EXPERT REVIEWS

Immunotherapy: is a minor god yet in the pantheon of treatments for lung cancer?

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Immunotherapy has been studied for many years in lung cancer without significant results, making the majority of oncologists quite skeptical about its possible application for non-small cell lung cancer treatment. However, the recent knowledge about immune escape and subsequent 'cancer immunoediting' has yielded the development of new strategies of cancer immunotherapy, heralding a new era of lung cancer treatment. Cancer vaccines, including both whole-cell and peptide vaccines have been tested both in early and advanced stages of non-small cell lung cancer. New immunomodulatory agents, including anti-CTLA4, anti-PD1/PDL1 monoclonal antibodies, have been investigated as monotherapy in metastatic lung cancer. To date, these treatments have shown impressive results of efficacy and tolerability in early clinical trials, leading to testing in several large, randomized Phase III trials. As these results will be confirmed, these drugs will be available in the near future, offering new exciting therapeutic options for lung cancer treatment.

KEYWORDS: belagenpumatucel-L • CIMAvax • GVAX • immunotherapy • ipilimumab • MAGE-A3 • non-small cell lung cancer • PD-1 • PD-L1 • racotumomab • tecemotide • TG4010 • vaccines

Despite several advances in lung cancer translational research, the survival outcomes for the majority of non-small cell lung cancer (NSCLC) patients still remain very poor [1,2]. Recently, the introduction of targeted therapies, including EGF receptor (EGFR) tyrosine kinase inhibitors (erlotinib, gefitinib, afatinib) for EGFR mutated tumors and EML4-ALK inhibitors (crizotinib, ceritinib) for ALK-rearranged tumors significantly improved both median progression-free survival (PFS) and quality of life (QoL) of metastatic NSCLC patients [3]. However, these personalized treatment options are limited to a minority of NSCLC patients whose tumors report the target activating mutations, while platinum-based doublet chemotherapy remains the unique choice for the majority of them. Therefore, the need for additional therapeutic options is widely shared in the scientific community. A promising field of research in oncology is represented by immunotherapy, which has been demonstrated to be a valid and effective treatment option for some other malignancies such as melanoma and prostate cancer [4,5]. The rationale of studying immunotherapy for lung cancer comes from the possibility of enhancing a non-efficient or inhibited immune response against cancer cells. A better understanding of the expression of tumor-specific peptides and the functional defects in immune pathways that allow tumors to escape immune-recognition/killing has led to the development of new immunotherapeutic strategies with promising activity in the clinical setting. This review provides the biological basis for immunotherapy in NSCLC, focusing on the new emerging immunotherapeutic agents currently under investigation in clinical trials.

Cancer immune-features & immunoediting

The immune response to cancer involves both innate and adaptive compartments. Mechanisms are not fully known, but they could be explained as follows. In the complex system of immune response to cancer, called immunoediting, three phases have been identified: elimination, equilibrium and escape (FIGURE 1). In the first two phases (elimination and equilibrium), T-lymphocytes play the principal role and as result of their activation, the immune

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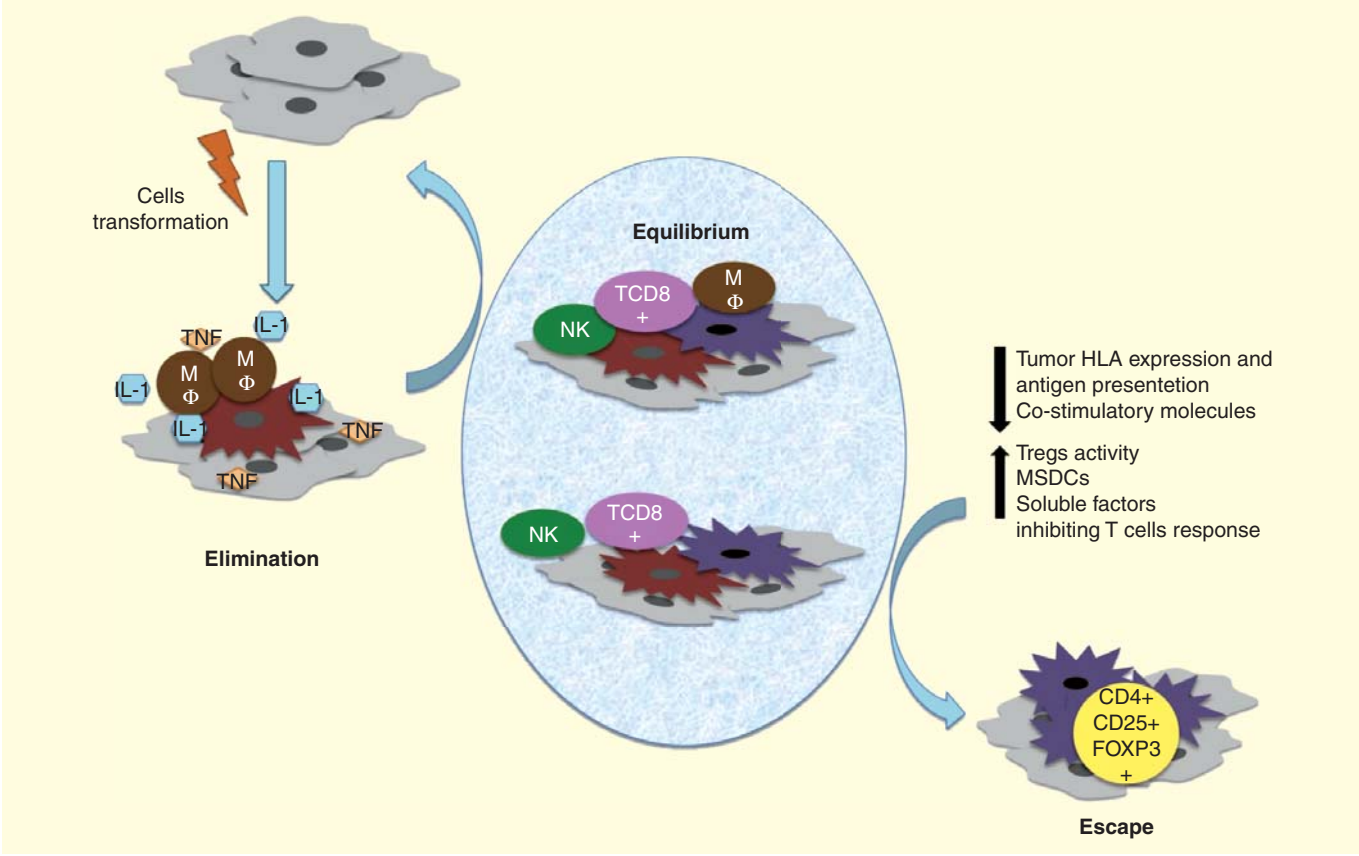

Figure 1. This image represents the immunoediting process that can be described by the three 'E': elimination, equilibrium and escape. In the elimination phase, innate and adaptive immune systems work to eradicate the tumor cells. Tumor cells undergo several changes and some cell variants could survive to the elimination phase and enter the equilibrium phase. Finally, tumor cells enter into the escape phase where different mechanisms lead to tumor growth and immune evasion.

system is able to recognize and eliminate cancer cells. In the escape phase, malignancies become clinically relevant because they bypass the immune system's control by suppressing it [6]. In the first phase, the tumor's growth induces the release of cytokines, which activates cells of the innate compartment (NK, γδ-cells and macrophages) and makes them interact with tumor's surface destroying its cells [7]. Dendritic cells (DCs) are called antigen-presenting cells (APCs) because they are able to internalize and process tumor antigens and present them as small fragmented peptides linked to class I and II MHC proteins. Activated DCs migrate to regional lymph nodes where they interact with naïve T-cells; this results in the activation of cancer-specific Th1 (CD4) lymphocytes, which are responsible for strengthening the immune response by the recruitment of CD8+ T-cells activated into cytotoxic T-lymphocytes (CTL). Then cancer-specific Th1 CD4+ and CD8+ lymphocytes migrate into the cancer site and kill its cells [8]. The presence of a lymphocytic infiltrate within the tumor masses is considered in fact the direct evidence of the activity of the immune system against malignant tumors, and in some cases, such as ovarian cancer, this seems to have a correlation with prognosis [9]. In the phase called equilibrium, which can last for years, various tumor cell clones grow and accumulate mutations, thus exposing the immune system to different antigenic phenotypes. These changes determine an alteration of cell death and proliferation in the tumor; the disease is not yet evident in this phase. In the last, so-called escape, stage, cancer cells gain the ability to evade the immune system. Immunotolerance can be induced through various mechanisms such as the activation of a class of T-lymphocytes with immune-suppressive functions (Treg) or tumor's production of inhibiting cytokines such as IL-10 and TGF-β [10]. A downregulation of NK activators such as NKG2D, overexpression of CD46, CD55 and CD59 and a reduced expression of class I MHC proteins are also implicated [11–13]. An altered expression of proteins that normally regulate a Fas-induced apoptosis mediated by NKs or CTLs (i.e., downregulation of Fas receptors and the secretion of Fas ligand) is another possible mechanism involved in immunotolerance to cancer [14,15].

Immunity in NSCLC

NSCLC has been considered a non-immunogenic malignancy for years, and this opinion probably has addressed the choice of research fields [16]. In fact, data from the analysis of NSCLC

tissues indicate that an immune reaction is present but the local immunosuppressive compartment limits its efficacy in controlling the tumor growth [17]. The presence of an intratumoral lymphocytic infiltrate has been demonstrated to have a positive prognostic significance also in NSCLC [18,19], while a high number of Treg cells in the tumor (e.g., squamous NSCLC patients) seem to be correlated with a high risk of recurrence [20]. Various antigens have been found in NSCLC and classified into diverse classes such as cancer/testis antigens (melanoma-associated antigen-A3 [MAGE-A3] and NY-ESO-1) expressed by squamous NSCLC cells and silent in normal tissues [21]. Moreover, gene mutations in cancer cells may produce mutated proteins which are exclusively expressed by tumors and function as tumor-associated antigens (TAAs). In fact, CTLs directed against mutated antigens have been found in NSCLC patients [22,23]. Some differences have been described between squamous and non-squamous NSCLC in terms of immunogenic profiles. Squamous-cell carcinoma expresses a multitude of molecules such as p63/CK5/6/34bE-12, but does not express TTF-1. Adenocarcinoma is instead characterized by a wider molecular heterogeneity [24]. Differences in antigen expression have been shown in several studies. For example, (MAGE)-A3, MAGE-A4 and NY-ESO-1 are more frequently expressed in squamous NSCLC than in non-squamous histology [25,26]. These data constitute the conceptual basis to suppose that manipulation of the immune system may be a viable treatment option for NSCLC.

Immunotherapeutic strategies for cancer

In a systematic classification of the possible strategies to improve the immune response against cancer and NSCLC, we can consider two different strategies: enhancing immune-stimulating components to start or maintain an effective response or inhibiting suppressing factors that block the immune response [27]. We can produce a positive effect on the immune system through two main strategies. The first is the administration of immunoglobulins directed against target-related antigens to induce passive immunization in the host. This kind of immunization is known to confer a rapid protection and is usually administered to immunocompromised patients. The second strategy confers an active and durable immunization through the administration of antigens that activate the host's immune system. In anticancer therapy, active immunization can be obtained by the creation of cancer-specific vaccines that are able to induce the activation of CD4+ and CD8+ T-lymphocytes and secretion of specific immunoglobulins against TAAs. In fact, anti-cancer vaccines can be intended as a class of preparates containing TAAs administered as peptides, recombinant proteins, gangliosides or whole tumor cells, in combination with an adjuvant and able to induce an active antitumor immunity in the host [28]. Another possible way is to modulate the immune system in a non-antigen-mediated way with the use of immunomodulators. For example, the administration of immunostimulating cytokines, such as IL-2 or IFN-α, or the use of inhibitors that block Treg cell functions.

Vaccines for NSCLC

Whole-cell vaccines

Belagenpumatucel-L

Belagenpumatucel-L is an allogeneic whole-cell vaccine produced from four cells lines derived from three different histologic types of NSCLC (one large cell carcinoma, one squamous cell carcinoma and two adenocarcinoma) whose DNA have been modified through the transfection of a plasmid containing the *TGF-β2* antisense transgene, which determines the downregulation of TGF-β2 (Figure 2A). TGF-β is considered as an anti-inflammatory cytokine that can suppress the activity of NK cells, CTLs and APC cells, determining a blockade of the anticancer activity of the immune system. TGF-β also works by inducing FOXP3, thus converting CD4+ T-lymphocytes cells into Treg cells with immunosuppressive activity. Moreover, a high TGF-β2 level seems to be associated with a poorer prognosis in NSCLC patients [29]. Belagenpumatucel-L has been studied by Nemunaitis *et al.*, in a Phase II dose variation trial in which 75 NSCLC patients with stage II, IIIA, IIIB and IV disease were randomized to receive 1.25, 2.5 or 5.0×10^7 cells/injection of the investigational drug administered in a monthly or every other month schedule for up to a total of 16 doses [29]. A total of 81% of the patients enrolled had non-resectable or metastatic disease and 15% of the assessable cases showed a partial response to belagenpumatucel-L. An important finding of the trial was the survival rate, which seems to be dose-related; those patients who received the dosage of $\geq 2.5 \times 10^7$ cells/injection showed an improvement in overall survival (OS) rate of 47%, while for those receiving 1.25×10^7 cells/injection, the survival rate was 18%. A subgroup analysis of this study performed by Fakhrai H *et al.* demonstrated a correlation between the presence of combined cellular and humoral immune responses and an improvement in OS in patients treated with belagenpumatucel-L. Patients with both types of responses, defined immune-response positive (n = 11), obtained in fact an advantage in terms of median OS compared to those patients (n = 24) who were defined as immune response-negative (32.5m 95% CI: 25.2–39.8 vs 11.6m 95% CI: 5.6–17.6; p = 0.011). This suggested that the activity of both immune system compartments is critical for the induction of a clinically significant antitumor immunity by this vaccine. Only one grade 3 toxicity has been registered, and the drug had a good safety profile [30]. Based on these promising data, the Phase III placebo-controlled, randomized, multinational STOP trial has evaluated the treatment with belagenpumatucel-L as maintenance therapy for IIIA, IIIB and IV NSCLC patients who did not progress after frontline chemotherapy and randomized between 4 and 17.4 weeks from the end of chemotherapy. A total of 532 eligible patients were enrolled (42 stage IIIA and 490 stage IIIB/IV) and randomized to receive 18 monthly and two quarterly intradermal injections of belagenpumatucel-L or placebo until disease progression or withdrawal. The two arms were balanced for age, disease stage and type; 57% of the patients had adenocarcinoma, 27% squamous carcinoma and 6% large-cell carcinoma. The primary

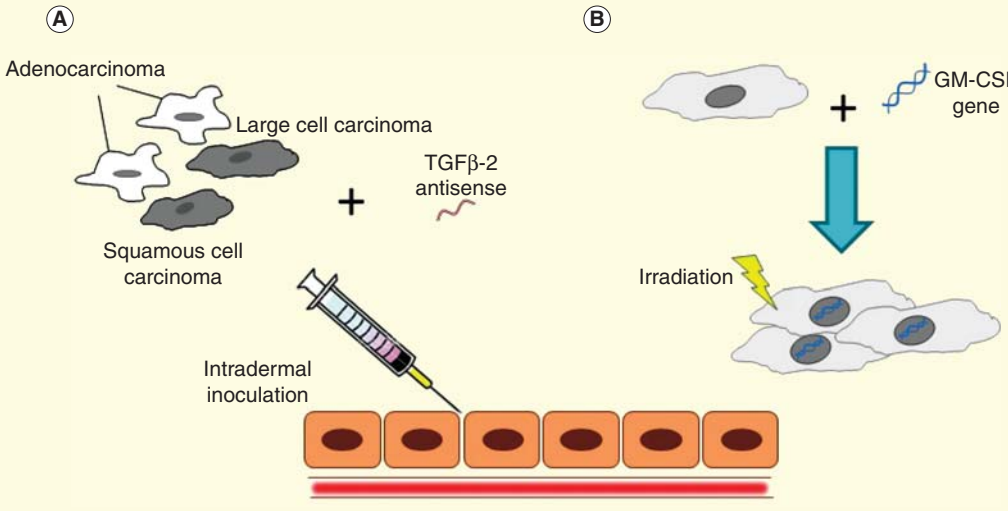

Figure 2. Whole-cell vaccines are composed of manipulated tumor cells that induce the immune system to react against tumor. (A) Belagenpumatucel-L is produced with four cells lines representing three different histologic types of NSCLC (large cell carcinoma, squamous cell carcinoma and adenocarcinoma) transfected with a plasmid containing TGF-β2 antisense transgene. The downregulation of TGF-β, considered as an anti-inflammatory cytokine, determines the activation of NK cells, CTLs and APC and CD4+ T-cells **(B)** GVAX is a gene therapy vaccine. *GM-CSF* gene is transfected in tumor cells that are inoculated in the patient after lethal irradiation. Secretion of GM-CSF by genetically modified tumor cells stimulates cytokine release at the vaccine site to activate antigen-presenting cells, which prime CD4+ and CD8+ T-cells to recognize circulating tumor-associated antigens.
APC: Antigen-presenting cells; CTL: Cytotoxic T-lymphocytes; GM-CSF: Granulocyte-macrophage colony-stimulating factor; NSCLC: Non-small cell lung cancer.

endpoint was OS. Recently, in the European Cancer Congress 2013 Presidential Session I, G. Giaccone presented the data of this study, which did not meet its primary endpoint with a median OS of 20.7 months for the belagenpumatucel-L arm compared to 13.4 months in the control arm (hazard ratio [HR]: 0.75; p = 0.083). Patients treated in the belagenpumatucel-L arm within 12 weeks of chemotherapy completion showed a non-significant improvement in median OS compared to those treated with placebo (20.7 vs 13.4months; HR: 0.75; p = 0.083). A relevant and statistically significant improvement in OS was observed in stage IIIB/IV non-adenocarcinoma patients (n = 99) who started belagenpumatucel-L within 12 weeks of the completion of frontline chemotherapy (9.9 vs 12.3m; HR: 0.55; p = 0.036). Moreover, radiotherapy pretreated patients, treated with the investigational vaccine within 12 weeks of chemotherapy completion, showed an advantage of 29.8 months in OS (40.1 vs 10.3months; HR: 0.45; p = 0.014). Belagenpumatucel-L showed a good safety profile in the study with only one serious adverse event (grade 2 allergic rash). Although the study did not meet its primary endpoint, it cannot be considered negative at all, because the analysis carried out by the investigators identified subgroups of patients who achieved a benefit in terms of OS [31]. These data, together with a good safety profile, support the development and the future approval of belagenpumatucel-L with appropriate patient selection.

GVAX

GVAX is a gene therapy vaccine that stimulates natural defenses against tumor cells. The vaccine employs *ex vivo* transfection of autologous or allogenic tumor cells with the *GM-CSF* gene, followed by lethal irradiation before inoculation into the patient (Figure 2B). The secretion of GM-CSF by genetically modified tumor cells stimulates cytokine release at the vaccine site to activate APCs, which prime CD4+ and CD8+ T-cells to recognize circulating TAAs, thereby inducing a tumor-specific cellular immune response [32]. It has been studied for various malignancies such as prostate cancer, melanoma, kidney cancer and NSCLC. Data on the use of this vaccine in prostate cancer are not promising and the negative results, together with a high toxicity, shown in two Phase III clinical trials limited further applications for this type of cancer [33,34]. Despite these non-promising data, GVAX has also been evaluated in NSCLC patients. A first Phase I study in which 34 patients with stage IIB-IV NSCLC were treated with an autologous vaccine showed disease stabilization in five patients, one mixed response and in two patients who had undergone surgery, there was no evidence of disease at 43 and 42 months post-operatively [35]. Subsequently, a second Phase I/II trial by Nemunaitis *et al.* has been conducted with GVAX produced with autologous NSCLC cells. A total of 83 patients, 20 with early-stage NSCLC and 63 with advanced-stage NSCLC were enrolled; however, the vaccine was

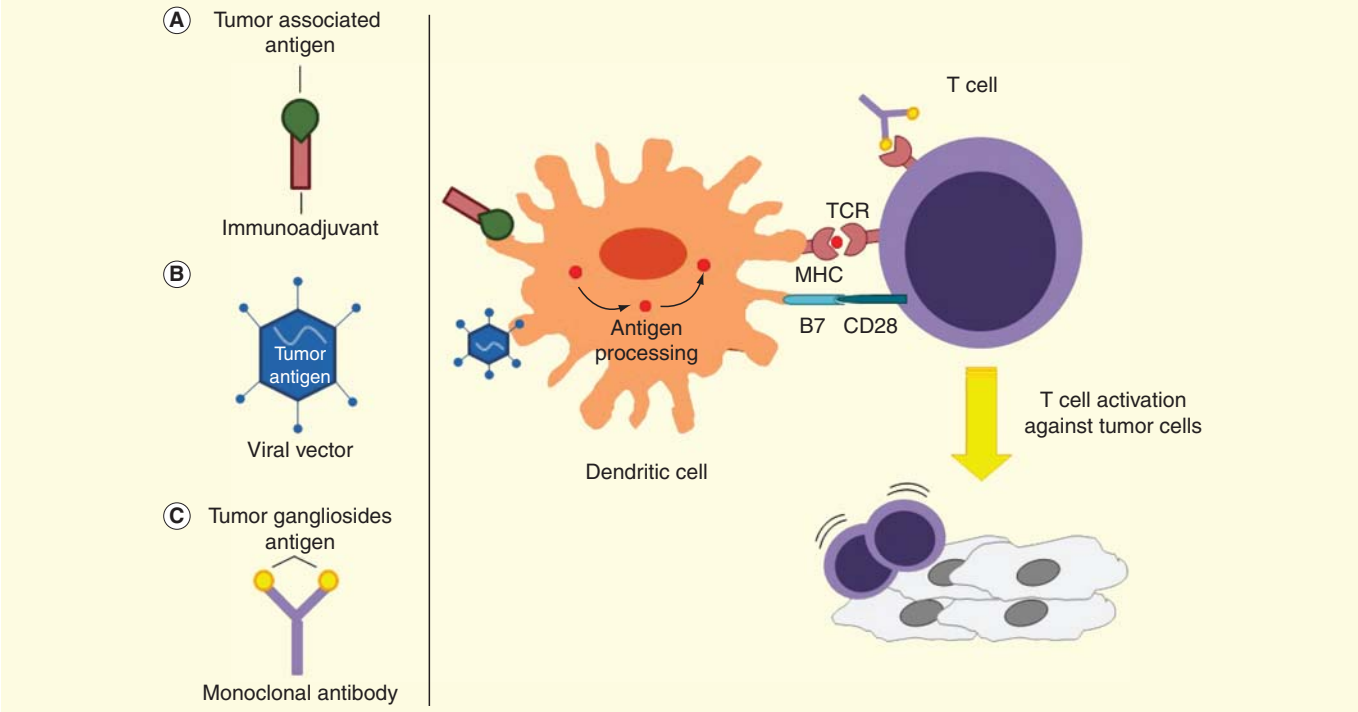

Figure 3. Another approach to induce immune response against cancer cells is to increase the immunogenicity of tumor-associated antigen. (A) Protein- or peptide-based vaccine (as melanoma-associated antigen-A3, Tecemotide and CIMAVax) are recombinant proteins obtained by the fusion of tumor associated antigens with an immunoadjuvant that is usually the immunogenic component of a virus or a bacterium. **(B)** TG4010 is a viral vector vaccine composed of a modified Ankara virus that expresses MUC-1 antigen and IL-2 gene. **(C)** Racotumomab is an anti-NeuGc-containing gangliosides anti-idiotype monoclonal antibody that induces a strong specific cellular and humoral response against NeuGc. Dendritic cells are responsible for the antigen processing and the presentation to T-cells through the interaction between MHC and TCR. In order to achieve T-cell activation, this first signal has to be followed by a second co-stimulatory signal mediated by B7 and CD28 interaction.
TCR: T-cell receptor.

successfully created and administered to only 43 patients (33 with advanced stage and 10 with early stage). The most frequent toxic event was skin reaction at the site of injection. Three of the 33 patients with advanced disease had a complete and durable tumor response and two of them maintained this response for more than 5 years [36]. These data, although arising from limited experiences on small number of patients, have been considered promising. Nevertheless, the negative studies previously conducted on prostate cancer inhibited the research and development of other studies using GVAX.

Peptide & protein vaccines

MAGE-A3/vaccine

MAGE-A3 is a TAA that represents a promising future target for cancer immunotherapy because it is not normally expressed in normal human cells except in male germline cells in which this antigen is not normally presented because of the lack of MHC class I molecules [37]. MAGE-A3 expression has been demonstrated in almost 35% of NSCLCs, with a trend of increase from early (30%) to late stages (55%) [38]. Moreover, although its biological role is not clear, a greater expression of MAGE-A3 in advanced NSCLC patients also correlates with a worse prognosis [39]. MAGE-A3 vaccine is composed of a recombinant protein obtained by the fusion of MAGE-A3 antigen and part of the protein D of *Haemophilus influenzae*, packaged with lipid adjuvants (Figure 3A). This vaccine has been evaluated by Vansteenkiste J. *et al.* in a multicenter, double-blind, randomized, placebo-controlled Phase II trial in which 182 MAGE-A3+ patients with stage I and II NSCLC were randomized to receive MAGE-A3 protein plus adjuvant or placebo after complete surgical resection. Patients were treated with an induction of five doses administered every 3 weeks, and then with eight doses every 3 months as maintenance therapy. After a 28-month follow-up, for patients in the experimental arm, there was a non-significant but clinically relevant improvement in the disease-free interval, disease-free survival and OS with HRs of 0.75 (95% CI: 0.46–1.23; p = 0.254), 0.76 (95% CI: 0.48–1.21; p = 0.248) and 0.81 (95% CI: 0.47–1.40; p = 0.454). Treatment showed a good tolerability profile that ensured a valid compliance of the patients in the study [40]. Despite the small number of patients in the study and the limited results, based on this data, a Phase III study (MAGRIT trial) was initiated in 2007. This double-blinded, placebo-controlled study recruited 2278 MAGE-A3-positive stage IB, II and IIIA NSCLC patients who were randomized to receive either MAGE-A3 vaccine as adjuvant therapy or placebo after complete surgical resection [41]. The

experimental treatment was composed of 13 injections in a 27-month period after the standard of care (surgery or surgery plus adjuvant chemotherapy). Primary endpoint of the trial was disease-free survival and secondary endpoints were OS, lung-cancer-specific survival, disease-free survival at 2-, 3-, 4- and 5-year, disease-free specific survival, anti-MAGE-A3 and anti-protein D seropositivity rate as well as adverse events occurrence. Patient accrual of the largest NSCLC clinical trial ever has been completed in 2011, but although expectations about its results with their potential therapeutic implications were eagerly awaited, through a recent press release, GlaxoSmithKline announced its decision to stop the trial because it did not meet its first and co-primary endpoints. Final results and publications are still not available.

MUC1 & L-BLP25 (tecemotide)

MUC1 is a protein normally produced by many epithelial cells and expressed on their apical surfaces and is overexpressed in an hyperglycosilated form by various cancer cells [42]. This type of tumor-associated MUC1 is immunologically distinct from the one produced by normal cells and its typical hyperglycosylation exposes the antigenic core of the protein to the action of the immune-competent elements [43]. It seems to be involved in tumor cell migration and resistance to chemotherapy-related apoptosis and is partially responsible for immune system suppression through CTL inhibition. Moreover, high levels of this protein have been found to be related to a poor prognosis in cancer patients [44]. On the other hand, a higher level of MUC1 natural antibodies has been found to be related to a better prognosis in NSCLC patients [45], such as in pancreatic adenocarcinoma [46] and in patients with early-stage breast cancer [47] L-BLP25 (tecemotide) is a peptide vaccine composed of the immunogenic portion of MUC1 protein-linked to a lipidic adjuvant (monophosphoryl lipid A) and three lipids (cholesterol, dimyristoyl phosphatidylglycerol and dipalmitoyl phosphatidylcholine) [48]. This vaccine targets the core of MUC1 when hyperglycosylated and elicits an immune response with the subsequent production of IFN-γ and tumor-specific CTL proliferation. This promising drug has been evaluated in 2007 by Butts *et al.* in a Phase IIB trial in which 171 stage IIIB/IV NSCLC patients were randomized to receive best supportive care (BSC) plus L-BLP25 (n = 88) or BSC alone (n = 83) after response or stable disease to first-line chemotherapy [49]. Patients in the L-BLP25 arm received a single intravenous dose of cyclophosphamide 300 mg/m^2 before the vaccine, followed by administration of the vaccine (1000 mcg) at 8-week intervals. Subsequent maintenance administrations could be given at 6-week intervals until disease progression. The primary endpoint of the study was median OS which resulted in 17.4 months for L-BLP25 patients versus 13.0 months for the control arm (adjusted HR: 0.739; 95% CI: 0.509–1.073; p = 0.112). Despite the lack of statistical significance of this advantage, a subgroup analysis of the 35 stage IIIB patients with loco-regional disease treated with L-BLP25 plus BSC showed a 17.3-month difference in median OS (30.6 vs 13.3 months, HR: 0.524; 95% CI: 0.261–1.052; p = 0.069) over the BSC group. The safety profile was good with only few adverse events like mild flu-like symptoms and injection site reactions [50]. Although these results were limited and non-statistically significant, the START trial, a multicenter Phase III randomized, double-blind placebo-controlled study was initiated in 2007. In this large trial, 1239 patients with stage IIIA/B unresectable NSCLC without progression following chemoradiotherapy were randomized to receive weekly administrations of L-BLP25 (tecemotide) 806 μg for 8 weeks then every 6 weeks thereafter plus BSC or placebo plus BSC; both arms continued until progression. Although there was a trend toward improved OS (the primary endpoint of the trial) with the vaccine, difference from placebo did not reach statistical significance; OS was 25.6 months in the L-BLP25 arm versus 22.3 months in the placebo group (adjusted HR: 0.88; 95% CI: 0.75–1.03; p = 0.123). The subgroup analysis of patients who received previous concurrent chemoradiotherapy (n = 538) showed a significant benefit of 10.2 months in OS with tecemotide (HR: 0.78; p = 0.016). This advantage was absent in the group of those who received tecemotide after sequential chemoradiotherapy (HR: 1.11; p = 0.38). Tecemotide has once again been demonstrated to be a very well-tolerated treatment and a possible valuable weapon for some patients on maintenance therapy after chemoradiotherapy [51]. Based on these data, another Phase III study of tecemotide has just been initiated – the START-2 trial: a randomized study of patients with unresectable stage III NSCLC who had either stable disease or objective response following primary concurrent chemoradiotherapy treated with L-BLP25 or placebo [52]. The INSPIRE trial is another similar Phase III trial with tecemotide for Asian patients with stage III unresectable NSCLC and is still recruiting patients [53].

EGF vaccine: CIMAVax

EGFR and its ligand EGF are considered key elements in many malignancies. Targeting EGFR pathway when mutated with TKIs is today a very well-known treatment modality in NSCLC [54]. Moreover, EGFR is overexpressed in a very high proportion of NSCLCs [55]. CIMAVax vaccine is composed by human recombinant EGF linked to a carrier protein, P64K from Neisseria meningitides, and administered with an adjuvant. The use of this vaccine induces the production of anti-EGF antibodies whose activity reduces the activation of EGFR and thereby inhibits tumor cell proliferation [56]. In a Phase II study, 80 patients with stage IIIB or IV NSCLC previously treated with first-line chemotherapy were enrolled. In this small trial conducted by Neninger *et al.*, patients were randomized to receive either vaccination plus BSC or BSC alone. The investigational vaccine was administered on days 1, 7, 14, 28 and monthly thereafter after administration of a priming dose of cyclophosphamide (200 mg/m^2) [57]. The measurement of EGF in patients' serum showed reduced EGF concentrations in those in the experimental arm, which indirectly confirmed that CIMAVax induced production of anti-EGF antibodies. Because of the small number of patients enrolled, the results of the

study showed a non-statistically significant survival benefit for patients treated with the vaccine (6.5 vs 5.3 months; p = 0.098), but the improvement in median OS reached significance in the subgroup of patients younger than 60 years (11.5 vs 5.3 months p = 0.0124). In addition, a statistically significant improvement in survival (11.7 vs 3.6 months; p = 0.002) was also seen for patients with EGF antibody titers ≥ 1:4000. No critical side effects occurred in this trial [57]. A larger Phase II/III (Malaysian trial) study was started but interrupted in 2011 due to poor enrollment [58].

Viral vector vaccines: TG4010

TG4010 is an anti-cancer vaccine composed by an attenuated Ankara virus, genetically modified to express MUC1 antigen and IL-2 [59]. The latter has been introduced to bypass the MUC1-related T-cell suppression (Figure 3B) [44]. This vaccine has been studied in a Phase II trial in which 65 patients with stage IIIB/IV NSCLC were randomized to receive either TG4010 in addition to cisplatin–vinorelbine chemotherapy or the vaccine alone (continued until progression) [60]. Those patients who progressed in the control arm were treated with a cross-over to the experimental arm, with the same chemotherapy regimen. The median OS was 12.7 months for the chemotherapy plus vaccine arm (Arm 1) and 14.9 for the vaccine only arm (Arm 2). One-year survival rate was 53% for Arm 1 and 60% for Arm 2; in the latter, two patients experienced stable disease for more than 6 months. A subsequent Phase IIb trial evaluated this vaccine in a larger number of patients in combination with cisplatin and gemcitabine. In this trial, 148 untreated patients diagnosed with stage IIIB or IV MUC1-positive NSCLC were randomized to receive cisplatin and gemcitabine, with or without TG4010. In the experimental arm, the vaccine was administered with subcutaneous injections each week for 6 weeks and then every 3 weeks until disease progression. Primary endpoint was 6-month PFS, with a target rate of 40% or higher in the experimental group. The results of the trial showed that the addition of TG4010 to chemotherapy confers a clinically relevant advantage in terms of PFS to the patients. In fact, PFS rate at 6 months was 43% (95% CI: 33–54) in the experimental arm and 35% (95% CI: 26–45) in the chemotherapy alone arm (p = 0.13), while no difference in OS was demonstrated. Overall response rates (ORRs) were 43% in patients receiving the vaccine versus 27% in patients receiving chemotherapy only. In addition, the subgroup analysis of patients in the chemotherapy plus TG4010 group (with normal levels of activated NK cells) showed an improvement in response rate and OS (58 vs 38%; p = 0.04 and 18 vs 11.3 months; p = 0.02, respectively) [61]. Based on these data, a Phase IIB/III randomized, double-blind, placebo-controlled trial started to evaluate this effect in a larger population and trying to answer the question if the addition of TG4010 to standard first-line chemotherapy confers an advantage to MUC1-positive stage IV NSCLC patients. Primary endpoint is OS and the investigators will try to prospectively validate activated NK level as a predictive biomarker with PFS as a primary endpoint by comparing the two treatment arms in two subgroups defined according to the level of aNK cells at baseline (normal or high) [62]. This trial is still active and recruiting patients [63].

Ganglioside vaccines: racotumomab

Gangliosides are part of the glycosphingolipids family present on the cell surface membrane, enabling cell communication, cell matrix adhesion, cell differentiation and regulation of the immune response and are expressed on the surface of tumor cells [64]. *N*-glycolylneuraminic acid (NeuGc) is a sialic acid which has been found, conjugated with GM3 ganglioside (NeuGcGM3) expressed by NSCLC cells and considered a possible target for immunotherapy (Figure 3C) [65]. Racotumomab (1E10) is an anti-NeuGc-containing ganglioside anti-idiotype monoclonal antibody (mAb). Preclinical data suggest a promising activity of this vaccine [66] also in combination with chemotherapy [67]. In a compassionate study published in 2007 by Alfonso *et al.*, 34 patients with stage IIIB and 37 stage IV NSCLC patients were treated with the anti-idiotype vaccine after they received standard chemotherapy and radiotherapy. Patients received 5 biweekly administration of the vaccine followed by monthly maintenance administration. The OS was 9.93 months (95% CI: 8.61–11.25) with a 1-year survival rate of 34%. Those patients who had ECOG performance status 1 (PS1) and started the experimental treatment after a PR or stabilization with first-line treatments had a more consistent benefit in OS (11.50 months; 95% CI: 7.97–15.03) and a 1-year survival of 39%. Toxicity profile was good, and the most common side effects were local reaction at the injection site and fever [68]. Currently, a prospective, randomized, open-label, parallel-group, multicenter Phase III study is still recruiting patients. This study will evaluate racotumomab for NSCLC patients with advanced disease who have achieved an objective response or stable disease with standard first-line treatment [69]. In the 2013 ASCO annual meeting, Gomez *et al.* presented the data of an open, non-randomized study to evaluate if racotumomab could be of benefit to patients with NSCLC in progression. A total of 180 patients were included, and after at least 10 months of follow-up, median survival was 8.06 months and OS rate at 24 months was 21%. A control group of 85 patients who did not receive second-line therapy or racotumomab showed a median survival of 6.26 months (p = 0.011) and OS rate at 24 months was only 7%.

Immunomodulators & NSCLC

As previously explained, a possible strategy to stimulate an immune response against cancer is to modulate the functions of the principal actors of the immune system. Obviously, the introduction of cancer-specific antigens leads the immune system to react against precise targets, while immunomodulation causes the enhancement of the existing mechanisms with a wider spectrum of consequences including the aggression of cancer cells but also autoimmunitary events. The enhancement of the immune system can be obtained either with the introduction of immune-stimulating factors such as DCs,

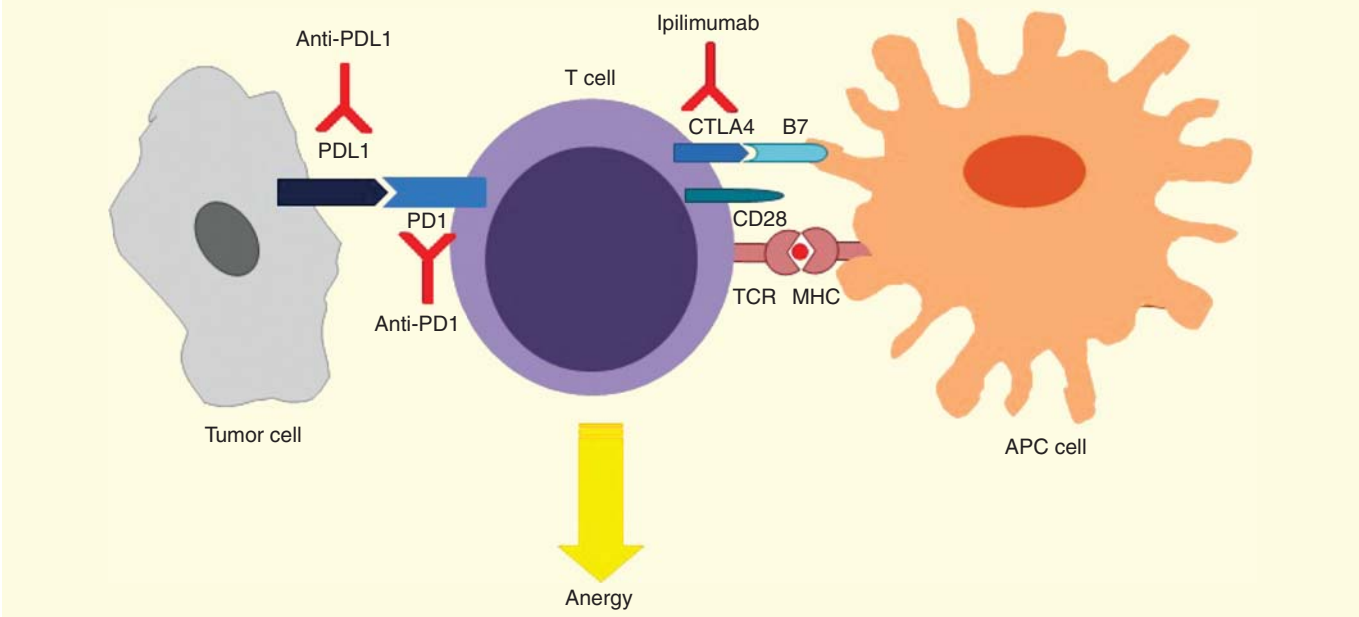

Figure 4. Immune system is able to inhibit self-reaction by means of several inhibitory receptors. Some examples of these receptors are CTLA-4 and PD-1. When CTLA-4 interacts with B7 T-cells are not appropriately stimulated and enter an anergic state. Ipilimumab (on the right) is able to block this mechanism, thereby determining T-cell activation. PD-1 receptor elicits T-cell inactivation by interacting with its ligand PDL-1. Tumor cells are able to overexpress PDL-1 enhancing the immune escape. Therefore, anti-PD1 and anti-PDL1 drugs exert their action by decoying receptor and ligand, respectively.
APC: Antigen-presenting cells; TCR: T-cell receptor.

injection of CTLs or activated NK cells, IL-2 or IFN-α or by the inhibition of the immune-inhibiting elements responsible for tolerance to cancer (e.g., inhibition of Treg cells and blocking inhibiting cytokines) [27]. Historically, but not clinically, relevant are the first attempts at using immunomodulators for lung cancer such as the administration of BCG [70] or mycobacterium-derived vaccine (SRL172) [71] alone or in association with chemotherapy. Negative data emerged also from clinical trials with PF-3512676, a synthetic TLR9-activating oligodeoxynucleotide, which works as the natural ligand of TLR9 which enhances maturation of DCs [72,73]. Also, lactoferrin, which is an iron-binding glycoprotein found in breast milk, has shown several immunomodulatory functions [74]. Talactoferrin-α is the recombinant human variant of lactoferrin, purified from *Aspergillus niger var. awamori*, administered orally and transported to the gut-associated lymphoid tissue where it induces DC maturation [75]. Based on positive data from Phase II trials [76,77], two Phase III studies tried to evaluate the activity of talactoferrin-α in patients with NSCLC. In the first one, the FORTIS-M trial, talactoferrin-α was administered to patients with refractory advanced NSCLC in comparison with placebo, and the results showed no improvement in efficacy with this drug [78]. The second trial (FORTIS-C trial) studied talactoferrin-α in association with chemotherapy as a first-line treatment in advanced or metastatic NSCLC. However, due to negative results of the FORTIS-M trial, Agennix AG decided to stop enrollment and analyze the results.

A promising immunotherapy approach for NSCLC is the blockade of inhibitory 'checkpoint pathways', and the following paragraphs focus on the possible therapeutic targets and on the clinical implications of this type of approach (Figure 4).

CTLA-4 inhibitor: ipilimumab in lung cancer

CTLA-4 is an immunomodulatory receptor expressed by activated CD4+ and CD8+ T-lymphocytes that works as a negative regulator of T-cell-mediated immune response. Ipilimumab is a fully human IgG1 mAb directed against CTLA-4 whose activity prevents the interaction between CTLA-4 and its ligands (CD80/CD86), thus producing a blockade of the inhibitory signal provided by CTLA-4 and enhancing the activation and proliferation of tumor-specific T-cells [79,80]. Based on the results of two randomized Phase III trial that showed an improvement in OS for ipilimumab compared with vaccine alone or standard dacarbazine chemotherapy, this mAb has been approved for treatment of metastatic melanoma, representing a real turning point for patients with such an aggressive type of cancer [81,82]. Ipilimumab has been evaluated in a Phase II trial for advanced NSCLC and SCLC patients who were randomized to receive as first-line therapy paclitaxel plus carboplatin with either placebo or ipilimumab administered concurrently (four doses of ipilimumab plus paclitaxel and carboplatin followed by two doses of placebo plus paclitaxel and carboplatin) or as a phased regimen (two doses of placebo plus paclitaxel and carboplatin followed by four doses of ipilimumab plus paclitaxel and carboplatin). Treatment was administered

intravenously every 3 weeks and not over 18 weeks (induction). Patients with no progression continued ipilimumab or placebo every 12 weeks as maintenance therapy until progression, death or intolerance. Results for NSCLC and SCLC were published separately. The trial met its primary endpoint, immune-related PFS (irPFS), for NSCLC patients (n = 204) but only for those treated with phased ipilimumab (5.7 vs 4.6 months; HR: 0.72; p = 0.05), while in the concurrent ipilimumab group, no significant improvement was demonstrated (5.5 vs 4.6 months; HR: 0.81; p = 0.13). In the three groups of patients, phased, concurrent ipilimumab and control, median OS was 12.2, 9.7 and 8.3 months, respectively. Two patients died from treatment-related toxicity and severe adverse events seemed to be more frequent in the concurrent group. In the phased ipilimumab group, patients with squamous cell carcinomas had a more significant improvement in irPFS (HR: 0.55) than patients with adenocarcinoma (HR: 0.82) and similar results were found in the concurrent arm [83]. Data for patients with advanced SCLC showed that phased ipilimumab, but not concurrent ipilimumab, improved irPFS versus control (HR: 0.64; p = 0.03), while no improvement in PFS (HR: 0.93; p = 0.37) or OS (HR: 0.75; p = 0.13) was obtained. In the phased, concurrent and control arms, median irPFS was 6.4, 5.7 and 5.3 months and median OS was 12.9, 9.1 and 9.9 months, respectively. Overall rates of grade 3–4 adverse events were 17, 21 and 9% for phased ipilimumab, concurrent ipilimumab and control, respectively [84]. These promising data led to two open Phase III trials to evaluate ipilimumab immunotherapy for lung cancer. The first study [85], presented at the 2013 ASCO meeting, is a randomized, multicenter, double-blind Phase III study comparing ipilimumab in combination with paclitaxel and carboplatin versus placebo in addition to paclitaxel and carboplatin for stage IV/recurrent NSCLC patients with squamous histology with OS as primary endpoint [86]. The second study [87], presented at the same congress, will evaluate the efficacy of the addition of ipilimumab to etoposide and platinum chemotherapy for patients with extensive-stage disease SCLC in comparison with etoposide and platinum therapy alone [88]. These two trials are still recruiting patients and obviously the expectations are great about the possible clinical implications of their results.

Anti-PD-1 antibodies

Programmed cell death protein 1 (PD-1) belongs to the B7-CD28 superfamily and works as a receptor, which can be expressed on T-lymphocytes, B cells, NK cells, activated monocytes and DCs. It normally binds two ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC) [89]. The interaction between PD-1 and its ligands results in diminished T-cell proliferation, altered cytokine production and initiation of T-cell exhaustion and/or apoptosis. These functions explain the immunosuppressive role of PD-1, which limits the risk of autoimmune reactions but also decreases the anti-tumor activity of CD8+ lymphocytes [90]. Blockage of this immune checkpoint is considered one of the most promising field of study to enhance the immune response against cancer cells, and several anti-PD-1 agents are in development: two of them are nivolumab and lambrolizumab (MK-3475). Nivolumab (BMS-936558 formerly MDX-1106/ONO-4538) is a fully human IgG4 mAb directed against the PD-1 receptor, which showed a promising spectrum of activity in a Phase I trial in 296 patients with refractory solid tumors including 129 patients with NSCLC [91]. Patients were treated with five escalation doses from 0.1 to 10.0 mg/kg every 2 weeks for up to twelve 8-week cycles. It has been recently shown that, of the 129 pretreated NSCLC patients (non-squamous n = 74, squamous n = 54, unknown histology n = 1) who received nivolumab 1, 3 and 10 mg/kg IV every 2 weeks, 22 patients (17%) had responses (CR/PR) with a median duration of 74 weeks (range 6.1+ to 133.9+ weeks). The highest ORR was at 3 mg/kg (24%) across the various histologies. Median OS across all doses was 9.6 months and 14.9 months at 3 mg/kg across all histologies. Median OS across all doses was similar for squamous (9.2 months) and non-squamous patients (10.1 months), while at the 3 mg/kg dose, a difference in median OS (9.5 months for squamous NSCLC; 18.2 months for non-squamous) was reported. Moreover, the 1-year survival rate was 43% for non-squamous NSCLC patients and 39% for squamous NSCLC patients across all doses. About the toxicity of nivolumab, any grade adverse events was seen in 53/129 patients (41%) while grade 3/4 events occurred only in 6/129 patients (5%) [92]. Data of activity and tolerability of nivolumab in strongly pretreated NSCLC patients led to the start of two Phase III trials which are evaluating nivolumab as second-line monotherapy versus docetaxel both in squamous (OS and ORR as primary endpoint) [93] and in non-squamous (OS primary endpoint) NSCLC [94]. These studies are still ongoing. Another Phase I trial is testing nivolumab in combination with three platinum-based doublet chemotherapy regimens (cisplatin/gemcitabine; cisplatin/pemetrexed and carboplatin/paclitaxel), with targeted drugs (bevacizumab and erlotinib), with ipilimumab in monotherapy and in maintenance in patients with advanced NSCLC [95]. Nivolumab is being evaluated also as third-line therapy in a Phase II trial with ORR as primary endpoint in patients with advanced or metastatic squamous NSCLC after failure of two prior systemic therapies [96]. Another Phase II trial is also evaluating the epigenetic priming with azacitidine and entinostat or oral azacitidine alone prior to nivolumab in recurrent metastatic NSCLC patients [97]. Lambrolizumab (MK-3475) is another anti PD-1 humanized IgG4 mAb which has been defined by the US FDA as 'breakthrough therapy' for the durable results obtained in the treatment of patients with advanced melanoma [98]. Preliminary data of MK-3475 in pretreated NSCLC patients have been presented at the 15th World Conference on Lung Cancer in Sidney, Australia. In this study, lambrolizumab was administered at a dose of 10 mg/kg every 3 weeks in 38 treatment-refractory patients with NSCLC: ORR was 24% and most of the responses were observed by the time of first planned assessment at week 9 [99]. The drug is being studied in comparison with both docetaxel in NSCLC patients after failure of a platinum-based regimen [100] and with chemotherapy or immunotherapy [101].

Anti-PD-L1 antibodies

Another way to block PD1-immune checkpoint is to reduce the activity of its ligand, and for this reason, mAbs directed against PD-L1 are in development. PD-L1 is a transmembrane protein expressed by various elements of the immune system and its expression in solid tumors seems to be associated with tumor grade, squamous histology, immune cell density and co-localization of PD-L1+ immune cells [102]. Data from preliminary studies suggest that the expression of PD-L1 in human cancers evaluated with immunohistochemistry in formalin-fixed paraffin embedded tissue samples could be a prediction test for response to anti-PD-1/PD-L1 therapy [103,104]. In a recent study by Velcheti *et al.*, antibodies and *in situ* mRNA hybridization were used to evaluate PD-L1 expression in NSCLC using quantitative fluorescence to determine the frequency of expression and prognostic value in two cohorts of patients from Greece (n = 340) and USA (n = 204). PD-L1 expression was 36% in patients from Greece and 25% in patients from the USA and it was related to the presence of a consistent lymphocyte infiltrate in tumors. Surprisingly, patients with protein and mRNA PD-L1 expression showed statistically better outcome in both groups, independent of the histology. These data are in contrast with previous studies which reported that the expression of PD-L1 protein in various malignancies, including in NSCLC, was associated with worse outcome [105–108]. BMS-936559 (MDX-1105) is a fully human IgG4 anti-PD-L1 mAb that blocks the binding of PD-L1 to both PD-1 and CD80. It has been evaluated in a Phase I trial for patients with various types of cancers including 75 NSCLC patients. Five of the 49 assessable NSCLC patients showed response with a range of duration of 1.6–2.3 months. Toxicity profile was acceptable with only 9% of grade 3/4 drug-related adverse events [104]. MPDL3280A is another anti PD-L1 mAb in development whose modified Fc domain seems to maximize the therapeutic effects by minimizing the antibody-dependent cellular cytotoxicity-mediated depletion of activated T-lymphocytes. This drug has been studied in a dose escalation Phase I trial in which 175 patients with solid tumors have been treated. Preliminary data for the 41 evaluable NSCLC patients first dosed at 1–20 mg/kg showed an ORR of 22% (9/41), and responses lasted from a range of 1+ to 214+ days. Curiously, the ORR was 23% (8/35) for former/current smokers versus 17% (1/6) for non-smokers, and it seems also to be associated with PD-L1 tumor status [109]. A Phase Ib trial will assess intravenous MPDL3280A in combination with erlotinib administered to patients with locally advanced or metastatic NSCLC [110]. MPDL3280A is also being evaluated in three Phase II trials: in two of them as monotherapy for patients with PD-L1-positive locally advanced or metastatic NSCLC [111,112] and in the third one in comparison with docetaxel for platinum refractory patients [113]. Moreover, a Phase III multicenter, open-label, randomized, controlled study has started to evaluate MPDL3280A in comparison with docetaxel in patients with locally advanced or metastatic NSCLC after failure with platinum-containing chemotherapy.

Expert commentary

It could be a long time before we see any hope of immunotherapy finally turning into an option for lung cancer therapy. Lung cancer tumors always had the reputation of not being very immunogenic, and the fact that we have not really discovered the antigen(s) that are key to generate an adequate immune response have made things worse. We have tried whole-cell vaccines over the years, we have tried several specific antigens and have done autologous vaccinations and allogeneic ones. Finally, in the last 3 years, information from the first four randomized Phase III clinical trials of promissory lung cancer vaccines have started coming in; unfortunately, most of them are unable to prove improvements in survival, but in some cases like BLP-25 (former stimuvax) the final story is not written yet and we will have a second chance with the new prospective randomized trial for concurrent chemoradiation patients. The new hope arrived with the checkpoint inhibitors that are new options for immunotherapy, and after the success of antiCTL4 in other tumors, there is a lot of expectation to see if this agent gets approval for lung cancer. Finally, the discovery of PD-1/PDL-1 as potential targets in lung cancer brings another option to the table, the original data the authors have review here is solid and very promissory.

Also, we cannot forget that, in general, immunotherapy has proven to be less toxic than chemotherapy giving even incurable patients a much better QoL, so the goal is not only to try to cure this terrible disease but also to prolong survival with excellent and decent QoL, making lung cancer a chronic disease as first step toward the cure.

After being for many years the God that was forgotten in the garden of the lords, the authors hope that immunotherapy will become a major divinity.

Five-year view

Perhaps, few years ago, it was still thought that immunotherapy would not impact lung cancer survival. Immunotherapy has had ups and downs in lung cancer treatment roadmap. Sometimes showing provocative and intriguing results, but without showing an improved OS in a large Phase III randomized, placebo-controlled trial. Nonetheless, the authors believe that soon this paradigm will change. The advances in bio-immunology have taught us how to target those intrinsic immune mechanisms which are shut down by tumor cancer cells. Novel agents such as anti-PD1 and PD-L1 antibodies are shaking all prior experiences that we had had in the past trying to elicit immune response against cancer cells using vaccines with different kind of antigens, adjuvants (carrier) and procedures.

As we move toward the concept of 'personalized' or individualized therapy by trying to minimize toxicity for the patient and attain maximum response which translates into survival advantage, perhaps the effective way to improve the immune response against NSCLC would be by inhibiting suppressing factors that block the immune response. These novel agents such as nivolumab, lambrolizumab, MPDL3280A and others are reporting good responses in advanced NSCLC. Due to their toxicity profile, it seems that we can combine them with chemotherapy and perhaps move

them into early stages. This is a new field and we have to wait for ongoing clinical trials to mature and report their results. One of the many questions that need to be answered in the next 5 years is which mechanism is more effective in blocking PD1 or its ligand to exert maximum response. Also, are they effective in the adjuvant setting? Or is this an area in which vaccination still may have an impact? Meanwhile, the efficacy and reported data from these immunomodulators are encouraging and giving rise to many new hypotheses and clinical uses and clinicians should stay tuned to developments unfolding over the next few years.

On the other hand, well-advanced clinical trials already completed or ready to start using vaccines are eagerly awaited, such as MAGRIT and START2 trials. At the end of this journey, the authors sense that we are finally close to the development of an immunotherapy approach that can help us to improve OS and cure rate in lung cancer.

Conclusions

Recently, a better understanding of the biology of immune system in lung cancer led to the development of several immunotherapeutic agents, resulting in two major proposed therapeutic strategies: antigen-specific immunotherapy or cancer vaccination and non-antigen-specific immunotherapy or cancer immunomodulation. Vaccine therapy has not proven effective to date, but both activity and very low-toxicity profile define a unique treatment opportunity. Immunomodulators, working as checkpoint inhibitors in the interaction between T-cells and cancer cells have shown impressive activity in early clinical trials, representing the best pathway to target for the achievement of an immunological response against cancer cells. Waiting for the confirmatory results from ongoing randomized Phase III trials, some issues still need to be resolved. We need to develop more accurate predictive biomarkers for patient selection, in order to treat subgroup of patients who could benefit more and have their disease controlled for a long period of time. Indeed, most of the patients who are benefiting from immunotherapy are disease progression free after a long time of treatment, suggesting that their immune system has been reset in a way that could allow them to live with their disease. Furthermore, the immune response may take longer time to become clinically evident compared with standard cytotoxic therapy, sometimes leading to a temporary increase of the tumor target lesions because of the inflammatory infiltrate, subsequently followed by the final tumor shrinking. Therefore, we need to validate specific immune response criteria for the assessment of immunotherapy activity, because the commonly used RECIST criteria might not be appropriate to evaluate the activity of the new immune drugs. Even if most of the trials so far have used immunomodulators alone in metastatic lung cancer, we also need to work out when it is preferable to use immunotherapy in the course of lung cancer, moving our interest on early disease. Ongoing trials are exploring the use of these agents in combination with other therapies, such as chemotherapy, radiotherapy and targeted agents, suggesting that combination treatments could increase the activity of immunotherapy for NSCLC. Finally, combining the different immunotherapeutic agents could also produce a synergistic effect. If these findings are confirmed, immunotherapy will most likely become part of daily practice for NSCLC in the near future, conferring the opportunity to finally enter in the pantheon of lung cancer treatments.

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Key issues

- Survival outcomes of advanced non-small cell lung cancer (NSCLC) patients are still poor for the majority of them, but the introduction of targeted therapies represented a critical turning point for the treatment of molecular selected patients.
- The immune system is widely involved in the complex mechanisms of development, growth and spreading of malignant tumors, including NSCLC.
- Although lung cancer has been considered non-immunogenic for years, some scientific evidences suggest the opposite, especially for squamous NSCLC.
- Two different strategies of generating an immune response against cancer: enhancing immune stimulating components to start or maintain an effective response or inhibiting suppressing factors limiting the immune response.
- Many vaccines have been evaluated using different targets and others are still under evaluation. Promising data are coming especially from belagenpumatucel-L, a whole-cell vaccine, melanoma-associated antigen-A3 vaccines and tecemotide, an anti-MUC1 vaccine.
- The inhibition of immune checkpoints is a possible strategy to enhance immune response against cancer.
- Ipilimumab showed promising activity in Phase II trials for patients with advanced squamous NSCLC and SCLC, improving both progression-free survival and OS.
- Anti PD-1 antibodies, nivolumab and lambrolizumab, works through the inhibition of the interaction of PD-1 and its ligands. Preliminary promising data encouraged the start of larger studies.
- Anti-PD-L1 antibodies BMS-936559 (MDX-1105) and MPDL3280A showed promising results, and they are currently being evaluated both in monotherapy and in combination with other anticancer agents.

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