REVIEW ARTICLE

Journal of Cancerology. 2014;1:23-31

Immunotherapy in Lung Cancer: Evolution and Future Perspectives

Lourdes Barrera[1] and Óscar Arrieta Rodríguez[2]*

[1]Laboratory of Integrative Immunology, National Institute of Respiratory Diseases, Mexico City, Mexico; [2]Lung Cancer Clinic, National Cancer Institute, Mexico City, Mexico

ABSTRACT

In the current decade, new insights in the interaction between tumors and the immune system have led to the development of immunotherapy as a fundamentally new concept for the treatment of non-small cell lung cancer. This type of cancer is a promising target for the next generation of immune-based strategies. The goal of immunotherapy in lung cancer is to induce or re-induce a cell-mediated immune response, mainly of T-cells that can selectively destroy cells that display tumor-associated antigens. Modern non-small cell lung cancer vaccine strategies rely on better identification of antigenic targets, the addition of strong immunoadjuvants, and use of more efficient delivery systems. These treatments have convincingly demonstrated to elicit potent immune responses and have shown promising efficacy signals and excellent tolerability in phase II randomized studies. This review examines the most promising active immunotherapy using protein or peptide vaccines, whole-cell vaccines, and dendritic cell vaccines and examines current phase I, II, and III clinical trial data on some novel immune checkpoint inhibitors such as anti-programmed death 1. (J CANCEROL. 2014;1:23-31)

Corresponding author: Óscar Arrieta Rodríguez, ogar@unam.mx

Key words: Immunotherapy. Lung cancer. NSCLC. Non-small cell lung cancer. Vaccine. Immune checkpoint.

Correspondence to:
*Óscar Arrieta Rodríguez
Clínica de Cáncer de Pulmón
Instituto Nacional de Cancerología (INCan)
Av. San Fernando, 22
Col. Sección XVI, Del. Tlalpan
14080 México, D.F., México
E-mail: ogar@unam.mx

Received for publication: 27-8-2014
Accepted for publication: 3-9-2014

INTRODUCTION

New therapeutic strategies have been developed over the past decade to treat lung cancer. Unlike first-line treatments such as platinum-based chemotherapy or radiotherapy, these new approaches have focused on the properties and functions of the tumor itself as well as on understanding the surrounding microenvironment. From this perspective, new drugs have been developed that enhance antitumor response while reducing toxic side effects in patients.

Immune surveillance is an essential mechanism in the prevention of cancer development and is dependent on the cells in the immune system recognizing the antigens expressed by tumor cells as foreign and as precursors of cancer and subsequently eliminating them[1]. Tumor antigens may be expressed as a result of the aberrant expression of mutated genes, viral antigens, or the overexpression of self-antigens[2]. The immunoediting hypothesis posits that once intrinsic tumor suppressor mechanisms such as apoptosis fail, neoplastic transformation begins. Immunoediting consists of three phases: elimination, equilibrium, and escape[3]. During the initial phase of elimination, the host's innate and adaptive immune system works to eliminate incipient subclinical tumors. If tumor cells survive beyond the elimination stage, they enter the equilibrium phase, in which tumor growth and metastasis are checked by the competent host immune system. However, some tumor variants can evade immune responses, either by defective antigen presentation or by mechanisms of resistance to cell-mediated cytotoxicity. These resistant cell clones lead to the development of clinically apparent disease[4]. During the escape phase, the mechanisms of tumor escape protect each other to allow tumor development and therapeutic interventions are ineffective.

The goal of immunotherapy in cancer is to induce or re-induce a cell-mediated immune response, mainly of $CD4^+$ T-cells and $CD8^+$ (cytotoxic T-cells) that can selectively destroy cells that display tumor-associated antigens[5-7].

Immunotherapy for non-small cell lung cancer (NSCLC) can be broadly classified into two categories: passive and active[8]. Passive immunotherapy consists in the administration of therapeutic monoclonal antibodies to patients so that they will recognize antigens expressed on the surface of tumor cells that are functionally involved in oncogenesis.

The immune system works by distinguishing antigens from other organisms such as viruses and bacteria or by detecting malignant transformed cells. This response can damage healthy tissue if left unchecked or if it is exaggerated. To avoid such damage, the immune system has multiple mechanisms to decrease the response, involving various molecules that are collectively called immune checkpoints[9]. Various monoclonal antibodies directed against these immune checkpoint molecules have been developed and are currently in various phases of clinical trials.

Alternatively, active immunotherapy seeks to stimulate the patient's own immune system so that it will recognize tumor cells more efficiently and eliminate them (Fig. 1)[8]. This approach includes vaccination with tumor cell lysate, dendritic cells, recombinant DNA vectors, or with peptides and proteins.

PASSIVE IMMUNOTHERAPY

Growth factor inhibitors

Under the concept of passive immunity, monoclonal antibodies that recognize different antigens that are important for tumor growth such as the epidermal growth factor receptor (EGFR) or insulin-like growth factor 1 receptor (IGF1R) have been developed. Others, such as vascular endothelial growth factor (VEGF), bind and neutralize ligands secreted by tumor cells. Generally speaking, these

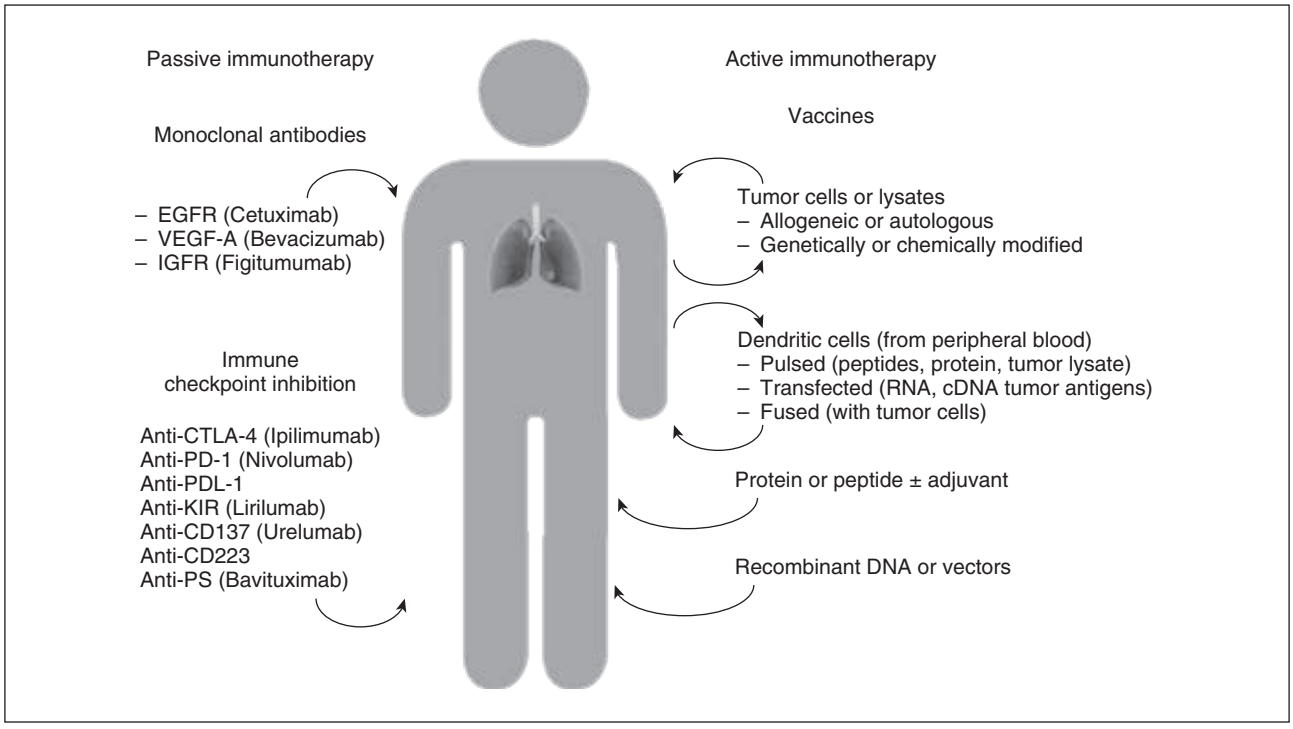

Figure 1. Passive and active immunotherapy for lung cancer. Passive immunotherapy involves the administration of monoclonal antibodies directed against extracellular domains of receptor tyrosine kinases (EGFR and IGFR) or vascular endothelial growth factor receptor ligands, VEGF-A, by modifying the signaling pathway required for tumor formation and development. Active immunotherapy seeks to stimulate the immune system so it will recognize known and unknown tumor antigens and eliminate tumor cells. These types of immunotherapies are based on the release of tumor antigens by different means to initiate the capture, processing, and presentation of these antigen-presenting cells. This leads to the activation and expansion of antigen-specific T-cells that recognize and attack tumor cells presenting peptides derived from tumor antigens on their surface.
EGFR: epidermal growth factor receptor; IGFR: insulin-like growth factor receptor; VEGF: vascular endothelial growth factor.

antibodies interfere with the activity of tyrosine kinase (TK) receptors, which play a key role in tumorigenesis. These adenosine triphosphate (ATP)-dependent receptors play an essential role in the transduction of extracellular signals as they cascade down the Ras/Raf/MAPK, PI3K/Akt and JAK/STAT intracellular signaling pathways that regulate cell proliferation, differentiation, metabolism, migration, or apoptosis. The aberrant activation of these tyrosine kinase receptors has been linked with the initiation and progression of several diseases. Monoclonal antibodies used in immunotherapy against these molecules bind to either the extracellular domain of the receptor or to the ligand itself, thus preventing ligand receptor binding or activation of the receptor and thereby limiting tumor growth.

Inhibition of the activity of growth factor receptors with tyrosine kinase activity (TKR) is done by using small-molecule inhibitors that have been studied during the past 10 years[10]. Members of the ErbB family, which includes EGFR, are among the most studied TK receptors. In human tumor cells, the EGFR pathway is constitutively activated due to mutation in the EGFR gene as a result of amplification, overexpression, or autocrine ligand production. In NSCLC, overexpression of EGFR has been reported in 62% of cases[11]. Aberrant signaling through EGFR has been associated with increased tumor proliferation, increased metastatic potential, resistance to chemotherapy, and a poor prognosis[12].

Two EGFR tyrosine kinase inhibitors (TKI) have been developed for the treatment of NSCLC, gefitinib

Table 1. Mechanisms of action of anti-tyrosine kinase monoclonal antibodies and clinical studies for lung cancer

Antibody	Target molecule	Mechanism of action	Clinical studies*
Cetuximab (Erbitux®; Bristol-Myers Squibb) chimeric human-mouse IgG1	EGFR	Prevents endogenous ligand from binding to its receptor, thus blocking activation. Binding of antibody induces receptor internalization, inhibits cell proliferation, inhibits invasion, angiogenesis, and metastasis and induces apoptosis of tumor cells as well as antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity.	FLEX phase III: First-line in combination in advanced NSCLC in patients with positive EGFR. BMS-099 phase III: First-line in combination in advanced NSCLC.
Bevacizumab (Avastin®; Genentech/Roche) humanized IgG1	VEGF	Neutralizes all isoforms of VEGF-A preventing it from binding to its receptors. Blocks the formation and growth of new blood vessels. Improves migration, maturation, number, and function of dendritic cells. Enhances the effect of dendritic cell-based immunotherapy. Modulates the production of regulatory T-cells.	ECOG 4599 phase III: First-line in combination in advanced non-squamous NSCLC. AVAIL phase II: First-line in combination in advanced non-squamous NSCLC.
Figitumumab	IGF1R	Prevents binding of IGF1 to its receptor, reducing the anti-apoptotic activity of IGF and inhibiting tumor growth.	ADViGO phase III: First-line treatment in combination.

EGFR: epidermal growth factor receptor; VEGF: vascular endothelial growth factor; NSCLC: non-small cell lung cancer; IGF: insulin-like growth factor. *Adapted from Gérard and Debruyne, 2009[8].

(Iresa®) and erlotinib (Tarceva®). Both agents bind competitively to ATP in the EGFR catalytic domain, suppressing receptor self-phosphorylation and subsequent downstream signaling. Both agents have been tested as monotherapy and in combination with other treatments with different results[13,14].

Immunoglobulin G antibodies bound to membrane receptors have the ability to activate antibody-dependent cellular cytotoxicity mechanisms. Cetuximab (Erbitux®), bevacizumab (Avastin®) and figitumumab (CP-751871) belong to this family of products[8]. Their mechanisms of action and clinical studies are summarized in table 1.

Immune checkpoint inhibitors

As with other epithelial tumors, lung cancer employs various mechanisms to evade immune surveillance and elimination carried out by the host immune system (Fig. 2). Lung cancer undergoes a slow process of immunoediting during which the precancerous cells gradually adapt and evolve to thwart immune surveillance. Tumor cells in this type of cancer secrete soluble proteins that prevent the processing and presentation of antigens such as transcription factor STAT3, indoleamine 2,3-dioxygenase, transforming growth factor beta, and interleukin 10[9]. Similarly, tumor cells decrease their expression of major histocompatibility complex class I molecules to avoid being recognized and eliminated by cytotoxic cells. They may also induce the aberrant expansion of $CD4^+$ $FoxP3^+$ regulatory T-cells or myeloid-derived suppressor cells, which inhibit the activity and proliferation of $CD4^+$ and $CD8^+$ T lymphocytes through the overexpression of proinflammatory factors such as PGE2[15].

On the other hand, as part of the regulatory mechanisms of cell activity, some surface receptors play a role in attenuating or inhibiting these signals to prevent excessive tissue damage when repair mechanisms are activated. These receptors are known as immune checkpoints. In cancer, these receptors and molecules exert these

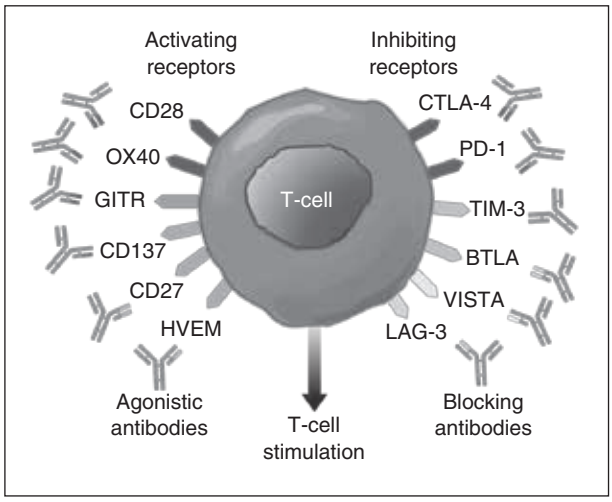

Figure 2. Therapeutic targets for T-cell immune regulation. T-cell activation is regulated through a balance between positive and negative signals provided by costimulatory receptors. These surface proteins are members of the tumor necrosis factor (TNF) family or the B7 family of molecules. Agonistic antibodies directed against activating molecules and antagonistic antibodies block inhibitory molecule signaling. Both types of antibodies are intended to increase T-cell stimulation to promote tumor destruction.

functions without any controls, resulting in exaggerated activation that promotes tumor development and maintenance.

The most noteworthy monoclonal antibodies that have been developed to block these immune checkpoints include anti-CTLA-4, anti-PD-1[16], anti-PD-L1, killer-cell immunoglobulin-like receptors antibodies, anti-CD137[17], anti-CD223[18] and anti-phosphatidylserine antibodies[19]. The mechanisms of action of these antibodies and clinical studies underway are summarized in table 2.

Due to their promising results in the field of immunotherapy, monoclonal antibodies directed against CTLA-4, PD-1, and PDL-1 stand out in particular.

Cytotoxic T-lymphocyte antigen 4 inhibition

Cytotoxic T-lymphocyte antigen 4 (CTLA-4; CD152). Once it is expressed on the cell surface, CTLA-4 competes with CD28 for ligands B7-1 (CD80) and B7-2 (CD86), which are expressed on the antigen-presenting cells. This balance restricts cytotoxic activity by limiting over activation of T-cells[20]. Lung cancer tumor cells stimulate abnormal expression of CTLA-4 by inducing anergy in the cells that express it[21]. This makes it a target for therapy in order to restore T-cell function.

Two anti-CTLA-4 antibodies, tremelimumab (CP-675.206) and ipilimumab (MDX-010) have been developed and tested in controlled clinical trials. In phase II clinical trials, tremelimumab has shown a 7% partial response rate and it is currently being tested in phase II studies for advanced mesothelioma (NCT01843374).

Ipilimumab has been the subject of several clinical studies, but results have been variable. Most notable have been those observed in squamous cell lung cancer subtypes, which showed a radiological response rate of 60% in 10 patients in combination with platinum-based chemotherapy (NCT01285609) (Table 2).

Programmed cell death receptor-1 inhibition

Programmed cell death receptor 1 (PD-1) plays a central role in the immune checkpoint pathway. Like CTLA-4, PD-1 is a cell-surface receptor belonging to the B7-CD28 superfamily. It is expressed on different cell types, including activated T-cells, B-cells and natural killer (NK) cells[22,23]. The PD-1 binds to its ligand PD-L1 (B7-H1, CD274) on antigen-presenting cells. This interaction inhibits nuclear factor kappa B (NFκB) transcription, down-regulating interferon gamma (IFN-γ) secretion and inducing T-cell tolerance[24]. The PD-1 also binds to PD-L2 (B7-DC, CD273) present in dendritic cells, although the precise functional consequences of this interaction are not known[25]. Overexpression of PD-L1 induces T-cell anergy, altering antigen recognition and processing. In lung cancer, PD-L1 overexpression has been identified in approximately 19% of patients with NSCLC, in particular those with adenocarcinoma, and has been associated

Table 2. Mechanisms of action of immune checkpoint inhibitor antibodies and clinical trials in lung cancer

Target molecule	Antibody	Mechanism of action	Clinical studies
CTLA-4	Ipilimumab (Yervoy®; Bristol-Myers Squibb) IgG1 Tremelimumab (CP-675,206) IgG2	IgG1 monoclonal antibody directed against CTLA-4. High binding affinity for inhibitory receptor CTLA-4. T-cell anergy is reversed when CTLA-4 is blocked.	NCT01285609 Phase III: NSCLC, stage IV/ recurrent. NCT01820754 Phase II: in combination with chemotherapy. NCT00527735 Phase II: in combination with chemotherapy. NCT01844505 Phase II: in combination with nivolumab. NCT01843374 Phase II for advanced mesothelioma.
PD-1	Nivolumab (BMS-936558) IgG4 Lambrolizumab (Pembrolizumab) (Merck)	IgG4 monoclonal antibody. It binds to PD-1 restoring cytokine secretion and $CD8^+$ T lymphocyte proliferation.	NCT01642004 Phase III: monotherapy in metastatic squamous NSCLC. NCT01673867 Phase III: monotherapy in non-squamous NSCLC. NCT01840579 Phase I: advanced NSCLC.
PD-L1	MPDL3280A (RG7446) Roche IgG1-kappa BMS-936559	Binding this antibody to PDL-1 restores T-cell-mediated tumor cell destruction.	NCT01846416 Phase II: advanced or metastatic NSCLC. NCT01903993 Phase II: advanced NSCLC, 2nd line after platinum failure. NCT00729664 Phase I: NSCLC.
CD137	Urelumab (BMS-663513) IgG4	Anti-CD137 agonist monoclonal antibody stimulates activated NK cells increasing antibody-mediated cytotoxicity.	NCT01471210 Phase I: solid tumors, monotherapy.
KIR	Lirilumab	Blocks KIR receptors leading to sustained activation of NK cells.	NCT01714739 Phase II: in combination with nivolumab in NSCLC. NCT01750580 Phase II: in combination with ipilimumab in NSCLC.
LAG3 (CD223)	BMS-986016	The binding of soluble LAG-3 to antigen-presenting cells induces a positive signal and activates regulatory molecules that enhance the immune response. Induces the immediate release of proinflammatory cytokines in NK and memory $CD8^+$ T-cells.	Preclinical.
PS	Bavituximab IgG3 chimeric 3G4	Blocks phosphatidylserine expression inhibiting stimulation of myeloid-derived suppressor cells and type 2 macrophages. Restores antigen presentation by dendritic cells.	NCT01323062 Phase II: in combination with chemotherapy in NSCLC.

CTLA-4: cytotoxic T-lymphocyte-associated antigen 4; PD-1: programmed cell death protein-1; PD-L1: PD-1 ligand; KIR: killer cell immunoglobulin-like receptor; LAG3: lymphocyte-activation gene 3 protein; PS: phosphatidylserine; NSCLC: non-small cell lung cancer.

with a worse prognosis[26]. Nivolumab (BMS-936558) is a monoclonal IgG4 antibody that has been tested in combination with different first-line treatment regimens (NCT01642004) and in combination with other immune checkpoint inhibitors (ipilimumab NCT01928394) (Table 2).

Programmed death-ligand 1 inhibition

Another immune checkpoint inhibition strategy is to block PD-L1.

Programmed death-ligand 1 is the ligand for PD-1 that is expressed in tumor cells. The anti PD-L1 antibody does not interfere with the PD-1 present in T-cells binding to antigen presenting cells through other ligands such as B7-H2 (ICOS-L)[27]. However, it has been reported that selective inhibition of PD-L1 induces overexpression of other PD-1 inhibitory ligands such as B7-DC (PD-L2)[24]. Four PD-L1 antibodies are currently being tested in different clinical trials, although whether inhibition of PD-1, PD-L1 or the combination of both can yield more efficient results in the reduction of solid tumors is still being studied. Of note have been the results seen in a phase I clinical trial of the BMS-936559 (human IgG4) antibody, which reported a 10% response rate and survival in 49 patients with NSCLC who were enrolled in the study[28] (Table 2).

ACTIVE IMMUNOTHERAPY

Vaccines for non-small cell lung cancer

Vaccines for treatment of NSCLC can be classified according to their main ingredient: those that contain peptides or proteins as an active ingredient and those containing whole cell lysates. The latter can be subdivided into: (i) autologous cell lines with or without genetic modifications such as cell suspensions from surgically removed tumors transfected with granulocyte-macrophage colony-stimulating factor, and (ii) autologous tumor cell lines.

There are currently six different vaccines for NSCLC that are in the latter stages of clinical development: MAGE-A3, liposomal BLP25 and TG4010 for early stage NSCLC and the EGF vaccine, belagenpumatucel-L and tergenpumatucel-L for advanced disease.

Melanoma-associated antigen A3 vaccine

Melanoma-associated antigen A3 (MAGE-A3) is a tumor-associated antigen that is expressed on the surface of certain cell types that are not normal, therefore making it a good candidate for vaccine development. Expression of MAGE-A3 has been associated with various solid tumors, including NSCLC. Studies indicate that between 17 and 50% of NSCLC tumors express MAGE-A3 on their surface and this has been associated with a worse prognosis[29,30]. This vaccine consists of purified recombinant MAGE-A3 protein in a liposomal formulation containing the AS15 adjuvant system.

Liposomal BLP25 vaccine

Mucin-1 (MUC1) is a membrane glycoprotein also considered to be a tumor-associated antigen that is found overexpressed or glycosylated in NSCLC tumors[31]. Two vaccines have been developed for MUC1: BLP25 and TG4010. Both have shown activity in clinical trials and are being tested in phase III clinical trials[32,33].

Liposomal BLP25 (L-BLP25, tecemotide, Stimuvax®) is a liposomal vaccine consisting of a chain of 25 amino acids from the variable region of MUC1 combined with immunoadjuvant monophosphoryl lipid A[34]. In a phase II study of patients with stage IIIB or IV NSCLC who were stable or responding to chemotherapy as first-line treatment, L-BLP25 was administered following a low dose of cyclophosphamide and was associated with a median survival of two years more than in the subgroup of patients with stage IIIB locoregional disease[35].

Vaccine TG4010 contains a genetically modified virus that expresses MUC1 and IL-2, which activates T lymphocytes and NK cells. This vaccine was evaluated in patients with stage IIIB or MUC1-positive NSCLC in conjunction with chemotherapy (cisplatin plus gemcitabine) in a phase IIB clinical study. Progression-free survival at six months was

higher with TG4010 compared to chemotherapy alone (43 vs. 35%)[36].

Epidermal growth factor vaccine

Epidermal growth factor receptor is overexpressed in many tumor types, including NSCLC. Signaling through this receptor has been associated with cell proliferation, decreased cell death, cell migration, and angiogenesis. This makes it an obvious therapeutic target in the development of vaccines. The vaccine was designed to reduce EGFR signaling and thus limit tumor growth. It is composed of recombinant EGF protein produced in yeast, chemically conjugated to a recombinant bacterial protein, P64k of *Neisseria meningitidis*, produced in *Escherichia coli* (E. coli). This vaccine has been tested in patients with advanced NSCLC as second-line treatment in patients younger than 60 years old who showed improvement in survival as compared with patients who did not receive the vaccine (median survival 11.6 vs. 5.3 months; p = 0.0124). This vaccine is well tolerated with no grade 3 or 4 adverse events reported[37]. Phase III clinical trials are now underway in the UK with estimated completion in 2015. There are currently no reports of clinical trials in the USA[38].

Belagenpumatucel-L

Transforming growth factor beta (TGF-β) is a multifunctional cytokine that promotes epithelial differentiation and inhibits cell growth both in normal and pathological conditions[39]. In cancer, defective TGF-β-mediated signaling has been associated with a more aggressive phenotype and poorer survival in advanced NSCLC, just as elevated levels of TGF-β have also been linked to a state of immunosuppression and worse prognosis[40]. Belagenpumatucel-L is an allogeneic whole cell vaccine composed of four irradiated NSCLC cell lines (two adenocarcinoma, one squamous, and one large cell), that have been transfected with a plasmid that contains the TGF-β2 antisense gene, which causes the inhibition of cellular expression of this molecule, resulting in an increased immunogenicity of the cancer cells.

Three different doses of the vaccine have been tested using the same treatment schedule in patients with NSCLC after prior chemotherapy[41]. In this study, a dose-dependent increase in survival was observed in patients receiving the higher doses compared to those who received lower doses (581 vs. 252 days; p = 0.0186).

Tergenpumatucel-L

The tergenpumatucel-L vaccine is composed of three allogeneic tumor cell lines that have been modified to express the a-galactosyltransferase enzyme (a-GAL), which enhances immune recognition of tumor cells. In a phase II study, 28 patients with advanced NSCLC received sets of eight shots every two weeks. Immunogenicity was measured by testing serum samples for presence of a-GAL and IFN-γ. Median survival was 11.3 months and 31% of patients who received the vaccine subsequently responded to chemotherapy. This vaccine is currently in a phase III clinical trial comparing it with second-line chemotherapy in advanced NSCLC[42].

CONCLUSION

Until recently, NSCLC was considered non-immunogenic or poorly immunogenic; however, immune-based strategies have shown initial promise for early and advanced-stage NSCLC. At the present time, there is limited data from phase III trials showing a clear clinical benefit for vaccines in lung cancer. Most of these trials are focused on patients with advanced-stage disease; however, the ideal candidates for lung cancer vaccines may be patients with stage I or II disease who are considered at high risk of recurrence post-resection. It is hoped that ongoing studies will provide additional information on optimal patient selection and vaccine administration needed to bridge the gap

between promising concept and therapeutic reality. On the other hand, clinical trials on immune checkpoint inhibitors have shown promising but limited results. The model of single molecules is no longer viable. It is becoming clear that being able to treat a cancer in a particular tumor setting is going to require a much more complex series of targeted molecules. The opportunity is that those multiple targets can much more accurately treat complex diseases such as lung cancer.

REFERENCES

1. Schreiber RD, Old LJ, Smyth MJ. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:565-70.
2. Finn OJ. Cancer immunology. N Engl J Med. 2008;358:2704-15.
3. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Ann Rev Immunol. 2011;29:235-71.
4. Forde PM, KellyRJ, Brahmer JR. New strategies in lung cancer: Translating immunotherapy into clinical practice. Clin Cancer Res. 2014;20:1067-73.
5. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund L-T. Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res. 2008;14:5220-7.
6. Hiraoka K, Miyamoto M, Cho Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer. 2006;94:275-80.
7. Zhuang X, Xia X, Wang C, et al. A high number of CD8+ T cells infiltrated in NSCLC tissues is associated with a favorable prognosis. Appl Immunohistochem Mol Morphol. 2010;18:24-8.
8. Gérard C, Debruyne C. Immunotherapy in the landscape of new targeted treatments for non-small cell lung cancer. Mol Oncol. 2009;3:409-24.
9. Creelan BC. Update on immune checkpoint inhibitors in lung cancer. Cancer Control. 2010;21:80-9.
10. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med. 2005;353:172-87.
11. Hirsch FR, Varella-Garcia M, Bunn PA, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol. 2003;21:3798-807.
12. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7:169-81.
13. Cataldo VD, Gibbons DL, Pérez-Soler R, Quintás-Cardama A. Treatment of non-small-cell lung cancer with erlotinib or gefitinib. N Engl J Med. 2011;364:947-55.
14. Gridelli C, De Marinis F, Di Maio M, Cortinovis D, Cappuzzo F, Mok T. Gefitinib as first-line treatment for patients with advanced non-small-cell lung cancer with activating epidermal growth factor receptor mutation: Review of the evidence. Lung Cancer. 2011;71:249-57.
15. Dieu-Nosjean M-C, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol. 2008;26:4410-17.
16. Fedorov VD, Themeli M, Sadelain M. PD-1 – and CTLA-4 – based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Sci Transl Med. 2013;5;172.
17. Melero I, Murillo O, Dubrot J, Herva S. Multi-layered action mechanisms of CD137 (4-1BB)-targeted immunotherapies. Trends Pharmacol Sci. 2008;137:383-90.
18. Escudier B. Emerging immunotherapies for renal cell carcinoma. Ann Oncol. 2012;23(Suppl 8):viii35-40.
19. Derose P, Thorpe PE, Gerber DE. Development of bavituximab, a vascular targeting agent with immune-modulating properties, for lung cancer treatment. Immunother. 2011;3:933-44.
20. Erfani N, Mehrabadi SM, Ghayumi MA, et al. Increase of regulatory T cells in metastatic stage and CTLA-4 over expression in lymphocytes of patients with non-small cell lung cancer (NSCLC). Lung Cancer. 2012;77:306-11.
21. Li L, Chao QG, Ping LZ, et al. The prevalence of FOXP3+ regulatory T-cells in peripheral blood of patients with NSCLC. Cancer Biother Radiopharm. 2009;24:357-67.
22. Agata Y, Kawasaki A, Nishimura H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol. 1996;8:765-72.
23. Sauce D, Almeida JR, Larsen M, et al. PD-1 expression on human CD8 T cells depends on both state of differentiation and activation status. AIDS. 2007;21:2005-13.
24. Liang SC, Latchman YE, Buhlmann JE, et al. Regulation of PD-1, PD-L1, and PD-L2 expression during normal and autoimmune responses. Eur J Immunol. 2003;33:2706-16.
25. Wang S, Bajorath J, Flies DB, Dong H, Honjo T, Chen L. Molecular modeling and functional mapping of B7-H1 and B7-DC uncouple costimulatory function from PD-1 interaction. J Exp Med. 2003;197:1083-91.
26. Mu C-Y, Huang J-A, Chen Y, Chen C, Zhang X-G. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol. 2011;28:682-8.
27. Bennett F, Luxenberg D, Ling V, et al. Program death-1 engagement upon TCR activation has distinct effects on costimulation and cytokine-driven proliferation: attenuation of ICOS, IL-4, and IL-21, but not CD28, IL-7, and IL-15 responses. J Immunol. 2003;170:711-18.
28. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455-65.
29. Gure AO, Chua R, Williamson B, et al. Cancer-testis genes are coordinately expressed and are markers of poor outcome in non-small cell lung cancer. Clin Cancer Res. 2005;11:8055-62.
30. Sienel W, Varwerk C, Linder A, et al. Melanoma associated antigen (MAGE)-A3 expression in Stages I and II non-small cell lung cancer: results of a multi-center study. Eur J Cardiothorac Surg. 2004;25:131-4.
31. Vlad AM, Kettel JC, Alajez NM, Carlos CA, Finn OJ. MUC1 Immunobiology: From discovery to clinical applications. Adv Immunol. 2004;82:249-93.
32. Clinical Trials.gov. (2013). BLP25 liposome vaccine and bevacizumab after chemotherapy and radiation therapy in treating patients with newly diagnosed stage IIIA or stage IIIB non-small cell lung cancer that cannot be removed by surgery. Available at: http://clinicaltrials.gov/ct2/show/NCT00828009?term=NCT00828009&rank=1
33. Clinical Trials.gov. (2013). Phase III Lucanix™ vaccine therapy in advanced non-small cell lung cancer (NSCLC) following front-line chemotherapy. Available at: http://clinicaltrials.gov/show/NCT00676507.
34. Decoster L, Wauters I, Vansteenkiste JF. Vaccination therapy for non-small-cell lung cancer: review of agents in phase III development. Ann Oncol. 2012;23:1387-93.
35. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol. 2005;23:6674-81.
36. Quoix E, Ramlau R, Westeel V, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled phase 2B trial. Lancet Oncol. 2011;12:1125-33.
37. Neninger Vinageras E, de la Torre A, Osorio Rodríguez M, et al. Phase II randomized controlled trial of an epidermal growth factor vaccine in advanced non-small-cell lung cancer. J Clin Oncol. 2008;26:1452-8.
38. Bioven Europe. (2014). No Title. A randomized trial to study the safety and efficacy of EGF cancer vaccination in late-stage (IIIB/IV) non-small cell lung cancer patients (NSCLC). [Accessed April 15, 2014]. Available at: http://clinicaltrials.gov/show/NCT01444118. NLM identifier: NCT01444118
39. Bierie, B., & Moses, H. L. (2006). TGF-beta and cancer. Cytokine & Growth Factor Reviews, 17, 29–40. doi:10.1016/j.cytogfr.2005.09.006
40. Malkoski SP, Haeger SM, Cleaver TG, et al. Loss of transforming growth factor beta type ii receptor increases aggressive tumor behavior and reduces survival in lung adenocarcinoma and squamous cell carcinoma. Clin Cancer Res. 2012;18:2173-83.
41. Nemunaitis J, Dillman RO, Schwarzenberger PO, et al. Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. J Clin Oncol. 2006;24:4721-30.
42. Clinical Trials.gov. NewLink Genetics Corporation. (2014). Immunotherapy Study in Progressive or Relapsed Non-Small Cell Lung Cancer. Available at: http://clinicaltrials.gov/show/NCT01774578.