Review Article
Immunotherapy for lung cancer: advances and prospects

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Abstract: Lung cancer is the most commonly diagnosed cancer as well as the leading cause of cancer-related deaths worldwide. To date, surgery is the first choice treatment, but most clinically diagnosed cases are inoperable. While chemotherapy and/or radiotherapy are the next considered options for such cases, these treatment modalities have adverse effects and are sometimes lethal to patients. Thus, new effective strategies with minimal side effects are urgently needed. Cancer immunotherapy provides either active or passive immunity to target tumors. Multiple immunotherapy agents have been proposed and tested for potential therapeutic benefit against lung cancer, and some pose fewer side effects as compared to conventional chemotherapy and radiotherapy. In this article, we discuss studies focusing on interactions between lung cancer and the immune system, and we place an emphasis on outcome evidence in order to create a knowledge base well-grounded in clinical reality. Overall, this review highlights the need for new lung cancer treatment options, with much ground to be paved for future advances in the field. We believe that immunotherapy agents alone or with other forms of treatment can be recognized as next modality of lung cancer treatment.

Keywords: Lung cancer, immunology, immunotherapy, vaccines, checkpoint inhibitor

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths. Particularly, lung cancer has an estimated incidence of 1.6 million new cases every year [1]. Lung cancer is categorized into two major subtypes depending on their histological feature: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC and SCLC constitute 85% and 15% of total lung cancer cases respectively [2].

Only 16.6% of lung cancer patients survive 5 years or more, with only 3.9% surviving in the metastatic setting [3]. Use of conventional therapeutic strategies has lots of unwanted side effects and drawbacks. For example, chances of missing micro metastasis and recurrence are common problems observed in surgically operated lung cancer patients; chemotherapy, radiotherapy, or concomitant chemo-radiotherapy for inoperable cases may not prevent recur-

rences. Recent introduction of molecular targeted therapies, including activating mutations of epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) translocations, in the metastatic setting led to improved treatment outcomes in selected subgroups of patients with advanced stage NSCLC [3]. Even with the latest advances, lung cancer prognosis remains dismal, and novel therapeutic approaches are needed.

In the last decade, there has been a better understanding of the interactions between immune cells and cancer cells, and the mechanisms that cancer evades the immune system, resulting in a new era of cancer immunotherapy protocols which overcomes the limitations of conventional therapeutic strategies [4]. Immunotherapy represents a broad class of treatment modalities designed to elicit immune-mediated destruction of tumor cells [3]. In this review, we will provide a comprehensive review

about the current understanding of immuno-therapy for lung cancer.

Lung cancer: specific immune responses

Lung cancer progression is a multi-step mechanism. Chronic inflammation mediated by cigarette smoking, chronic obstructive pulmonary disease, chronic bronchitis, and occupational exposure are some of the many factors that lead to an imbalance in cytokine secretions and inflammatory responses, which may favor the malignant transformation of normal epithelial cells [5]. Lung cancer employs several methods to evade surveillance and elimination by the host immune system. Here in this section, we present a brief introduction of lung cancer immunology.

Innate immunity

Innate immunity is a nonspecific first-line of defense, involving natural killer (NK) cells, macrophages and neutrophils [6]. A chronic inflammation state activates innate immunity with subsequent release of cytokines, which may promote tumor destruction but can also lead to oncogenesis [7]. In addition, lung cancer cells can re-educate M1 macrophages to M2 macrophages [8]. Sentinel cells from the innate branch of immunity may recognize relatively non-specific structurally preserved molecules, which are distinguishable from the host's molecules through Toll-like receptors on their surfaces.

Tumor microenvironment (TME)

The TME is facilitated by many distinct cell types, including endothelial cells and their precursors, pericytes, myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), T and B lymphocytes, NK cells, dendritic cells (DCs), neutrophils, eosinophils, basophils, and mast cells [9] (**Figure 1**). The TME along with its contents are crucial for driving malignant transformed cells into solid masses which include resistance to apoptosis, proliferation, invasion, angiogenesis and metastasis.

MDSCs comprise a major type of immunosuppressive leukocyte population that inhibits host-protective anti-tumor responses. MDSCs are capable of suppressing multiple phases of the immune response by promoting tumor local invasion and metastases by secreting factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinases [10, 11]. MDSCs contribute to the development of an immuno-suppressive TME that blocks the action of cytotoxic anti-tumor T effector cells. The TME also possesses the potential to induce regulatory T cell function by secreting tumor growth factor (TGF)-β and interleukin (IL)-10 and plays a major role in immune tolerance, resulting in a major obstacle to efficient cancer immunotherapy [12, 13].

Fibroblasts are distorted during cancer progression. Particularly, CAFs are present in abundance in the tumor stroma of the TME, where they release the hepatocyte growth factor (HGF). HGF released promotes proliferation and invasion by modulating the HGF/c-Met pathway. Some studies have supported the role of CAFs in angiogenesis via demonstrating the capacity of CAFs to secrete pro-angiogenic factors like VEGF-A, platelet derived growth factor, and bFGF [14].

Similarly, macrophages with normal innate phagocytic functions are recruited to tumor cells and become TAMs. TAMs support tumor growth by promoting angiogenesis, immuno-suppression, invasion, and metastasis. Specifically, TAMs mediate tumor growth through ILs (-6, -4, -13), leukocyte inhibitory factor, prostaglandin E2, CCL17, CCL22, and CCL24 [15-17].

Immunosurveillance

Immune system invasion and/or escape is essential for lung cancer propagation. Several mechanisms are proposed for immune invasion by lung cancer cells. Key mechanisms proposed are: (i) resistance of tumor cell lysis due to deficient expression of major histocompatibility complex (MHC), (ii) expression of poorly immunogenic epitopes, (iii) release of immuno-suppressive cytokines, and (iv) T cell apoptosis. Alternatively, escape may result from the establishment of an immunosuppressive state within the TME [18].

Cytotoxic CD8+ T cells present in the TME of lung cancer patients were observed to be less effective. Furthermore, these cells were found to be hyporesponsive to activation via the T cell receptor (TCR). This deficiency in T cells is due
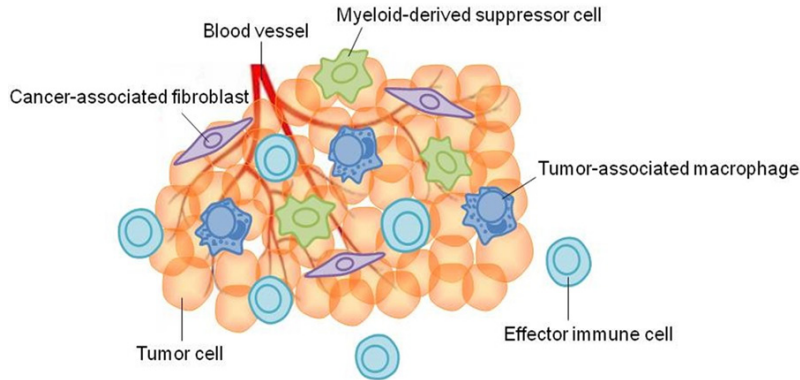
Figure 1. The tumor microenvironment (TME) is facilitated by many distinct cell types, including endothelial cells and their precursors, pericytes, myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), T and B lymphocytes, natural killer (NK) cells, dendritic cells (DCs), neutrophils, eosinophils, basophils, and mast cells.
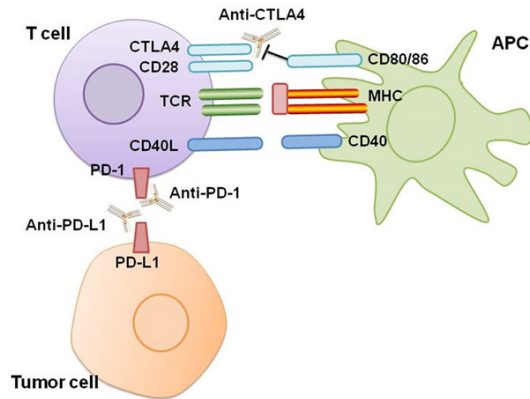

Figure 2. Improved therapeutic efficacy can be achieved by modulating immune checkpoints, including activation of effector cells by blocking CTLA4 or PD-1.

to a TCR signaling arrest partially dependent on membrane-associated TGF-β1 present in the TME. Alterations in IL-10-producing CD8+ T cells were observed in patients with early stage lung cancer compared to patients with advanced stage lung cancer. IL-10-producing CD8+ T cells were at significantly higher proportions in early stage lung cancer; such an observation may provide some insights about slower tumor progression or a longer overall survival (OS). Changes in the proportion of CD8+ T cells subsets (naïve, effector/memory) in the tumor stroma also explain its alteration in lung cancer [19-21]. Functions of NK cells are also impaired in lung cancer. They possess less cytotoxicity along with a decreased ability to degranulate and produce interferon (IFN)-γ variation in the

NK cell receptors [22, 23]. Immature or dysfunctional DCs are also seen in lung cancer and are mediated by IL-10, VEGF, and TGF-β. These dysfunctional DCs are shown to have role in cancer migration, invasion, and epithelial-mesenchymal transition [24].

Immune checkpoints

Besides the antigen-MHC-TCR interaction, additional co-activation signals must also be present (**Figure 2**), namely interaction of the T cell's CD28 molecule with the antigen presenting cell's (APC's)/tumor cell's B7 surface molecules (CD80 or CD86).

In order to prevent autoimmunity, immune checkpoints are set in place. Activated CD8+ T cells express a protein receptor named cytotoxic T-lymphocyte antigen-4 (CTLA-4), which also binds B7 with high affinity, limiting further T cell activation by CD28 [25, 26]. Programmed cell death-1 (PD-1) is another T cell surface receptor that upon binding its cognate ligand (Programmed cell death - ligand 1, PD-L1) in the APC/tumor cell, it inhibits the immune response (**Figure 2**). While CTLA-4's action focuses on limiting the initiation of T cell activation in the lymph nodes, PD-1 acts later by limiting T cell activity in the TME [25].

Immunotherapy options for lung cancer

Given this myriad of immunosuppressive tools, it is of little wonder that traditional immunotherapy approaches have largely failed to eradicate lung cancer. Nevertheless, the fruits of this scientific effort are beginning to be realized in lung cancer. The aim of immunotherapy is to enhance the immune response specifically directed to the tumor. A description of current investigational immunotherapies for lung cancer is provided in the following sections below and summarized in **Figure 3**.

Cytokines

The first immunotherapies developed for NSCLC were recombinant cytokines, namely those secreted by Th1 cells, such as IL-2 and IFN.
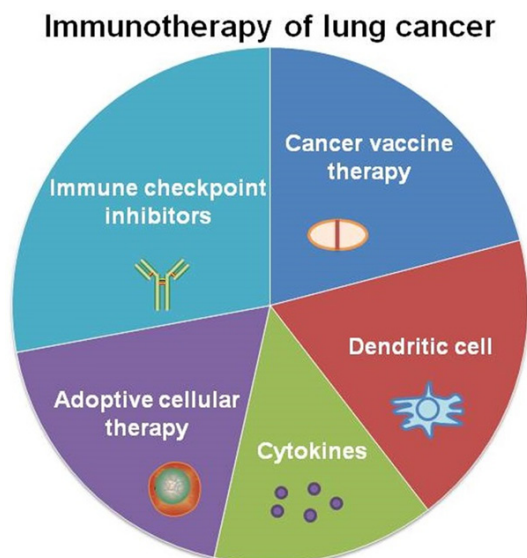
Figure 3. Immunotherapeutic strategies for lung cancers: administration of immune checkpoint inhibitors, cancer vaccine therapy, adoptive transfer of immune cells generated in vitro, immunotherapy with dendritic cells (DCs), and recombinant cytokines.

Phase II trials were not suggestive of clinical benefit for human recombinant IL-2 administration (with or without IFN) [27]. In fact, therapy was not well tolerated, yielding grade 3–4 cardiac and pulmonary toxicity. A phase II trial by Correale et al. showed that addition of IL-2 to chemotherapy (gemcitabine plus docetaxel) in patients with advanced NSCLC improved response rates (58.3% vs. 28.6%) with good tolerability [27, 28]. However, these findings were not replicated in a phase III randomized trial of IL-2 in combination with chemotherapy with a cisplatinum doublet [27, 29]. These results were further challenged by a subsequent study reporting 20% partial response and 50% stable disease among 20 advanced NSCLC patients when IL-2 was administered with the pineal neurohormone melatonin [27, 30]. Blood concentration of IL-2 seems to follow a circadian pattern, which must be taken into account when defining a therapeutic strategy [31].

Cancer vaccine therapy

Cancer vaccine therapy for treatment of lung cancer has recently re-emerged as a potential therapeutic approach. Vaccine therapy of lung cancer is based on the fact that all the malignancies, including those found in lung cancer,

express either mutated proteins that can be recognized as foreign antigens, over-express normal proteins, or re-express fetal antigens not present in the normal, non-cancerous adult. These tumor associated antigens help to recognize malignant tissue as foreign particles, thus stimulating APCs [32]. Different vaccination strategies have been investigated for treating lung cancer (**Table 1**). Therapeutic lung cancer vaccines include whole cell vaccines and vaccines directed against specific antigens [33]. Below, we discuss different therapeutic lung cancer vaccines.

Belagenpumatucel-L vaccine

Belagenpumatucel-L (Lucanix™) is an allogenic lung cancer tumor cell vaccine derived from four cancer cell lines, including SK-LU-1 (adenocarcinoma), NCI-H460 (large cell carcinoma), NCI-H520 and Rh2 (squamous cell carcinoma), that are genetically modified. Particularly, each cell line is transfected with the antisense gene for TGF-β2, which decreases the expression of this immunosuppressive cytokine, thereby enhancing the immunogenicity of the vaccine [34, 35].

A randomized phase II trial in 75 patients with stage II–IV NSCLC was performed by Nemunaitis et al. [35]. Three different doses of belagenpumatucel-L (1.25×10^7, 2.5×10^7, or 5.0×10^7 cells) were tested in these patients. The drug showed a good safety profile at all three doses. The results showed that there was a dose-dependent survival advantage. OS of the two higher dose groups was significantly better than that of the low-dose patients (581 days vs. 252 days; P = 0.0069).

A double-blind, randomized, phase III study (STOP clinical trial) was conducted involving advanced NSCLC patients pretreated with a first line platinum-based chemotherapy and then treated with belagenpumatucel-L (2.5×10^7 cells/intradermal monthly injection). The results revealed a median OS of 20.3 and 17.8 months in Lucanix™ and placebo groups, respectively [hazard ratio (HR) = 0.94; P = 0.594]. Although OS was larger, the STOP trial did not meet the primary endpoint. In addition, the results showed the improved OS in the non-adenocarcinoma and the stage IIIB/IV patients who were treated with this vaccine therapy within 12 weeks of finishing the initial chemotherapy [4].

Table 1. Clinical trials of cancer vaccines for lung cancer

Agents	Study design	N	TNM staging	Results
Belagenpumatucel-L	Phase II, randomized, 3 dose cohorts (1.25, 2.5, or 5 × 10^7 cells/injection)	75	II–IV NSCLC	Better OS in higher dose group, P = 0.0069
	Phase III, randomized, double-blind, Belagenpumatucel-L vs. placebo (STOP trial)	532	IIIB-IV NSCLC	Median OS: 20.3 months vs. 17.8 months, P = 0.594
TG4010	Phase II, multicenter, randomized, TG4010 + chemotherapy vs. TG4010 until progression, followed by chemotherapy	65	IIIB-IV NSCLC	Median OS: 12.7 months vs. 14.9 months, 1-year survival rate: 53% vs. 60%
	Phase IIB, multicenter, open-label, randomized, TG4010 + chemotherapy vs. chemotherapy	148	IIIB-IV NSCLC	6-month PFS: 43.2% vs. 35.1%
L-BLP 25	Phase I, 2 dose cohorts (20 or 200 mg)	17	IIIB-IV NSCLC	Well tolerated, primary cellular immune response
	Phase II, open-label, parallel-group, randomized. L-BLP25 + BSC vs. BSC	171	IIIB-IV NSCLC	Well tolerated, OS: 17.4 months Vs. 13 months, 3-yr survival rate: 31%.
MAGE-A3	Phase II multicenter, double-blind, randomized, MAGE-A3 vs. placebo	182	IB/II NSCLC	No significant improvement in PFS, OS
EGF	Phase II, randomized, EGF vs. BSC	80	IIIB-IV NSCLC	Median OS: 11.7 months vs. 5.33 months, well tolerated
Talactoferrin	Phase II, randomized, double-blind. talactoferrin vs. placebo	110	IIIB-IV NSCLC	ORR: 44% vs. 29%, P = 0.05
	Phase II, randomized, double-blind. talactoferrin vs. placebo	100	IIIB-IV NSCLC	Median OS: 13.7 months vs. 6.1 months
	Phase III, multicenter, randomized, double-blind, talactoferrin vs. placebo (FORTIS trial)	742	IIIB-IV NSCLC	OS: 7.49 months vs. 7.66 months, P = 0.6602
BEC2/BCG	Phase III, randomized, open-labeled (SILVA study)	515	Limited stage SCLC	No benefit for survival or quality of life
Racotumomab	Phase III, multicenter, randomized, racotumomab vs. placebo	176	IIIB/IV NSCLC	Median OS: 8.23 months vs. 6.8 months
GVAX	Phase I/II	49	IIIB-IV NSCLC	Stable disease in 7 cases

NSCLC: Non-small cell of lung cancer; OS: Overall survival; PFS: Progression-free survival; BSC: Best supportive care; EGF: Epidermal growth factor; ORR: Objective response rate; SCLC: small cell lung cancer.

Mucinous glycoprotein-1 (MUC1)

MUC1 is a tumor-associated antigen (TAA) that is commonly expressed in NSCLC, and often aberrantly expressed or glycosylated [36]. Two MUC1 vaccines, TG4010 and L-BLP25 have shown evidence of activity in clinical trials.

TG4010 vaccine: The TG4010 vaccine is a suspension of modified vaccinia Ankara (MVA strain) that expresses the MUC1 and IL-2 [37]. TG4010 has been designed to amplify a cellular immune response directed against tumor cells expressing MUC1 [38].

A multicenter randomized phase II trial of 65 advanced stage (stage IIIB/IV) NSCLC patients with MUC1 antigen expression has explored two schedules of the combination of TG4010 with first-line chemotherapy. In arm 1, TG4010 was combined with cisplatin (100 mg/m^2 day 1) and vinorelbine (25 mg/m^2 day 1 and day 8). In arm 2, patients received subcutaneous injection of TG4010 monotherapy until disease progression followed by TG4010 plus the same chemotherapy regimen as in arm 1. The median time to progression was 4.8 months, median OS was 12.7 months, and the 1-year survival rate was 53%. In arm 2, only 2/14 evaluable patients responded, median OS was 14.9 months, and the 1-year survival rate was 60%. This regimen was not progressed further [39]. Further in a multicenter, open-label phase IIB randomized study, 148 untreated patients with MUC1 antigen expression in stage IIIB/IV NSCLC were randomized to receive up to six cycles of cisplatin-gemcitabine with or without TG4010. The results showed 6-month progression-free survival (PFS) was 43.2% in the TG4010 plus chemotherapy group and 35.1% in the chemotherapy alone group. In addition, increased numbers of CD16+, CD56+, and CD69+ NK cells prior to treatment correlated negatively with OS. The data suggest that TG4010 augments the therapeutic effect of chemotherapy for advanced stage lung cancer [40].

A phase IIb/III trial of TG4010 (TIME clinical trial, NCT01383148) with first-line therapy in 1,000 patients with stage IV NSCLC is currently on-going. This is a randomized, double-blind, and placebo-controlled study comparing first-line therapy with or without TG4010. PFS and OS will be evaluated [41].

L-BLP25 (Tecemotide) vaccine: The BLP25 liposome vaccine (L-BLP25) consists of a 25 amino acid sequence that provides specificity to the exposed core peptide of MUC1 [42]. Even though MUC1 is also present in normal epithelial tissues, it differs structurally when expressed by malignant cells [43].

An initial phase I study in patients with NSCLC showed that the vaccine could be administered with minimal toxicity [44]. Survival patterns in patients with advanced NSCLC who received L-BLP25 were sufficiently encouraging to proceed with a phase II randomized study. In addition, an open-label, non-randomized phase I study combined with a double-blind, randomized, placebo-controlled phase II study was conducted in Japanese patients with unresectable stage III NSCLC after primary chemoradiotherapy. Their preliminary phase I safety data reported that L-BLP25 was well tolerated in Japanese patients, and the safety profile was consistent with that seen in previous studies.

In a phase II trial of L-BLP25, consisting of 171 patients with stage IIIB/IV NSCLC who are either stable or responding to fist-line chemotherapy were randomly assigned to receive L-BLP25 plus best supportive care (BSC) or BSC alone. Patients in the L-BLP25 arm received a single intravenous dose of 300 mg/m^2 cyclophosphamide followed by eight weekly subcutaneous immunizations with L-BLP25. Subsequent immunizations were administered at 6-week intervals. The results demonstrated a median survival time of 4.4 months longer for patients randomly assigned to the L-BLP25 arm than patients assigned to the BSC arm. Survival benefits were especially seen in patients with loco-regional advanced stage IIIB NSCLC. The drug was well tolerated with no adverse reactions observed during the study period [45]. This study was followed by a subsequent update on survival analysis of these patients, which showed that the 3-year survival rate was 31% in patients receiving L-BLP25 plus BSC and 17% in those receiving BSC alone (P = 0.035) [45]. Furthermore, a subset of patients with locally advanced NSCLC showed a significantly better survival rate after vaccination than those in the control group (30.6 months vs. 13.3 months) [46].

Three phase III trials are currently underway and in development in order to assess the effi-

cacy of L-BLP25 in NSCLC. The first phase III trial (START trial) is a multicenter, randomized, double-blind placebo-controlled study which will enroll 1,513 stable or responding unresectable stage IIIB patients with completion of first-line chemotherapy and/or radiotherapy. OS in the L-BLP25 group was 25.6 months, compared with 22.3 months in the placebo group (HR = 0.88, 95% CI: 0.75–1.03, P = 0.123). However, in the subgroup analysis, the survival of patients in the vaccination group was significantly better than that of those in the placebo group of patients receiving concurrent chemoradiotherapy. In the sequential chemoradiotherapy group, OS was not significantly different (19.4 months in the vaccination group vs. 24.6 months in the placebo group). The reason for this result was unclear, but concurrent chemoradiotherapy may have induced a strong, nonspecific immune activation or antigen presentation. The START2 trial (NCT02049151), which will enroll 35 patients with completed, concurrent chemoradiotherapy for unresectable stage III NSCLC, is ongoing. The other phase III study of L-BLP25 cancer vaccine study for stage III unresectable NSCLC in the Asian population (INSPIRE, NCT01015443) has been terminated with an aim to evaluate efficacy and drug safety in 40 patients. The study is a multinational, double-blind, placebo-controlled, randomized trial.

Melanoma-associated antigen (MAGE)-A3 vaccine

MAGE is a TAA, which is silent in normal tissues except for the testes [47]. MAGE-A3 is expressed in about 35% of NSCLC cases and may be associated with worse prognosis [48]. The MAGE-A3 vaccine is the first vaccine to be evaluated in the postoperative adjuvant setting, which suggests that operable patients with early-stage NSCLC may prove to be better candidates for this vaccination.

In a multicenter, double-blind, randomized, placebo-controlled phase II study of the MAGE-A3 vaccine, efficacy was assessed in 182 resected stage IB/II NSCLC patients. The patients were randomly assigned to MAGE-A3 treatment or placebo groups. There was a 25% relative risk reduction for relapse after a median post-resection period of 44 months, but there were no significant benefits for OS or PFS [49]. Based on this result, a large-scale phase III trial, similar in design to the previous phase II trial, has started in patients with completely resected stage IB–IIIA NSCLC (MAGRIT study; NCT-00480025). The MAGRIT trail will include 2,270 patients with stage IB, II, or IIIA NSCLC. The primary aim of the study is to assess the disease-free survival (DFS) and further evaluate adverse effects and the OS and DFS at 2, 3, 4, and 5-year intervals. However, the study was terminated following assessment of the lack of efficacy of the study's product.

Epidermal growth factor (EGF) vaccine

The EGFR is a well-known oncogene. Over expression of EGFR by tumor cells is related to the aggressiveness of tumor cell growth [50]. The EGFR pathway is involved in cell proliferation, apoptosis, angiogenesis, and metastasis. CIMAvax-EGF vaccine is composed of human recombinant EGF produced in yeast and chemically conjugated to the P64K *Neisseria meningitides* recombinant protein produced in *Escherichia coli*. The vaccine is developed in Cuba and is already in practice for treatment of advanced stage lung cancer patients.

CIMAVax-EGF was tested in patients with advanced NSCLC who had finished first-line therapy and showed significant survival improvement in patients 60 years of age as compared with patients who did not receive the vaccine (median survival: 11.7 months vs. 5.33 months, P = 0.0124). The vaccine was well tolerated, with no grade 3 or 4 adverse events reported [51]. An international phase III trial is ongoing which is to determine whether the recombinant human EGF cancer vaccine is safe, immunogenic, and effective in the treatment of stage IV NSCLC patients who are positive in the selective EGF biomarker and wild type EGFR compared to standard treatment and supportive care (NCT02187367).

Talactoferrin

Talactoferrin is a recombinant human lactoferrin isolated from Aspergillus niger var. awamori, which can suppress tumor growth through the recruitment of DCs into the intestinal lymphoid tissue, activating immune effector cells such as NK and CD8+ T cells [52].

Table 2. Clinical trials of immune checkpoint inhibitors for lung cancer

Target	Agents	Study design	N	TNM staging	Results
CTLA-4	Ipilimumab	Phase II, double-blind, randomized, Ipilimumab (phased and concurrent) vs. placebo	204	IIIB-IV NSCLC	irPFS (5.7 and 5.5) months vs. 4.6 months, median OS (12.2 and 9.7) months vs. 8.3 months
CTLA-4	Ipilimumab	Phase II, multicenter, double-blind, randomized, Ipilimumab (phased and concurrent) vs. placebo	130	Extensive stage SCLC	Phased group improved irPFS
CTLA-4	Tremelimumab	Phase II, randomized	87	IIIB-IV NSCLC	PFS at 3 months: no significant improvement
PD-1	Nivolumab	Phase I, 3 dose cohorts (1, 3, 10 mg/kg)	76	IIIB-IV NSCLC	Cumulative response rate: 18%
PD-1	Pembrolizumab	Phase Ib	38	NSCLC	ORR: 21%, median OS: under a year
PD-L1	MPDL3280A	Phase I	53	NSCLC	ORR: 23%
PD-L1	BMS-936559	Phase I, multicenter	75	IIIB-IV NSCLC	ORR: 10%

CTLA-4: Cytotoxic T-lymphocyte antigen-4; NSCLC: Non-small cell of lung cancer; OS: Overall survival; SCLC: small cell lung cancer; irPFS: Immune-related progression-free survival; PFS: Progression-free survival; PD-1: Programmed cell death-1; ORR: Objective response rate; PD-L1: Programmed cell death - lignad 1.

In a placebo-controlled, randomized trial in 110 patients with stage IIIB/IV NSCLC, objective response rate (ORR) was significantly better in the talactoferrin-carboplatin/paclitaxel group than in the placebo-carboplatin/paclitaxel group (44% and 29%, respectively; P = 0.05). The difference in OS was also promising, although not statistically significant (10.4 months in the talactoferrin group and 8.5 months in the placebo group; P = 0.11) [53]. On the contrary, Parikh *et al.* showed that oral talactoferrin-α as monotherapy significantly increased OS compared with placebo (3.7 months vs. 6.1 months; one-tailed P = 0.04 log rank) [54]. The international, multicenter, randomized, double-blind phase III trials (FORTIS-M trial) failed to show improved survival after talactoferrin [55]. The FORTIS-C trial (NCT00706862), a randomized, double-blind study of talactoferrin combined with first-line chemotherapy, is currently ongoing.

Ganglioside vaccines

One of the earliest attempts at an antitumor vaccine used the GD3 ganglioside as an antigen and the Bacillus Calmette-Guérin (BCG) vaccine as an immunoadjuvant. It was used as a treatment option SCLC rather than NSCLC. After a very promising pilot study [56, 57], this BEC2/BCG vaccine did not provide survival or quality of life benefit in a phase III trial with 515 patients by the European Organization for the Research and Treatment of Cancer (Silva Study) [56, 58].

Racotumomab is a vaccine that consists of a monoclonal antibody (mAb) that mimics gangliosides with a glycosylation pattern almost exclusive of neoplastic cells. A phase III trial by Alfonso et al. showed a median OS of 8.23 months in NSCLC patients treated with racotumomab when compared to a median OS of 6.8 months in patients treated with a placebo, P = 0.004 [59].

GVAX

An autologous vaccine, named granulocyte macrophage colony-stimulating factor gene-transduced tumor vaccines (GVAX), was isolated from 49 NSCLC patients in a phase I/II trial. Seven patients attained stable disease during 12 weeks or more following the first vaccina-

tion, but no patients attained remission (complete or partial) [56, 60].

DC-based therapies

DC-based vaccines work by administering activated autologous DCs to the patient, producing a specific immune response against the neoplasia. A phase III trial demonstrated a lower recurrence rate in patients treated with surgery with an adjuvant DC vaccine than in patients treated with surgery alone (10% vs. 25%, respectively) [61]. A translational study was conducted during the aforementioned trial in order to detect valid biomarkers for successful DC vaccine therapy such as reduction of macrophage inflammatory protein-1α, increase of RANTES mRNA expression levels, increase of NK cell counts, and a normal CD4+/CD8+ ratio [61].

Immune checkpoint inhibitors

One of the most promising approaches in immunotherapy for lung cancer is to inhibit the immune checkpoints in order to harness an effective immune response against the tumor. In theory, immune checkpoint inhibitors should "remove the brakes" on most T cell-mediated immune responses. The current data on the activity of immune checkpoint inhibitors in lung cancer are reviewed below and in **Table 2**.

Anti-CTLA-4 checkpoint inhibitors

CTLA-4 is a member of the immunoglobulin superfamily. Once a cytotoxic T cell becomes active, it expresses CTLA-4 on its cell surface and then competes with the costimulatory molecule CD28 for their mutually shared ligands, B7-1 (CD80) or B7-2 (CD86), on the APC. CTLA-4 regulation takes place in the early activation phase of immune induction, occurring in the regional lymph nodes at the level of the APC and unprimed T cell interaction. Lung cancer can stimulate abnormal expression of CTLA-4 in T cells, and these CTLA-4 aberrant T cells exhibit an anergic phenotype. Currently, two human mAbs to CTLA-4, tremelimumab and ipilimumab, are being tested in lung cancer.

Ipilimumab: Ipilimumab is a mAb, designed to target CTLA-4, which inhibits T cell activation; the blockage of CTLA-4 leads to an increased immune response against tumor cells. The rationale of ipilimumab as an immunotherapy agent is based on the notion that blocking

CTLA-4 may produce an increased immune response against tumor cells.

Two concurrent randomized phase II trials used ipilimumab in combination with chemotherapy (carboplatin/paclitaxel) for extensive stage SCLC (n =130) and advanced stage NSCLC (n = 204) [62, 63]. In the second study, patients were randomized to receive one of three regimens. All treatment arms received up to six cycles of paclitaxel and carboplatin. The first arm (early) received ipilimumab on day 1 of cycle 1–4 and a placebo for the remaining two cycles. The second arm received a placebo for the first two cycles and ipilimumab on day 1 of cycle 3–6 (delayed). The third arm (control) received only the placebo. Maintenance ipilimumab was given in patients in the first two treatment groups once every 12 weeks until progression. In the delayed arm, the immune-related PFS (irPFS) was 5.7 months vs. 4.6 months (HR = 0.72; P = 0.05). In the early arm, no improvement in irPFS was seen (5.5 months vs. 4.6 months; HR = 0.81; P = 0.13). In the delayed group, a non-statistical improvement in OS was also seen (12.2 months vs. 8.3 months; HR = 0.87; P = 0.23). Although not statistically significant, patients with squamous histology had longer OS (HR = 0.55; 95% CI: 0.27–1.12).

A larger phase III trial is currently being conducted, aiming specifically at the squamous subtype NSCLC (NCT01285609). Ipilimumab is also being studied in combination with EGFR and ALK tyrosine kinase inhibitors (NCT-01998126). The role of ipilimumab is also being investigated in additional SCLC trials (NCT01331525, NCT01450761, NCT020467-33).

Tremelimumab: Tremelimumab is another humanized mAb that binds to CTLA-4 and thus inhibiting this immune checkpoint. In a randomized phase II trial, 87 patients with locally advanced or metastatic NSCLC were enrolled. PFS at 3 months was not significantly improved by tremelimumab as compared with BSC, even though there was a 4.8% radiological response rate [64].

Anti-PD-1 checkpoint inhibitors

PD-1 is another immune checkpoint. Like CTLA-4, PD-1 is a surface receptor member of the B7-CD28 superfamily. It is expressed on many cell types, including activated T cells, B cells, and NK cells [65]. Known ligands of PD-1 include PD-L1 (CD274, B7-H1) and PD-L2 (CD 273, B7-DC). The binding of PD-1 with PD-L1 or PD-L2 leads to decreased cytokine production, reduced proliferation and cell lysis. In many tumors, PD-1 is up-regulated in lymphocytes infiltrated in tumor, and many tumors have increased PD-L1 expression [66]. It is proposed that through this mechanism, tumors can induce T cell anergy and avoid the processing tumor antigens by APCs that lead to recognition.

Nivolumab: Nivolumab (formerly known as BMS-936558 or MDX1106b) is a human antibody that inhibits PD-1 receptors expressed on activated T cells [67]. As PD-L1 is only expressed on selected tumor cells, the adverse effect of the drug is expected to be less than ipilimumab.

A phase I trial for nivolumab at three different doses (1, 3, and 10 mg/kg every 2 weeks) in NSCLC treatment refractory patients reported that the cumulative response rate (all doses) was 18% (14 of 76 patients) [68]. Follow-up of an expanded NSCLC cohort across all dosages showed a 1-year survival rate of 42%, a 2-year survival rate of 24%, and median OS of 9.9 months. Additional phase II studies are also ongoing, with ORR as their primary endpoint, testing nivolumab monotherapy as a third-line treatment in patients with advanced or metastatic squamous NSCLC, nivolumab plus ipilimumab in advanced or metastatic solid tumors (including NSCLC), and nivolumab following azacitidine and entinostat vs. oral azacitidine in patients with recurrent metastatic NSCLC. Phase III trials of nivolumab vs. docetaxel in patients with NSCLC (NCT01642004, NCT-01673867, NCT02041533, NCT02477826) have completed accrual, and results of these trials are eagerly anticipated.

Pembrolizumab: Pembrolizumab (formerly lambrolizumab or MK-3475) is a mAb targeting PD-1 with significant antitumor activity in melanoma [69]. In patients with NSCLC who were previously treated with two systemic regimens, MK-3475 was administered at 10 mg/kg every 3 weeks. In an interim analysis of 38 patients, the ORR was 21%, and most responses had occurred by 9 weeks [67]. New trials with pem-

Table 3. Clinical trials of adoptive cellular therapy for lung cancer

Agents	Study design	N	Patient	Results
LAK cell	Intrapleural transfer of LAK cells combined with rIL-2	121	IIIB-IV NSCLC with malignant pleural effusion	Disappeared rate: 58.6%, decreased rate: 36.2%
LAK cell	Randomized, LAK + chemo/radiotherapy vs. chemo/radiotherapy	105	Noncurative resection of primary lung cancer	7-year survival rate: 39.1% vs. 12.7%
LAK cell	Phase III, randomized, LAK + rIL-2 after chemo/radiotherapy vs. chemo/radiotherapy	174	Primary lung carcinoma after surgery	5-year survival rate: 54.4% vs. 33.4%
CIK cell	Phase II, CIK + chemotherapy vs. chemotherapy	87	I-IV NSCLC	3-year OS rate: 82% vs. 66%, median OS: 73 months vs. 53 months
CIK cell	DC + CIK + chemotherapy vs. chemotherapy	122	NSCLC	1-year OS rate: 57% vs. 37.3%
NKT cell	Phase I	6	Advanced and recurrent NSCLC	Well tolerated, sage
γδ T cell	Phase I, one-way, open	10	Recurrent NSCLC	Well tolerated, safe, feasible
γδ T cell	Phase I	15	Recurrent or advanced NSCLC	Median OS: 589 days, median PFS: 126 days

LAK: Lymphokine-activated killer; rIL-2: Recombinant interlukin-2; NSCLC: Non-small cell of lung cancer; CIK: Cytokine-induced killer; OS: Overall survival; DC: Dendritic cells; NKT: Natural killer T; PFS: Progression-free survival.

brolizumab for NSCLC have opened (NCT-02220894, NCT02359019, NCT02402920, et al.).

Anti-PD-L1 checkpoint inhibitors

PD-L1 (also known as CD274 or B7-H1), the ligand for PD-1, is a member of the B7 super-family and is involved in the negative regulation of the immune response. PD-L1 is expressed in T and B cells, macrophages, and DCs and is up-regulated in a range of solid tumors, including NSCLC. PD-L1 expression has been reported to be associated with vascular invasion and high-er-grade differentiation [70]. PD-L1 expression is associated with increased macrophages, DCs, and inflammatory infiltrate. Given the key role PD-L1 has in lung cancer, the inhibition of PD-L1 is an attractive therapeutic approach.

MPDL3280A: MPDL3280A (MDX-1105) is a human mAb that targets PD-L1 and thus blocks PD-L1 from binding its receptors, including PD-1 and B7.1. In a phase I study of MPDL3280A in pre-treated patients with advanced NSCLC, the ORR was 23% [71]. Additional trials with MPDL3280A are ongoing. One trial is monitor-ing objective responses and safety in patients with PD-L1-positive locally advanced or meta-static NSCLC receiving MPDL3280A monother-apy. Another study is evaluating response rates and safety of MPDL3280A compared to docetaxel in patients with advanced or meta-static NSCLC in whom platinum therapy has failed.

BMS-936559: BMS-936559 is a high-affinity, fully human, PD-L1-specific IgG mAb [72]. In a phase I study, 5 of 49 evaluable NSCLC patients had an objective response; response duration ranged from 2.3+ months to 16.6+ months. Six of 49 patients had stable disease lasting 24 weeks, and 31% of patients had PFS at 24 weeks [73].

Adoptive cellular therapy

Adoptive cell transfer is a form of passive immunotherapy that involves identification, iso-lation, expansion, and subsequent re-infusion of autologous lymphocytes with anti-tumor activity into patients. This form of therapy has been used with or without administration of appropriate growth factors to enhance T cell survival and expansion in vivo. Such an ap-proach also has the theoretical advantage in that identification and isolation of only a few tumor reactive lymphocytes is sufficient for therapy as these cells can be expanded signifi-cantly ex vivo prior to reinfusion. The genetic modification of isolated cells and the introduc-tion of TCRs with high avidity for tumor specific antigens also creates exciting therapeutic pos-sibilities. **Table 3** shows the most important studies involving adoptive cellular therapy in lung cancer.

Lymphokine-activated killer (LAK) cells

LAK cells were first reported in 1982 [74]. In vitro, lymphocytes can be stimulated by IL-2 to kill tumor cells insensitive to CTLs or NK cells. The application of LAK cells in the treatment of advanced tumors can be traced back to 1985.

To enhance the power of LAK cells, patients receive recombinant IL-2 (rIL-2) during the treat-ment. Intrapleural transfer of autologous or allogeneic LAK cells combined with rIL-2 was used in the treatment of 121 patients with malignant effusion associated with advanced lung cancer. The effusion disappeared in 71 patients (58.6%) and was significantly decre-ased in 45 patients (36.2%). No serious side effects were observed [75]. In another clinical trial, 105 patients who had undergone non-curative resection of primary lung cancer were randomly divided into two groups. The group receiving rIL-2 and LAK cells combined with radiation therapy or chemotherapy showed a better 7-year survival rate than the control group, in which patients were treated by radia-tion therapy or chemotherapy alone [76]. A sim-ilar result was obtained in a randomized phase III study [77]. Because of the large amount of IL-2 associated with the clinical application of LAK cells, serious side effects have been re-ported such as capillary leak syndrome, which can lead to hypotension, oliguria, pulmonary edema, and dyspnea. These effects constitute an important obstacle limiting the development of LAK cells for clinical application.

Cytokine-induced killer (CIK) cells

CIK cells are generated from peripheral lym-phocytes by a cytokine cocktail of CD3 mAbs, IL-2, and IFN-γ. CIK cells possess a T cell-NK cell phenotype and MHC-independent antitu-

mor action [78]. They possess an enhanced cytotoxicity and proliferate more than LAK cells.

In a phase II clinical study, autologous CIK cell immunotherapy improved the efficacy of conventional chemotherapy in advanced stage NSCLC patients [79]. DC-activated CIK cells enhanced antitumor effects, and chemotherapy combined with CIK/DC cells improved the clinical outcomes of advanced NSCLC patients [80]. Furthermore, combination treatment with CIK cells and endostatin or DC-based cancer vaccines may have a synergistic effect on improving clinical outcomes. In recent years, CIK cells have been widely used as immunotherapy for many cancers because of their high proliferation rate and cytotoxic activity, especially after activation by DCs [81].

Natural killer T (NKT) cells

NKT cells, which were first identified in 1986, share properties of both T cells and NK cells. NKT cells are characterized by the expression of unique invariant TCRs encoded by Vα24Jα18 in humans [82], and they can recognize α-galactosylceramide (α-GalCer) presented by CD1d. Invariant NKT (iNKT) cells can rapidly produce large amounts of IFN-γ, which is a hallmark of inflammatory cytokines and is critical for NK cells to attack MHC-negative tumors and for CTLs to kill MHC-positive tumors.

In a phase I study, activated NKT cell administration was well tolerated and performed safely with minor adverse effects in patients with advanced and recurrent NSCLC (n = 6). A clinical trial of iNKT cell-based immunotherapy showed that the infusion of ligand-pulsed APCs and/or activated iNKT cells was safe and well tolerated [83]. The administration of α-GalCer-pulsed APCs induced an activation of iNKT cells in the TME and augmented IFN-γ production by the α-GalCer-stimulated TILs. Another study investigated induced pluripotent stem (iPS)-derived NKT cells. iPS cells are developed into functional NKT cells in the presence of IL-7 and Flt3L in vitro. Although the function of iPS-derived NKT cells is well defined in vivo, further research is necessary before clinical application.

γδ T cells

Human γδ T cells comprise 1% to 10% of peripheral blood T cells. Unlike αβ T cells, whose activation requires antigen processing MHC-restricted peptides displayed by APCs, γδ T cells recognize tumor antigens directly through the γδ TCR and exhibit potent MHC-unrestricted lytic activity against microbial pathogens and tumors.

In a phase I clinical study, 10 patients with recurrent NSCLC were treated with intravenous infusion of autologous γδ T cells cultured with zoledronic acid and IL-2. γδ T cell therapy was given 3 to 12 times every 2 weeks. Median follow up of patients was 401 days. The regimen was well tolerated [84]. Recently a phase I study was conducted to evaluate the safety and potential anti-tumor effects of re-infusing ex vivo expanded γδ T cells in patients with recurrent or advanced NSCLC. In this study, ex vivo expansion of γδ T cells from peripheral blood mononuclear cells was achieved by culturing with zoledronic acid (5 μM) and IL-2 (1000 IU/ml) for 14 days. Harvested cells, mostly γδ T cells, were given intravenously every 2 weeks without additional IL-2 for a total of six times. The cumulative number of transferred γδ T cells ranged from 2.6 to 45.1 × 10^9 (median, 15.7×10^9). Fifteen patients were treated, and an increase in the number of peripheral γδ T cells was observed with the increase in number of infusions. All patients remained alive during the study period with a median survival of 589 days and median PFS of 126 days. The drug was thus well tolerated [85].

Cytotoxic T lymphocytes (CTLs)

CTLs are CD8+ αβ T cells, which are the main force of anti-tumor immunity. These cells have the ability to recognize MHC class I molecules, present TAAs, and release granzymes and perforins to lyse tumor cells. Hence, methods to increase the number of CTLs are being developed. Re-infused CTLs are not effective because of the downregulation of MHC and co-stimulatory molecules. Under these circumstances, there are no successful cases of treatment of lung cancer by CTL reinfusion. The identification of crucial factors potentially associated with the loss of MHC expression may provide a new direction for the development of immunotherapy strategies [86]. Injection of peptide vaccines, which are similar to TAAs, stimulate T cells, resulting in an increase in the number of CTLs available to attack tumor cells whose TAAs are positive.

Tumor-infiltrating lymphocytes (TILs)

TILs, which were first identified in 1986, are isolated from tumor samples, draining lymph nodes, or malignant effusion. One specimen is processed into a single cell suspension that is exposed to high-dose IL-2 to increase the number of lymphocytes and then re-infused into the patient.

Studies have shown the effectiveness of TIL therapy in NSCLC. High levels of intratumoral TILs are associated with a decreased risk of disease recurrence and improved DFS [87]. The infusion of in vitro expanded TILs derived from surgical samples is feasible and has been shown to prolong OS and control residual disease in patients with advanced NSCLC. In 1996, TILs engineered by the IL-2 gene were re-infused into 10 advanced lung cancer patients with pleural effusions. The pleural effusions did not re-accumulate for at least 4 weeks in six patients, and the size of the original tumor decreased in one patient [88].

Re-infusion of TILs into lung cancer patients has certain advantages such as its specificity and safety. However, limitations include the difficulty in obtaining samples from surgeries, adverse effects associated with the combination of high-dose IL-2, and long culture periods (e.g., 5 weeks). In contrast to the modest success of cell transfer therapy for melanoma, clinical experience in lung cancer has been far from satisfactory [89].

Engineered T cell therapy

Adoptive T cell therapy with engineered T cells to target tumor antigens is an attractive and powerful strategy for cancer therapy. With further modifications in the laboratory and an increased number of clinical trials to test these approaches, engineered T cell therapy for cancer may provide significant improvements to cancer immunotherapy.

Chimeric antigen receptor (CAR) T cell immunotherapy

T cells genetically engineered with CAR vectors can specifically target the surface antigen of cancer cells and kill cancer them in an MHC-independent manner [90]. An objective tumor response was obtained for CAR T cells. CAR T cells were first translated for hematologic malignancies because the antigen expression on hematologic cells was better understood and there were fewer barriers for honing in T cells on hematologic organs. The use of CAR T cells targeting CD19 has led to remarkable outcomes in the treatment of B cell malignances such as chronic lymphocytic leukemia, acute lymphoblastic leukemia, and other indolent lymphomas [91].

Zhou et al. constructed a CAR targeting EGFR on the cell membrane of T lymphocytes [92]. A xenogeneic mouse model of advanced lung metastatic (A549) cancer was established through tail vein injection in order to evaluate the functional activity of CAR-modified T cells. CAR-modified T cells were administered at days 3, 6, 9, 12, 15, and 18 after tumor injection. A549-derived tumor lung metastasis was significantly decreased when mice were treated with CAR-modified T cells. The mice treated with CAR-modified T cells had a very low tumor metastasis index ($P < 0.05$).

TCR-modified T cell immunotherapy

T cells express a heterodimeric αβ receptor on their surface called the TCR. This receptor recognizes antigenic peptides presented by MHC proteins. Genes that encode the α- and β-chains of TCRs can be identified and isolated from the T cells of the rare patients who respond to tumors. These chains are then introduced into T cells, usually by means of viral or non-viral technologies. In this manner, large numbers of antigen-specific T cells can be rapidly generated. The modified TCR-expressing T (TCR-T) cells respond to tumor cells expressing the target antigen.

An et al. designed a study to investigate the cytotoxicity of normal CD8+ T lymphocytes retrovirally transduced with Wilms tumor gene 1 (WT1) peptide-specific TCR genes against human lung cancer cells [93]. The results demonstrate the feasibility of adoptive immunotherapy with TCR-redirected T cell for the treatment of lung cancer.

Conclusions

Immunotherapies represent a novel approach to treat lung cancer and offer the potential for extended benefits even in advanced disease. At present, mAbs targeting immune check-

points and antitumor vaccines are the most promising representatives of this treatment modality. An improvement in the understanding of the immune system in tumor immunosurveillance has resulted in the development of a new generation of immunotherapeutic agents. Many clinical trials are ongoing and will eventually give further insight into immunotherapy's proper place in lung cancer treatment. Combination with chemotherapy, molecular-targeted therapy, and other vaccine therapies could also be viable treatment options.

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Disclosure of conflict of interest

None.

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