILs	Prognosis
<i>CTLA-4</i>									
Salvi [34]	81	Mixed	I–III	IHC anti CTLA-4/14D3	Cell surface, cytoplasm	50.6	Non-squamous	Not reported	OS: neutral 5 year survival 64.8 vs. 45.9%
Zheng [35]	89	Mixed	I–IV	IHC anti-CTLA-4	Not reported	86.5	Older age, poorer differentiation	Not reported	Not reported
<i>PD-L1</i>									
Yang [45]	163	ADC	I	IHC anti-PD-L1/Proteintech Group Chicago, IL	Membrane	39.9	Vascular invasion, higher grade differentiation	No association with TILs	RFS: improved, OS: neutral
Velchetti [46]	204 (US)	Mixed	I–IV	QIF/5HI	Membrane	36.1	SCC	Increased inflammatory infiltrate	OS: improved 60 v 27months
	340 (Greece)	Mixed	I–IV	QIF/5HI	Membrane	24.8	Lower stage	Increased inflammatory infiltrate	OS: improved NR v 31months
	173 (US)	Mixed	I–IV	mRNA	Not applicable	50.8	None	Increased inflammatory infiltrate	OS: improved
	314 (Greece)	Mixed	I–IV	mRNA	Not applicable	53.2	None	Increased inflammatory infiltrate	OS: improved
Chen [47]	208	Mixed	I–IV	IHC anti-PD-L1	Cytoplasm, membrane	65.3	Non-smokers, less LN metastasis	Increased macrophages	Not reported
Velchetti [52]	445	Mixed	I–IV	QIF/5HI	Not reported	27.4	Not reported	Not reported	Not reported
	13	Sarcomatoid	I–IV	QIF/5HI	Not reported	69.2	Not reported	Not reported	Not reported
Chen [48]	120	Mixed	I–III	IHC anti-PD-L1/236A/E7	Cytoplasm, membrane	57.5	Not reported	Not reported	OS: reduced
Boland [49]	214	SCC	I–IV	IHC anti PD-L1/5HI	Membrane	19.6	Not reported	No association with TILs	OS: neutral
Mu [50]	109	Mixed	I–III	IHC anti-PD-L1/not reported	Cytoplasm, membrane	53.2	ADC	Increased dendritic cells	OS: reduced PD-L1 + <3 year survival 46%, >3Y survival 12%
Konishi [51]	52	Mixed	I–IV	IHC anti-PD-L1/M1HI	Cytoplasm, membrane	27.2	None	Reduced TILs	OS: neutral 5 year survival 59% v 48%

Ab: antibody; ADC: adenocarcinoma; IHC: immunohistochemistry; LN: lymph node; NR: not reached; OS: overall survival; PD-L1: programmed death-1 ligand; QIF: quantitative fluorescence; RFS: relapsed free survival; SCC: squamous cell carcinoma; TILs: tumor infiltrating lymphocytes.

Table 3

Selected ongoing studies of immune checkpoint mediators.

Phase	Patient population	N	Regimen	Control	Primary endpoint	Clinical trials.gov
<i>CTLA-4</i>						
I	ALK/EGFR mutant NSCLC 1st line	46	Ipilimumab + erlotinib Ipilimumab + crizotinib	None	Toxicity	NCT01998126
II	NSCLC 1st line	204	Ipilimumab + Carboplatin/paclitaxel Concurrent vs sequential	Carboplatin/paclitaxel	irPFS	NCT00527735
III	Squamous NSCLC 1st line	920	Ipilimumab + Carboplatin/paclitaxel	Carboplatin/paclitaxel	OS	NCT01285609
I	EGFR mutant NSCLC 1st line	24	Tremelimumab + Gefitinib	None	Toxicity	NCT02040064
II	NSCLC after 1st line, maintenance	87	Tremelimumab	BSC	PFS	NCT00312975
<i>PD-1</i>						
I	EGFR mutant NSCLC 1st line	24	Tremelimumab + Gefitinib	None	Safety, RP2D	NCT02040064
I	1st line, Squamous, non-squamous NSCLC	250	Nivolumab in combination with platinum doublet, bevacizumab maintenance, erlotinib, ipilimumab, or nivolumab alone	None	Toxicity	NCT01454102
II	Squamous NSCLC, 3rd or more line	100	Nivolumab	None	ORR	NCT01721759
III	Nonsquamous NSCLC, 2nd line	574	Nivolumab	Docetaxel	OS	NCT01673867
III	Squamous NSCLC, 2nd line	264	Nivolumab	Docetaxel	ORR	NCT01642004
I	NSCLC 1st line	30	Lambrolizumab (MK-3475) monotherapy or in combination with platinum doublet	None	Toxicity	NCT01840579
I	Pre-treated NSCLC, PD-L1+	24	Lambrolizumab	None	ORR	NCT02007070
I	Advanced stage melanoma/NSCLC	1137	Various doses of Lambrolizumab	None	Toxicity	NCT01295827
I/II	NSCLC, any line	320	In combination with platinum doublet ± bevacizumab, erlotinib, gefitinib, or ipilimumab	None	PFS/ORR	NCT02039674
II/III	NSCLC 2nd line after platinum doublet	920	Lambrolizumab low dose/high dose	Docetaxel	OS	NCT01905657
<i>PD-L1</i>						
I	Advanced stage solid tumors	20	MSB0010718C	None	DLT	NCT01943461
I	Advanced stage selected solid tumors	590	MSB0010718C	None	DLT	NCT01772004
IB	EGFR mutant NSCLC 1st line	32	MPDL3280A + Erlotinib	None	DLT	NCT02013219
II	PD-L1 + NSCLC, any line	130	MPDL3280A	None	ORR	NCT01846416
II	PD-L1 + NSCLC, any line	300	MPDL3280A	None	ORR	NCT02031458
II	NSCLC, 2nd line after platinum doublet	300	MPDL3280A	Docetaxel	OS	NCT01903993
III	NSCLC, 2nd line after platinum doublet	850	MPDL3280A	Docetaxel	OS	NCT02008227
IB	NSCLC, any line	208	MEDI4736 +tremelimumab	None	DLT, MTD	NCT02000947

Phase	Patient population	N	Regimen	Control	Primary endpoint	Clinical trials.gov
<i>LAG3</i>						
I	Advanced stage solid tumors		BMS-986016± nivolumab	None	Safety, DLT	NCT01968109
<i>KIR</i>						
I	Advanced stage solid tumors	150	Lirilumab + nivolumab	None	DLT, MTD	NCT01714739
I	Advanced stage solid tumors	125	Lirilumab + ipilimumab	None	DLT, MTD	NCT01750580
<i>CD27</i>						
I	Advanced solid tumors	170	CDX-1127	None	Toxicity	NCT01460134

ALK: anaplastic lymphoma kinase; BSC: best supportive care; DLT: dose limiting toxicity; EGFR: epidermal growth factor receptor; irPFS: immune related progression free survival; MTD: maximally tolerated dose; NSCLC: non-small cell lung cancer, ORR: overall response rate; OS: overall survival; PFS: progression free survival; RP2D: recommended phase II dose.