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CLINICAL

# Low immunogenicity in non-small cell lung cancer; do new developments and novel treatments have a role?

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**Abstract** Approximately 1.6 million new cases of lung cancer are diagnosed annually (Jemal *et al. CA: A Cancer Journal for Clinicians*, *61*, 69–90, 2011) and it remains the leading cause of cancer-related mortality worldwide. Despite decades of bench and clinical research to attempt to improve outcome for locally advanced, good performance status patients, the 5-year survival remains less than 15 % (Molina *et al.* 2008). Immune checkpoint inhibitor (ICH) therapies have shown a significant promise in preclinical and clinical trails to date in the treatment of non-small cell lung cancer (NSCLC). The idea of combining these systemic immune therapies with local ablative techniques is one that is gaining momentum. Electrochemotherapy (ECT) is a unique atraumatic local therapy that has had very promising objective response rates and a number of advantages including but not limited to its immunostimulatory effects. ECT in combination with ICHs offers a novel approach for dealing with this difficult disease process.

**Keywords** NSCLC · Electrochemotherapy · Immunotherapy · Immunomodulators PD-1 CTLA-4

Abstracts presented at the American Society of Clinical Oncology, the Melanoma Bridge meeting, and the European Society of Medical Oncology annual meetings up until 2014 were reviewed with the relevant reports extracted.

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## 1 Introduction

Lung cancer imparts a heavy burden throughout the developed and developing world. Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related mortality worldwide. Its accounts for more deaths each year than colon, breast, and prostate cancer combined.

In the USA, lung cancer has the second highest predicted number of new cases (224,210) and the highest incidence of estimated cancer deaths (159,260) for 2014 [1]. Eighty to eighty-five percent of lung cancer tumors are non-small cell lung cancers (NSCLCs), which include adenocarcinomas, squamous cell, and large cell carcinomas. The remaining 15–20 % are small cell lung cancers (SCLCs) [2]. Histology is now an important consideration for treatment selection in NSCLC. Currently, patients with squamous NSCLC have more limited treatment options compared with patients with NSCLC of non-squamous histology. The role of surgery in small cell lung cancer is very limited.

Overall survival (OS) is the gold standard when assessing success in any cancer treatment. Lung cancer has one of the poorest predicted outcomes. Surgical resection remains the only therapeutic option with proven long-term cure and survival. However at presentation, up to 20–30 % of patients will qualify for potentially curative resection [3]. Despite optimal surgical management, more than half of these patients are destined to develop systemic recurrences of the disease in spite of the benefits of adjuvant chemotherapy. The 5-year survival post resection following gold standard optimum treatment protocols remains between 69–89 % for IA, 52–75 % for 1B, 45–52 % for IIA, and 33 % for IIB [4]. With clinical stage IIIA-N2 disease, the 5-year overall survival rate is 10–15 % but drops to 2–5 % with bulky mediastinal involvement.

In patients in whom surgical resection is not an option, the current standard chemotherapy treatment of choice is platinum-based agents. These combination platinum-based

regimens have been associated with improved survival compared with best supportive care. However, the median overall survival still remains less than 1 year with a 5-year survival of 3–7 % for IIIB and less than 1 % for stage IV [5–7].

In a small subset of patients with NSCLC whose tumors display anaplastic lymphoma kinase gene (ALK) or epidermal growth factor receptor (EGFR) mutations, small molecule inhibitors such as crizotinib and erlotinib can be utilized. These relatively non-toxic agents deliver longer PFS when compared to traditional chemotherapy. Unfortunately, even in the minority of patients with these mutations who get a measurable response, it is generally short-lived [8, 9]. This demonstrates that novel approaches targeting specific pathways are necessary.

It is clear that we need to advance our understanding of this cancer to help overcome it. Unlike renal cell carcinoma and malignant melanoma, spontaneous regression of NSCLC is rare and the response to vaccines has been poor to date [10]. This review article will evaluate current systemic immunomodulator therapies being used in NSCLC and the possibility of combining these with local ablative techniques to enable a more thorough immune response. This could theoretically allow us to advance survival in this disease. This review will be based on the data provided by clinical trials and translational research.

## 2 Immunogenicity

The immune system plays a critical role in identifying and destroying foreign or abnormal cells in the body, including the suppression of tumor growth [11, 12]. The initial definition of immunogenicity was "the discrimination between self and non-self" [13] based on Burnet's seminal ideas [14]. However, this is too simple a definition given our current level of understanding.

The ability to react with a poorly functioning or aberrant "self" is an essential mechanism of an efficient immune system. It must be remembered that tumor cells, except perhaps those caused by oncogenic viruses, are self-cells, in that they come from the genome of the individual. More recently, a modification for the definition of immunogenicity has been suggested to conclude that the immune system does not respond to non-self, but rather to abrupt modifications of the antigenic patterns with which it is in contact [15].

Tumor immunogenicity can be simply defined as the ability of a tumor to induce an immune response that can prevent its growth. Tumor cells like any antigen can trigger an immune response when the antigenic patterns they display vary significantly from normal. Genetic and epigenetic aberrations occur commonly in human tumors and produce altered antigenic profiles that can be selectively recognized as an antigenic discontinuity stimulating an adaptive immune response [16].

The process by which cancers such as NSCLC arise has been attributed to "immunoediting." This is illustrated by preclinical data that has shown that tumors developing in immunodeficient mice are inherently more immunogenic and consequently less able to develop independently than tumors that arise in immunocompetent hosts [17]. It can be inferred from this that the presence of an immunocompetent host forces the tumor cell to adapt to survive and overcome our innate and adaptive immune defenses. It is this ability of resilient tumor cells to evade immune recognition that determines the success of the tumor and ultimately the clinical course of the disease [18, 19].

One of the outstanding characteristics and challenges presented by NSCLC is low immunogenicity and the induction of immune tolerance. NSCLC has repeatedly been shown to be a poorly immunogenic tumor due to its proven lack of response to agents such as bacillus Calmette-Guerin (BCG) [20], IL-2 [21], and interferon [22]. As well as the ability to evolve chemotherapy-resistant clones [23], NSCLC can lie dormant in a microenvironment rich in immunoglobulins, rendering NSCLC a difficult target.

The question that needs to be answered is how we can manipulate the various immune escape mechanisms used by NCSLC to force its unmasking and enable an efficient adaptive immune response to be deployed against it. The combination of immunomodulators and locoregional ablative techniques could prove a fruitful combination to induce durable responses and improve overall survival.

## 3 Immune checkpoint inhibitors (ICHs)

As discussed previously the aberrant antigenic patterns on tumor cells enable tumor recognition. Tumor antigen presentation to T cells leads to T cell activation and then cell kill can occur. In the case of T cells, a balance between co-stimulatory and inhibitory signals regulates the ultimate amplitude and quality of the response. Immune co-stimulatory molecules include CD28, CD137, glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR), and OX-40. Negative regulatory molecules, also known as immune checkpoint inhibitors (ICHs), include cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and its ligands PD-L1/PD-L2. It is this last group of molecules that are of interest because they have been linked to tumoral immune escape.

These ICHs function in a normal physiological state, to prevent overstimulation of immune responses leading to autoimmune disease and inflammation. For example the expression of specific CTLA-4 polymorphisms [24–26], PD-I receptor deficiency [27], and PD-1/PD-L1 blockade [28] have all

been shown to correlate with a higher incidence of certain autoimmune diseases. In a neoplastic state, dysfunction of these ICHs can lead to tumor tolerance and eventually allow for tumor "escape."

Targeting the molecules that regulate the immune response using antibodies has been the subject of much research and has yielded some promising results. Preclinical cancer models demonstrate that inhibitory signals mediated by co-receptors on tumor-specific T cells impede anti-tumor immunity. This would suggest that blockade of such interactions can release the brakes on immune responsiveness leading potentially to tumor elimination. In this review, ICHs have been empirically divided into first- and second-generation ICHs based on their time of introduction. This article will provide an overview of the potential of combining systemic ICHs with locoregional modalities based on published research to date.

## 4 First-generation immune checkpoint inhibitors (ICHs)

### 4.1 CTLA-4

CTLA4 binds to B7 on antigen presenting cells (APCs) resulting in signals that revert an activated T cell into an inhibited T cell and is only upregulated following T cell activation [29] (Table 1). CTLA-4 engagement encourages peripheral tolerance in antigen specific T cells. It also results in down-regulation of T cell activation, as demonstrated in multiple experimental systems, including knock-out mouse models and T cell lines [36]. Inhibiting CTLA-4 allows unleashing of suppressed immune responses, primarily at the level of the APC-T cell interaction, and potentially depleting regulatory T cells in the tumor microenvironment [37, 38]. Regulatory T cells are naturally occurring CD25+ CD4+ regulatory T cells (Tregs) that suppress aberrant immune responses including autoimmune diseases and allergy [39]. CTLA-4 is highly expressed on Tregs and is instrumental in their function. CTLA-4 blockade has been shown to promote T cell activation, as well as depletion of intratumoral Tregs [40].

CTLA-4 expression in various histological subtypes of NSCLC has been evaluated by Salvi *et al.* [41]. This study demonstrated a 1.5-fold increase in CTLA-4 over expression in non-squamous as compared to squamous histological type [41]. This finding has not been reproduced in subsequent reports to date. Overall, CTLA-4 has been shown to be expressed in 51–87 % of cases of NSCLC [42]; however, its expression has not demonstrated any statistically significant prognostic value to date [41].

Given its powerful inhibitory effect, the blockade of CTLA-4's physiological function in T cells, by means of CTLA-4-specific humanized monoclonal antibody (mAbs) was the next logical step. CTLA-4 inhibitors, ipilimumab

**Table 1** Known co-inhibitory molecules

| Name | Expression | Therapeutic agent | Binding site (BS) | BS expression | BS therapeutic agent |
|---|---|---|---|---|---|
| CTLA-4 (aka CD-152) [29] | Activated T cells, Tregs | Ipilimumab (aka MDX-010 and MDX-101) tremelimumab (CP675) | CD80 (aka B7-1), CD86 (aka B7-2) | Activated B cells, monocytes | |
| PD-1 [30] | Activated CD4, CD8, Tregs, Activated B cells, and NK cells | nivolumab, lambrolizumab, pidilizumab (aka CT-011), AMP-224 pembrolizumab (aka MK-3475) | PD-L1 (aka B7-H1), PD-L2 | Heart, liver, pancreas, thymus, endothelium, small intestine | PD-L1 antagonists: MDX-1105, MEDI4736, MPDL3280A |
| KIR [31] | NK cells | Lirilumab | MHC-II or MHC-I | B lymphocytes, antigen presenting cells | |
| A2aR [32] | Ubiquitous | | Adenosine, B7-H3, B7-H4 | Hematopoietic cells | MGA271 |
| TIM3 [33] | Monocytes, NK cells, CD4 TH1 cells | | Galectin-9 | Small intestine | |
| LAG3 (CD223) [34] | CD4, CD8, □T cells, Tregs, B cells, NK cells plasmacytoid DC | IMP321 | MHC-II | Antigen presenting cells, B lymphocytes | |
| BTLA [35] | TH1 | | HVEM | Peripheral T and B cells | |

*A2aR* A2A adenosine receptor, *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, *MHC* major histocompatibility complex, *PD-1* programmed death-1, *NK* natural killer, *PD-L1/2* programmed death receptor ligand 1/2, *TIM3* T cell immunoglobulin and mucin domain-3-containing molecule 3, *Tregs* T regulatory cells

and more recently tremelimumab are the only two CTLA-4 antagonists to be developed clinically to date and are the best characterized [43–45].

Ipilimumab demonstrated significant improved survival in phase III clinical trials [44, 45] in patients with advanced melanoma. It was first approved by the FDA in 2011 for treatment of metastatic melanoma and was promptly added as a category 1 recommendation in the National Comprehensive Cancer Network. In 2013, the European Commission approved ipilimumab as a first-line treatment for advanced (unresectable or metastatic) melanoma. The mechanism of action of ipilimumab is not specific to one tumor type and has been trialed as a single agent [46] or in combination with other therapies [47] across multiple malignancies. However, only approximately 20 % of patients will achieve a prolonged disease control even in melanoma, a tumor whose vulnerability to immune-mediated therapy has been explored.

The novel aspect of agents like ipilimumab is that they do not work *via* conventional cytotoxic mechanisms but rather they prevent tumor immune evasion, thereby allowing destruction of recognized foreign tumor tissue by innate and adaptive immune responses.

As originally predicted by murine models, anti-CTLA-4 therapy in humans has resulted in objective tumor regressions including durable complete responses (CR) in some patients. However, as anticipated from the uncontrolled lymphoproliferation observed in CTLA-4 null mice [48], anti-CTLA-4 therapy has been associated with a significant frequency of serious immunologic adverse events (AEs) [49–53]. Standard treatment of these AEs include topical or systemic corticosteroids, or in severe cases anti-metastatic and anti-TNF antibodies [54]. Trials evaluating the efficacy of administration of concurrent prophylactic budesonide did not demonstrate any additional benefits [55].

## 5 Trial data for ipilimumab

Ipilimumab as a monotherapy has exhibited some clinical activity with encouraging long-term survival in previously treated patients within the advanced melanoma population [56]. However to date, anti-CTLA ICHs have shown very little efficacy as a monotherapy in NSCLC. In a phase II trial in advanced NSCLC, tremelimumab was given as a maintenance therapy following four cycles of chemotherapy *vs.* best supportive care (BSC) [57]. There was no demonstrable difference in progression-free survival (PFS) but there was a 4.8 % objective response rate (ORR) seen only in the investigational arm. However, treatment-related adverse events (AEs) were significantly higher in the tremelimumab group at 61.4 *vs.* 7 % with BSC.

The majority of trials have combined these ICHs with commonly administered chemotherapeutic regimes. The rationale is based on the hypothesis that chemotherapeutic agents favoring the release of tumor-specific antigens could promote the anti-tumor activity of anti-CTLA-4 monoclonal antibodies [58]. Also, it has been shown in preclinical models that certain chemotherapeutics including paclitaxel enhance the anti-tumor activity of anti-CTLA-4 monoclonal antibodies [59].

Lynch *et al.* [60] coupled ipilimumab with paclitaxel/carboplatin (the common standard of care for NSCLC) in the first-line setting in patients with advanced chemotherapy naïve NSCLC. It was a large ($n$=204) randomized, double-blind, multi-center phase II trial. A sequential combination therapy (two doses of chemotherapy followed by ipilimumab plus chemotherapy) showed significantly improved immune-related progression free survival (irPFS) ($P$=0.03), when compared with chemotherapy alone [60]. This finding was significant according to the Response Evaluation Criteria [61] in Solid Tumors (RECIST) but did not meet significance by the World Health Organization (WHO) progression-free survival (PFS) criteria ($P$=0.37). Phased ipilimumab showed improved efficacy over chemotherapy only for those patients with squamous cell histology.

This was the first study to adopt immune-related progression-free survival (irPFS) as its primary endpoint. This is defined as the time from random assignment to immune-related progression or death. New criteria were introduced based on observations made in melanoma clinical trials. It was noted that the pattern of response to immunotherapies differs from the pattern of response seen with traditional cytotoxic agents [62] (Table 2). In addition there is some evidence that response to immunotherapeutic agents, such as ipilimumab, could be preceded by an apparent disease progression due to inflammatory reaction in the tumor area. In clinical trials, approximately 20–30 % of responders treated with ipilimumab exhibit an apparent increase in total tumor burden after 3 months of treatment followed by prolonged tumor control or regression [64, 65].

In 2000, the RECIST group published a set of standardized response definitions based on a large international collaboration. These RECIST guidelines have since been revised and version 1.1 was published in January of 2009 [66]. The WHO criteria was subsequently evolved to characterize the patterns of response in melanoma to immunotherapies, the so-called immune-related response criteria (irRC) (see Table 2).

These findings subsequently lead to the design of two large international phase II trials, which are seeking to evaluate stage IV or recurrent squamous cell NSCLC. Both are evaluating a very similar phasing schedule combining ipilimumab with chemotherapy *vs.* chemotherapy alone (paclitaxel and carboplatin) but with varying doses of ipilimumab [67, 68] for first-line treatment. The primary endpoints of both studies will be OS, with irPFS as a key secondary endpoint. It is yet to

**Table 2** WHO criteria *vs.* immune-related response criteria (irRC)

| | WHO | RECIST (irRC) |
|---|---|---|
| Complete response (CR) | All tumors cleared. Must be observed in two consecutive appraisals ≤4 weeks apart [63] | All tumors cleared. Must be observed in two consecutive appraisals ≤4 weeks apart [61] |
| Partial response (PR) | SPD reduced in all index lesions by greater than 50 % (compared to baseline) in two consecutive appraisals ≤4 weeks apart with no evidence of new lesions or disease progression in non-index lesions [63] | Tumor burden reduced by greater than 50 % in two consecutive appraisals ≤4 weeks apart [61] |
| Stable disease (SD) | When a ≥50 % reduction in SPD cannot be demonstrated (compared with baseline) or a 25 % increase *vs.* nadir and without evidence of new lesions or progression in non-index lesions [63] | When a ≥50 % reduction in tumor burden cannot be demonstrated (compared with baseline) or a 25 % increase *vs.* nadir [61]. |
| Progressive disease (PD) | There must be a ≥25 % expansion in SPD *vs.* nadir or progression demonstrated *via*<br>• increase in non-index lesions<br>• new lesions [63] | There must be a ≥25 % expansion in SPD *vs.* Nadir observed in two consecutive observations at least 4 weeks apart [61] |
| New, measurable lesions (≥5×5 mm) | Taken as an indication of PD [63] | Incorporated into existing tumor burden [61] |
| New, non-measurable lesions (<5×5 mm) | Taken as an indication of PD [63] | Does not define progression (but precludes irCR) [61] |
| Non-index lesions (NIL) | Clearance of all NILs with normalization of tumor marker levels in conjunction with non-pathological LNs indicates CR<br>The Persistence ≥1 NIL lesion(s) and/or maintenance of tumor marker level above normal limits seen as non-PD and non-CR<br>PD is defined as progression of existing non-target lesions [63] | Relevant to irCR classification which requires complete disappearance of all lesions observed in two consecutive appraisals ≤4 weeks apart [61] |

*SPD* the sum of the products of the two largest perpendicular diameters, *NIL* any tumor lesion other than the largest tumor lesion targeted by therapy, *LNs* lymph nodes, *icCR* immune-related complete response

be seen if stratification of treatment based on histological subtype may be feasible or necessary.

The unquestionable (if modest) success of ipilimumab in melanoma has not been reproduced in NSCLC. However, there are a number of second-generation immunomodulators being developed of which programmed cell death-1 (PD-1)-directed therapies have shown the greatest success. Whether ipilimumab will play a role in combined immune checkpoint inhibition in conjunction with PD-1-directed therapy or with other modalities such as electrochemotherapy in the treatment of NSCLC remains a question to be answered.

## 6 Second-generation immune checkpoint inhibitors (ICHs)

### 6.1 Anti-programmed cell death-1 (anti-PD-1) and anti-programmed cell death ligand-1/2 (PD-L1/L2) -directed therapies

The most prominent and potentially promising group of second-generation checkpoint inhibitors include antibodies directed against PD-I and its endogenous ligands PD-L1/PD-L2. PD-1 is a transmembrane receptor of the immunoglobulin (Ig) super family that in its normal physiological state functions to limit the activity of T cells in the periphery during an inflammatory response to infection and autoimmunity. The periphery in this context refers to all T cells outside of the thymus and bone marrow. It achieves this by acting as a co-inhibitory ICH which is expressed on the surface of T cells, tumor-infiltrating lymphocytes [69], and some subsets of immature dendritic cells (DCs). Compared to the restricted expression of CTLA-4, this wide expression of PD-1 suggests a broader role in immune regulation. Additionally, unlike CTLA-4's mode of regulation, PD-1/PDL-1 functions in the peripheral tissue during the effector phase of T cell activation and demonstrates distinct immune-inhibitory signals.

The PD-1:PD-L1 pathway is an essential method of tumor immune evasion. The upregulation of this pathway leading to immune tolerance and allows tumor progression [69]. PD-1 is highly expressed on induced regulatory T cells (Tregs) and the PD-1:PD-L1 interaction appears to promote the induction, conversion, and maintenance of Tregs. PD-L1 may also act to inhibit antigen-specific dendritic cell (DC) activation and immune function [70] which plays a role in establishing and maintaining T cell tolerance. This suggests an additional mechanism for immunosuppression in a tumor microenvironment rich in PD-1 ligands.

Anti-PD-1-directed agents block the interaction of PD-1 to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC), activating previously functionally exhausted immune responses. Tumors have been demonstrated to escape immune surveillance by suppressing tumor-infiltrating lymphocytes *via* PD-1/PD-L1/PD-L2 interactions [71]. Inhibition of these

interactions with therapeutic antibodies has been shown to enhance T cell response and stimulate anti-tumor activity [72].

Two distinct groups of second-generation ICHs directed against PD-1 have been developed. The first group, anti-PD-1 antibodies, includes nivolumab and pembrolizumab. These agents block the binding of PD-1 receptor to its two endogenous ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). The second group, the anti-PD-L1 inhibitory antibodies, includes BMS-936559, MPDL3280A, and MedI-4736. Similar to the anti-PD1 antibodies, these function principally by blocking PD-1/PD-L1 signaling. However, this group is distinct in that they do not block interactions between PD-L2 and PD-1 but do block interactions between PD-L1 and CD80 [73]. The clinical significance of these interactions remains to be determined.

In preclinical models, antibody blockade of PD-1 or its ligands induces anti-tumor activity in murine cancer models through enhancement of T cell activity [74, 75]. Both PD-1 and PD-L1 inhibitors have shown clinical activity and safety in phase I trials [76–78]. Early trial results suggest these anti-PD-1-directed agents appear to have more tumor-specific activity across malignancies and produce fewer immune-related adverse events as compared with anti-CTLA-4 therapy [27, 79]. Hopefully, the results of ongoing and future trials will shed further light on this.

In addition, while anti-CTLA-4 antibodies have not demonstrated much, if any success as a monotherapy in the treatment of NSCLC, anti-PD1-directed therapies have resulted in a significant single-agent activity in advanced pretreated NSCLC, in terms of overall response (OR), stable disease (SD), and associated long-term survival.

## 7 Trial data for anti-PD-1 therapies

### 7.1 Nivolumab

Nivolumab (aka BMS-936558 aka MDX-1106), a fully human IgG4 blocking monoclonal antibody against PD-1 was the first of this class to be evaluated clinically [76]. Two phase I dose escalation trials assessing various solid tumors including NSCLC demonstrated that nivolumab was safe. Objective responses were seen in 16–31 % of the solid tumor types, with most responses being durable for >1 year [80, 81].

A separate phase I dose-escalating trial assessing nivolumab in advanced solid pretreated tumors, including NSCLC was completed [76]. An objective response rate (ORR) of up to 32 % was achieved in NSCLC with 3.0 mg/kg dosing. Similar rates of objective responses were observed across all NSCLC histologic types but a prolonged response (≤24 weeks) was only observed in the non-squamous group. Of note, three patients (two with NSCLC) in this study experienced fatal pneumonitis representing 1 % of the study population. The frequency and severity of drug-related adverse events appeared to be independent of nivolumab dose or histological subset. Because of this complication, treatment protocols now advocate the early use of immunosuppressive therapy when a complication is suspected.

A study by Brahmer *et al.* [82] was completed which evaluated nivolumab as a monotherapy in ($n$=129) in advanced generally heavily pretreated NSCLC patients. They demonstrated a median survival of 9.9 months with OS of 42 % at 1 year and 24 % at 2 years. This represented a significantly improved survival outcome relative to reports of other salvage therapies applied to this population. The significant response and disease stabilization rate in advanced chemotherapy-refractory patients achieved in this trial and the durability of responses was unprecedented. Pneumonitis was reported in 6 % of patients with two deaths [82]. The ORR in patients with PD-L1-positive and PD-L1-negative tumors was 16 and 13 %, respectively. The authors concluded that when assessing for PD-L1 expression in a pretreated group, archival tumor tissue might not be the most suitable specimen to use. Based on these findings, further investigations are being conducted with nivolumab (3.0 mg/kg) in NSCLC [83].

The ongoing and currently recruiting Checkmate 012 trial is evaluating nivolumab as a monotherapy and in various treatment combinations for patients with stage IIIB/IV NSCLC [84]. This trial is evaluating some novel combinations including nivolumab in combination with targeted agents such as erlotinib and ipilimumab and as a monotherapy in patients with asymptomatic brain metastases. The interim results were presented as an abstract and showed evidence of increased activity in patients with squamous NSCLC who received combination therapy [85]. No firm conclusions can be drawn, as of yet; however, this innovative trial will hopefully provide further insight into the most efficient use of nivolumab. Nivolumab has also entered a phase III clinical investigation as a single agent compared with second-line single-agent chemotherapy for advanced squamous and non-squamous NSCLC [83, 86].

### 7.2 Pembrolizumab (aka MK-3475 aka lambrolizumab)

Pembrolizumab is a humanized anti-PD-1 antibody. To date, it has demonstrated an improved anti-tumor activity in PD-L1-positive advanced treatment naïve and pretreated NSCLC [87]. In a phase I study, pembrolizumab was administered at 10 mg/kg every 3 weeks to NSCLC patients previously treated with two systemic regimens. The objective response rate (ORR) was 21 % by RECIST criteria with no demonstrable difference based on histological subtype. Rapid (≤9 weeks) and long duration of responses were reported. Drug-related adverse events (AEs) were reported at (9 %) with the most common being pneumonitis.

It has been demonstrated that patients with strong PD-L1 tumor expression may derive particular benefit from treatment with pembrolizumab. As a result, a large proportion of the ongoing trials are assessing pembrolizumab in only PD-LI-positive NSCLC. These trials are focusing on pembrolizumab treatment of PD-L1-positive NSCLC *vs.* standard of care in advanced treatment-naive tumors [88], pretreated tumors [89], and as a monotherapy in those patients who have experienced disease progression after platinum-based systemic therapy [90]. However, not all trials are using PD-L1 expression as a stratification tool for treatment with pembrolizumab [91].

### 7.3 PD-L1

PD-L1 is upregulated on many cell types (hematopoietic, endothelial, and epithelial) in response to pro-inflammatory cytokines, notably interferon gamma. PD-L1 activates PD-1 on T cells causing downregulation of T cell effectors. PD-L1 is broadly expressed in non-small cell lung cancers (27–57.5 %) [92, 93]. This expression has been shown to be largely confined to the tumor microenvironment with higher levels demonstrated in adenocarcinomas *vs.* squamous cell carcinomas (65.2 *vs.* 44.4 %) [94]. The strong expression of PD-L1 on various tumors is believed to play an important role in immune evasion of cancer cells [71, 95]. This pathway seems to mediate tumor immune resistance at the level of the tumor microenvironment potentially promoting tumor-specific immune responses, limiting widespread T cell activation [96] and impairing DC maturation [94].

The prognostic value of PD-LI has been investigated in many studies with very disparate conclusions.

In various studies it has been shown to be a poor prognostic indicator [71, 94], to not correlate to prognosis [97] and that it is associated with improved survival [93]. Studies evaluating its relevance with more specific aspects of NSCLC have shown an association with increased vascular invasion, higher-grade differentiation, and a negative correlation to lymphatic metastasis [92, 98]. It is becoming ever more clear that the relationship between PD-L1 expression and disease progression is not a simple one.

A previously reported study used automated immunohistochemical (IHC) assays to investigate the relationship between PD-L1 expression and clinical benefit from anti-PD-1-directed therapies. It concluded that although PD-L1-positive tumors may be more likely to respond, the lack of PD-L1 expression did not preclude the possibility of benefit from nivolumab [99, 100] or MPDL3280A [99]. In reality, it is likely that the mechanisms of response to PD-1 and PD-L1-directed immunomodulators are complex and elucidating markers of response may require an improved understanding of both the host and tumor response to immune checkpoint inhibition.

Several agents that target PD-L1 are in development which only block the PD-1:PD-L1 interaction. Three anti-PD-L1 antibodies, BMS-936559, MPDL3280A, and MedI-4736 have been or are being currently evaluated in NSCLC.

### 7.4 BMS-936559

BMS-936559 was the first PD-L1 antibody to be assessed in a phase I trial of patients with advanced cancer evaluating multiple different dose levels [77]. In the NSCLC arm, there was a 10 % objective response and 18 % demonstrated stable disease at 24 weeks. The PFS at 24 weeks was 43 % and 26 % for patients with squamous and non-squamous NSCLC. There were no drug-related deaths but 6 % of patients were withdrawn due to drug-related adverse events.

### 7.5 MPDL3280A

MPDL3280A is an anti-PD-L1 monoclonal antibody containing an engineered IgG Fc domain designed to optimize efficacy and safety and prevent antibody-dependent cell-mediated cytotoxicity (ADDC) in other immune cells expressing PD-L1. MPDL3280A was studied as a monotherapy in a phase I dose-ranging study for pretreated advanced and metastatic NSCLC. The ORR was 24 % and the 24-week PFS was 46 % with patients showing clinical response regardless of histology, EGFR mutation status, or number of prior therapies. The ORR in patients with PD-L1-positive and PD-L1-negative tumors was 100 % (4/4) and 15 % (4/26), respectively [101]. This study also noted the ORR in smokers was 25 % (8/31) *vs.* 16 % (1/6) in non-smokers. This was a small study and no significant conclusions can be drawn from these interesting findings as of yet.

An additional phase I study, currently recruiting patients, will evaluate MPDL3280A in combination with carboplatin/paclitaxel, with carboplatin/pemetrexed, and with carboplatin/nab-paclitaxel in patients with advanced or metastatic NSCLC [102]. Two additional phase II studies in patients with advanced or metastatic NSCLC are ongoing. One trial is evaluating objective responses in patients with PD-L1-positive NSCLC receiving single-agent MPDL3280A therapy [103]and the other trial is evaluating OS and safety of MPDL3280A compared with docetaxel after platinum therapy failure [104]. A phase III trial of similar design, comparing MPDL3280A with docetaxel, began in early 2014 [105].

### 7.6 MEDI4736

MEDI4736 is also an anti-PD-L1 antibody with an engineered Fc domain designed to avoid ADCC. A phase 1 study on solid tumors including NSCLC reported initial clinical activity with tumor shrinkage reported as early as first assessment

(6 weeks) and durable disease stabilization. No dose-limiting toxicity has been reported to date [106]. A maximum tolerated dose of anti-PD-L1 therapies has not been reached in any clinical trial to date. In a phase 1 trial examining solid tumors including NSCLC, adverse events of any grade were reported in 91 % but only 5 % had serious adverse events related to treatment [77]. Trials to date suggest that anti-PD-L1 is somewhat less active than anti-PD-1 but may also be associated with slightly lower toxicity [76, 77].

Given that multiple anti-PD-1 and anti-PD-L1 antibodies are under development, an important question is whether there are fundamental efficacy and toxicity differences between antibodies targeting the ligand *vs.* the receptor. Ongoing studies with the anti-PD-L1 antibodies MPDL3280A and MedI-4736 as well as the anti-PD-1 antibodies nivolumab and lambrolizumab will ultimately shed light on whether there are target-specific differences in activity or toxicity in NSCLC.

### 7.7 PD-L2

While PD-L1 has very broad expression, PD-L2 is inducibly expressed in a more restricted fashion. PDL2 is expressed more broadly than PDL1 on healthy tissue, so the PD-L1-targeting agents may cause less toxicity to healthy tissue. PD-L2 is upregulated on dendritic cells and macrophages in response to different proinflammatory cytokines such as IL-4 [107]. While the PD-L1:PD-1 interaction is considered the most important mediator of tumor immune resistance, the importance of the binding of PD1 to PD-L2 and its second binding partner B7.1 (CD80) are not well studied. Blocking the PD-L2 could play a role in distinguishing the clinical activities of anti-PD-1 *vs.* anti-PD-L1 antibodies.

## 8 Toxicity of anti-PD-1-directed therapies

On the basis of the role of the PD-1 pathway in down-modulating tissue inflammation, it is generally believed that organ-specific immune toxicities observed in patients receiving blockers of this pathway reflect underlying subclinical inflammation that is exacerbated upon initiation of therapy. There is also a strong possibility that opportunistic autoimmune disorders could be potentiated by ICHs [108]. The suggested management algorithms for patients with suspected grades 3–4 drug-related AEs involve discontinuation of the drug, prompt systemic steroid therapy, and consideration of adjunctive immunosuppressant therapy including infliximab, mycophenolate mofetil, or cyclophosphamide. Also, empiric antibiotics should be initiated because of the challenges involved in differentiating infective *vs.* drug-induced pneumonitis [80]. It is hoped that the toxicity profile of anti-PD-1-directed therapies are more favorable than anti-CTLA-4. However, it must be borne in mind that currently with second-generation ICHs, there is an increased awareness of the potential for such toxicity issues. Subsequently, there are more aggressive interventions being undertaken when such AEs are suspected.

### 8.1 CTLA-4 and anti-PD-1 combination therapy

The combination of nivolumab and ipilimumab makes sense given the distinct roles of the CTLA-4 and PD-1 pathways in regulating the initiation and execution of immune responses within tumors. Preclinical melanoma studies showed that blockade of both the CTLA-4 and PD-1 checkpoint pathways results in a significantly increased anti-tumor activity compared with blocking either checkpoint alone [109, 110]. A phase I study of nivolumab plus ipilimumab in metastatic melanoma has reported the combination is substantially more effective than either agent as monotherapy. In 52 patients who received combination therapy ORR was 40 %, 10 % of responders had complete responses, and 31 % had near-complete responses defined as 80 % or greater tumor reduction [111]. While these results are incredibly promising, there is a down side, namely increased incidence of immune-related adverse events. The frequency of severe adverse events was much higher with concurrent nivolumab plus ipilimumab treatment with 53 % of patients experiencing grade 3 or 4 adverse events [111]. As immunotherapies boost the immune response, they tend to induce autoimmune AEs that in early trials of ipilimumab and nivolumab have even led to death.

It is clear that modulation of the immune system may be necessary for durable recognition of tumor-associated antigens. Checkpoint inhibition is emerging as a powerful new therapeutic approach in the fight against lung cancer. However, simply blocking the pathway to immune cell suppression through checkpoint inhibition may not be enough to induce tumor control. As mentioned previously, there may be potential benefit from the synergy of immunomodulators and selected ablative techniques in facilitating this immunomodulation.

### 8.2 Ablative techniques

The next step in developing these immunotherapies is evaluating which other modalities could be combined with them to achieve a better outcome. To date, these ICHs have been used with traditional chemotherapy regimes with moderate success in NSCLC as described above. Ablative therapies involve local treatment in a variety of forms. The idea of combining a systemic immunotherapy with various local ablative techniques is an intriguing thought that is gaining momentum [112–115]. We are particularly interested in ablative therapies that aid the immune targeting of tumors. This will be discussed below.

There are a number of ablative techniques currently used with varying degrees of success in NSCLC, namely radiofrequency ablation (RFA), microwave ablation (MWA), cryoablation, and irreversible electroporation (IRE). RFA and MWA bring about focal hyperthermic injury to ablated cells, causing rapid protein denaturation, which is immediately cytotoxic and leading to coagulative necrosis [116]. RFA has increasingly being applied to intrathoracic malignancies [117, 118], with a reported 3-year survival rate of 15–46 %, local recurrence rate ranging 3–38.1 % (median of 11.2 %) [119], and a procedure-related mortality of 2.6 % [120]. The efficacy of RFA is limited by size with several studies showing a higher failure rate in tumors greater than 3.0 cm [121, 122], with tumor proximity to large vessels which is associated with a pronounced heat-sink effect [121, 123] and some protocols require the tumor to be at least 1 cm from vital structures [124].

MWA has greater efficacy with larger tumor volumes and a lower susceptibility to a heat-sink effect [125]. MWA is a weak stimulator of local inflammation, as well as innate and acquired anti-tumor immunity. There have been significant complications reported in conjunction with this technique including a third-degree burn, acute respiratory distress syndrome [126], and major hemoptysis secondary to the erosion of an abscess formed post MWA into a blood vessel [127]. The major limiting factor in using any form of thermal ablation for the treatment of lung cancer is the size of the ablation zone obtained after treatment [128]. Due to the destructive nature to the lung parenchyma, patients with compromised respiratory reserve are often excluded from receiving thermal ablative treatment [121]. Additionally, the subsequent necrosis and scarring left after thermal ablation imparts significant difficulties in identifying residual or recurrent disease in follow-up imaging.

Cryoablation is performed using an argon-helium-based system that inflicts cold injury to kill tumors (−20 and −40 °C) and as with RFA, the peripheral zone has limited cytotoxicity and, therefore, 3- to 5-mm margins are recommended [129]. Such a size requirement limits the tumors that can be targeted. It has been demonstrated that there is increased uptake of TAA by dendritic cells (DCs) in the lymph nodes following cryoablation allowing for more efficient antigen presentation and T cell priming [130]. So while cryoablation is an attractive alternative, its scope is limited.

It is imperative that we identify the ablative therapies that promote TAA presentation with expected immune cell response. This potentially allows for local control and a systemic response, which will theoretically improve outcome.

### 8.3 Electrochemotherapy (ECT) as an ablative therapy

ECT works by the application of an electric pulse (electroporation) which acts as a vector to increase the internalization of the chemotoxic drug (bleomycin or cisplatin most commonly) [131, 132]. It achieves this by causing a transient increase in tumor cell membrane permeability without causing any damage to the underlying tissue architecture [133]. This mechanism of action ensures a very localized effect on rapidly dividing cells and requires a much lower doses of a cytotoxic drug to achieve greater results with a low side effect profile [134]. Since the first clinical study in 1990 [135], ECT has had consistently reported complete response rates of between 60–70 % and objective response rates of about 80 % [134, 136–139] across multiple solid tumor types including NSCLC. Unlike other ablative techniques, ECT is not limited by the size of the tumor; additionally, it has been shown to be well tolerated and allows for immediate recovery [140, 141].

Electroporation may be used in two ways, irreversible electroporation or reversible electroporation as used in ECT. This depends on the voltage applied [142]. However to date, the use of IRE in NSCLC has failed to demonstrate efficacy and the tissue damage experienced equates to the thermal damage seen in RFA. A recent study investigating the safety of IRE in NSCLC had to be terminated prematurely. In this study, IRE failed to demonstrate efficacy with a local control rate of only 39 % in tumors 8 to 27 mm in size [143].

All of the above ablative techniques, which rely on extremes of temperature, suffer from a "heat-sink effect." This involves the dissipation of heat and cold when these are carried out near large vessels. This is particularly problematic in NSCLC as there is a considerable blood flow in this area originating from the right side of the heart, and the lung parenchyma itself is made up of air-filled pockets, which are constantly being ventilated. This causes significant dissipation of the applied heating during thermal ablation treatment. Reassuringly, this does not occur with ECT. Therefore, the safety and efficacy of ECT is not compromised when it is carried out near large blood vessels. This has been demonstrated with the treatment of liver metastasis with ECT that is located between the major hepatic vessels [144].

Further advances are also being made with the use of calcium rather than chemotherapeutic agents. The efficacy of this has been established in preclinical studies [145], and the first clinical trial on calcium use in electrochemotherapy has been initiated [146]. This approach has the obvious advantage of avoiding the associated ill effects of chemotherapeutics agents but, additionally, it would significantly reduce cost and allow the application of ECT outside of oncology centers. The potential to preserve healthy lung tissue is particularly pertinent in those with advanced NSCLC with a limited respiratory reserve in whom preservation of lung function and capacity is a priority.

In addition to its local cytotoxic effects, ECT also induces a vasoconstricting effect, known as the "vascular-lock" phenomenon which has been demonstrated in preclinical models [147]. This is a reflex constriction of vessels induced by

electric pulses and is believed to be responsible for a temporary reduction in perfusion of tumor tissue and an interstitial edema. The vascular-lock effect lasts longer in tumor tissue and brings about a prolonged exposure of tumor cells to the given chemotherapeutic agents [148, 149]. This effect has been successfully utilized clinically for the treatment of bleeding tumors [150, 151].

ECT's use in NSCLC has been limited to date due to issues relating to accessibility. ECT has been shown to be very effective in the treatment of superficial metastatic disease, such as melanoma and chest wall breast cancer recurrence [152–154]. However, the scope for ECT is ever expanding with the development of new methods and types of electrodes that serve to increase its adaptability for use in tumors located in more difficult-to-reach sites. Specific instruments including finger-borne and endoluminal electrodes [155, 156] are currently being developed for the treatment of deep-seated tumors [144, 157]. The successful treatment of bone metastases [158], metastases from colon cancer localized to the liver [144], and brain metastases [159, 160] with ECT have demonstrated that its use in non-superficial organs is both feasible and safe. This careful application of ECT allows for the treatment of tumors adjacent to or invading vital structures that would not be fully amenable to radiation therapy or surgical resection.

ECT has also been applied with success in a neoadjuvant setting with facial melanoma patients in order to reduce the tumor size making the lesion more amenable to surgical resection and enabling a less invasive procedure [161]. This application of ECT in a neoadjuvant setting will identify new operational challenges and could open up a new avenue of surgical oncology.

Another issue identified during early-phase clinical trials is how to go about monitoring the effect of ECT. With deep-seated tumors, the use of imaging will be mandatory, and it must be borne in mind that the healing process is different from thermal ablation technologies inducing tissue necrosis and thus image interpretation and time interval for repeated examinations will need to be further explored. With the more widespread application of ECT, new radiological guidelines will need to be designed and implemented to monitor post procedural progress.

### 8.4 Electrochemotherapy (ECT) and immunomodulation

ECT has been shown to cause a number of local potentially exploitable immune responses.

It is possible that under the right conditions, these local responses could be translated into a systemic anti-cancer response. Due to the inherent lack of homogeneity of tumor tissue, not all of the cells in a given tumor can be effectively eradicated by electrochemotherapy [162]. However, ECT-induced cell death facilitates the release of tumor-associated antigens (TAA), which can be captured by local dendritic cells and presented to tumor-specific cytotoxic T cells in draining lymph nodes [163]. Additionally, it is thought that the application of an electric field directly in the tissue results in an inflammatory response that aids in the priming of immune responses [164]. The electroporation (EP) employed is also believed to activate antigen presenting cells through danger signals released during EP allowing for a more efficient antigen presentation [133].

Preclinical studies looking at CT26 murine cancer cells showed that the injection of dying ECT-treated cells elicited an anti-tumor immune response that prevented the growth of a subsequent administration of viable cancer cells. This same study demonstrated that ECT treatment was much more efficient in immunocompetent animals than in immunodeficient ones, with complete regressions only seen in the immunocompetent animals [165]. A clinical study examining the inflammatory infiltrate of ECT-treated melanoma metastases was carried out recently. It demonstrated a high number of plasmacytoid and dermal dendritic cells. Furthermore, the results suggested that ECT induces a rapid migration of tumor-associated epidermal Langerhans cells to draining lymph nodes [166].

However, despite bringing about two of the essential factors for generating systemic anti-cancer immunity, namely dendritic cell recruitment and TAA availability at the tumor site, ECT in its use as a monotherapy has failed to show any regression of untreated distant metastases and has shown only local effect to date [91]. As the prior discussion illustrated ICHs may be the key to unlocking a systemic response [167]. It is quite possible that the massive reduction in tumor burden provoked by ECT may be accompanied by a significant decrease in the levels of immunosuppressive factors. This combined with local infiltration of dendritic cells, and TAA availability poses a significant potential to facilitate a systemic anti-cancer response. Therefore, it is reasonable to attempt to exploit this immune response to ECT and to seek to enhance it with the addition of immunomodulators and potentiate the systemic response to the same. Therefore, ECT can be thought of as a tool to facilitate the effect of ICHs.

A current ongoing animal study (OncoSec® medical) is examining the use of electroporation to introduce plasmid IL-12 in melanoma in combination with immune checkpoint inhibitors. They are examining CTLA-4, PD-1, or both at varying concentrations. Their preliminary data showed a 100 % regression with no deaths due to toxicity with further results eagerly awaited. Additionally, the preliminary results of an ongoing clinical study examining the combination of the superficial treatment of melanoma lesions with ECT using bleomycin and the systemic administration of ipilimumab at 3 mg/kg every 3 weeks for 4 cycles in patients with advanced melanoma (IIIC/IV) was presented at the Melanoma Bridge meeting. The preliminary results of this small study ($n$=15)

demonstrated a local objective response rate of 67 %. A response in distant untreated lesions was observed in 28 % at 24 weeks and a decrease in Tregs was seen in all responders. These results are incredibly promising and highlight the very real potential of combining ECT with immunomodulators to bring about both a local and systemic anti-cancer response.

## 9 Discussion

The area of immunotherapy is rapidly expanding and the data to date has been very promising.

Immunotherapies are predicted to play a very significant role in the cancer treatment of the future. However, there are clear limitations in their use as a monotherapy.

Many ablative techniques being currently used cause a degree of inflammation and variable immune responses. Of the ablative techniques available, we see particular benefit with ECT. It is a relatively novel therapy that has many advantages over currently employed ablative therapies as discussed above.

Given that ICHs work by blocking immuno-inhibitory pathways, it would follow that administering ICHs during a period of local stress to a tumor such as with ECT would be beneficial. This form of treatment targets rapidly diving cells and causes chemotherapy-associated necrosis. ECT represents a local stress on tumors, which is followed by a period of time with enhanced infiltration of dendritic cells and an inflammatory response that aids in the priming of immune responses, as discussed previously. This local immune response could be significantly improved by the functional removal of the immuno-inhibitory pathways CTLA-4 and PD-1 with monoclonal antibodies. In the absence of these barriers to immune recognition, there is great potential for the immune reaction to ECT to allow for tumor-associated antigen recognition. This treatment can also be repeated as many times as is necessary due to its atraumatic nature increasing the likelihood of a systemic immune recognition of the TAA.

We see the exploration of immunotherapy and suitable ablative techniques, particularly ECT, as the next step in finding a satisfactory therapeutic outcome in NSCLC. The ultimate goal of combining these therapies is to enable an effective, self-driven, anti-tumor immune response. Currently, the use of ECT is largely confined to Europe with is routine application limited to only 130 centers [168] and with predominantly superficial tumors. To date, significant progress has been made in the use of ECT and its potential applications are constantly expanding. However, further work needs to be done before a more widespread acceptance of this modality into treatment guidelines can be established.

## 10 Conclusion

We see the future treatment of NSCLC as being a combination of surgery in conjunction with chemotherapeutic and immune-related therapies. We see immune stimulation by local ablative therapies as being a very promising and exciting adjunct to these therapies. Further research will allow us to define their specific role. We expect that once these roles are more clearly defined, we will see a dramatic improvement in survival for NSCLC.

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