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Review

Immunotherapy in the landscape of new targeted treatments for non-small cell lung cancer

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ABSTRACT

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death worldwide. Active immunotherapies and molecules targeting tyrosine kinase receptors both offer new avenues for the treatment of NSCLC. Furthermore, their combinations or their administration along with standard treatments enlarges the potential for clinical benefit. Moreover, the discovery of biomarkers predicting the response to these new therapies should allow a better selection of patients susceptible to optimally benefit from these treatments. In this paper, we review the most promising active immunotherapies, antibodies and small molecules in the context of NSCLC management, focusing on compounds in phase III clinical development.

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1. Introduction

With more than 1.2 million new cases worldwide each year, lung cancer is the most common cause of cancer death in men and women. Non-small cell lung cancer (NSCLC) accounts for approximately 81% of all cases of lung cancer and they are often fatal, primarily because about 60% of the patients are diagnosed at advanced and usually incurable stage IIIB or IV (Katzel et al., 2009). Long-term survival for patients with advanced/metastatic disease is typically less than one year (Jemal et al., 2008; Paccagnella et al., 2006). However, even in the early stages, more than 50% of the patients will relapse within the two years following complete surgical

resection. According to the analysis done by the IASLC lung cancer staging project, the 5-year survival rate by pathological stage using the new AJCC staging V.7 is 73% in stage IA, 58% in IB, 46% for IIA while it drops dramatically to 36% in stage IIB and 24% in stage IIIA tumors (Rami-Porta et al., 2007). Besides the need for earlier diagnosis, there is a great need for additional effective adjuvant therapy. In resected NSCLC, the only approved therapy is chemotherapy, providing a limited clinical benefit. In completely resected NSCLC, an absolute benefit of 5-year overall survival of 5.4% was shown as demonstrated by a pooled analysis of five large trials of adjuvant cisplatin-based chemotherapy carried out by the LACE collaborative group. Moreover, 34% of the patients in these

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trials did not complete the planned number of cycles due to toxicity (Pignon et al., 2008). The benefit of chemotherapy is still under debate for stage I.

Over the last decade, new strategies to treat cancer patients have emerged. Unlike traditional cytotoxic therapies, they focus more on the tumor itself and its supportive micro-environment. The aim is to improve anti-tumor activity while reducing toxic side effects for the patients.

This paper reviews the emerging treatments for NSCLC, focusing on the most advanced products. Among these new treatments, we will discuss the promising recent advances in cancer immunotherapy, setting the scene for future development.

2. New strategies for the treatment of NSCLC

2.1. Cancer immunotherapy

Among the new approaches developed to fight NSCLC, immunotherapy has evolved considerably over the last twenty years and is now re-gaining importance. Cancer immunotherapy can either be passive or active. Passive immunotherapy in the context of NSCLC consists in transferring to the patients therapeutic monoclonal antibodies that recognize tumor antigens expressed at the surface of the lung cancer cells which are functionally implicated in oncogenesis. In contrast, active immunotherapy strives to stimulate the patient's own immune system to recognize tumor antigens expressed by the tumor and eliminate it. Recently, the field has benefited from increased understanding of tumor immunology and genetics, which identified the tumor microenvironment and the cross-talk between the tumor and its stroma as key parameters to be considered in the development of an active immunotherapy. The discovery of new tumor antigens, along with advances in immunological adjuvant technology and the identification of the key role played by the toll-like receptors (TLR), have paved the way to more successful immunotherapeutic approaches. Indeed, complex adjuvants are now being developed which contain multiple molecules able to bind and activate different TLRs. This leads to the initiation of innate and adaptive immune responses, which both contribute to the elimination of tumor cells (Pasare and Medzhitov, 2005).

2.1.1. Tumor antigens in NSCLC

Tumors express antigens which can be used to harness the immune system towards cancer cells. The targets are tumor antigen-derived peptides generated by intracellular processing of the proteins during their synthesis. These peptides are presented at the cell surface by human leukocyte antigen (HLA) molecules, and are recognized by helper T cells and cytotoxic T cells, which can mediate tumor cell lysis. In contrast to what was originally believed, it is now clear that NSCLC, like other tumor types, expresses many different tumor antigens which can be targeted by the immune system. Spontaneous attempts of the patient immune system to eliminate the tumors occur in NSCLC patients, and tumor lymphocyte infiltration has been reported as a good prognostic factor for NSCLC in a number of studies (O'Callaghan et al., 2009).

Tumor antigens can be classified into several main categories (Lucas and Coulie, 2008). Some are expressed in a percentage of NSCLC and in a variety of other tumors. One group of such shared tumor antigens is encoded by cancer-germ line genes or 'cancer-testis' genes, which include the MAGE family. MAGE-A3, a member of this family, is a tumor-specific antigen. It is not expressed in normal adult cells, except in the testis and in the placenta, but the positive cells in these two organs have no antigen presentation capabilities. MAGE-A3 is expressed in 35–50% of stage IB/II/IIIA NSCLC (Vansteenkiste et al., 2007).

Another type of shared tumor antigens is encoded by genes that are also expressed in a restricted set of normal cell types. One example is PRAME (PReferentially expressed Antigen of MElanoma) which was discovered in melanoma but is present in about 70% of NSCLC and other tumors (Ikeda et al., 1997). An additional group comprises tumor antigens that are encoded by genes overexpressed in tumor cells as compared to their expression in normal epithelial cells. The best-known examples of such antigens are the epidermal growth factor receptor (EGFR), the HER2-neu oncogene, mucin-1 (MUC1), the telomerase reverse transcriptase (hTERT) and survivin. Increased levels of EGFR and HER2-neu are found in both serum and broncho-alveolar lavage of NSCLC patients, especially with stage III and stage IV (Ludovini et al., 2009; Salam et al., 2009). MUC1 is a mucosal transmembrane glycoprotein present and aberrantly glycosylated in 80% of NSCLC (Butts et al., 2005). hTERT is overexpressed in more than 85% of NSCLC and is associated with poor prognosis (Bolonaki et al., 2007). Overexpression of survivin has been correlated with more aggressive tumors that have a high potential to metastasize into the lymph nodes (Yamashita et al., 2009).

Other antigens which are frequently expressed in NSCLC are abnormally expressed or mutated genes like p53 or k-RAS. Overexpression of p53 is a prognostic marker in NSCLC, especially in patients with adenocarcinoma (Mitsudomi et al., 2000; Yamashita et al., 2009), while mutations in the k-RAS gene are found in 40% of adenocarcinomas. Both are particularly common (60%) in tumors from smokers and they are associated with unresponsiveness to chemotherapy and EGFR tyrosine kinase inhibitor treatment (Linardou et al., 2008).

2.1.2. Passive immunotherapy

This approach consists in transferring to the patients therapeutic monoclonal antibodies that recognize antigens important for the tumor growth, like the epidermal growth factor receptor (EGFR), or the insulin growth factor receptor-1 (IGFR-1). Others bind and neutralize ligands secreted by tumor cells such as vascular endothelial growth factors (VEGF), thus blocking signaling by its cognate tyrosine kinase receptors (VEGFR).

Overall, the antibodies thus interfere with the activity of tyrosine kinase (TK) receptors involved in key steps of tumorigenesis. These receptors are essential ATP-dependent transducers of extracellular signals that cascade down along Ras/Raf/MAPK, PI3K/Akt and JAK/STAT pathways, regulating cell proliferation, differentiation, metabolism, migration or apoptosis. Aberrant activation of a number of TK receptors is indeed implicated in the initiation and progression of several types of human malignancies. (See also Section 2.2 for more

Table 1 – Overview of phase III trials in NSCLC evaluating passive immunotherapy.

Agent	Study	Disease setting	n	Treatment	mPFS HR (95% CI) p value	mOS HR (95% CI) p value	RR (%)
Cetuximab	FLEX ⁽¹⁾	First-line EGFR-expressing Advanced NSCLC	1125	CV + cetuximab versus CV	4.8 versus 4.8 HR = 0.943 (0.825–1.077), p = NS	11.3 versus 10.1 HR = 0.871 (0.762–0.996), p = 0.044	
	BMS-099 ⁽¹⁾	First-line Advanced NSCLC	676	C + P or D + cetuximab versus C + P or D	4.4 versus 4.2 HR = 0.902 (0.761–1.069), p = NS	9.7 versus 8.4 HR = 0.89 (0.75–1.05), p = NS	
Bevacizumab	ECOG 4599 ⁽²⁾	First-line Advanced NSCLC non-squamous	878	Bevacizumab (15 mg/kg) + CP versus CP	6.2 versus 4.5 HR = 0.66 (0.57–0.77), p < 0.001	12.3 versus 10.3 HR = 0.079 (0.67–0.92), p = 0.003	35 versus 15, p < 0.001
	AVAIL ⁽²⁾	First-line Advanced NSCLC non-squamous	1043	Bevacizumab (7.5 mg/kg) + CG Bevacizumab (15 mg/kg) + CG	6.7 versus 6.1 HR = 0.75 (0.62–0.91), p = 0.03 6.5 versus 6.1 HR = 0.82 (0.68–0.98), p = 0.031	Not yet reached	34 versus 20, p < 0.0001 30 versus 20, p = 0.0017

Abbreviations: NSCLC: non-small cell lung cancer; CV: cisplatin/vinorelbine; C + P or D: carboplatin + paclitaxel or doxorubicin; CG: cisplatin/gemcitabine; CP: carboplatin + paclitaxel; NS: not significant; mPFS: median progression-free survival; HR: hazard ratio; mOS: median overall survival; RR: response rate. (1) Adapted from (Stinchcombe and Socinski, 2009). (2) Adapted from (Reck et al, 2009)

detailed information on the activities of tyrosine kinase receptor signaling in NSCLC). By binding to either the extracellular domain of the receptor or to the ligand itself, these monoclonal antibodies prevent receptor-ligand interaction or receptor activation, which affects tumor growth and progression. Receptor-bound IgG antibodies also have the potential to trigger tumor killing through mechanisms of antibody-dependent cellular cytotoxicity (ADCC). Three antibodies belonging to this family of products, are now being tested in phase III trials: cetuximab, bevacizumab and figitumumab.

2.1.2.1. Cetuximab. Cetuximab (ErbixTM) is a chimeric human-murine monoclonal antibody that binds competitively and with high affinity to EGFR, preventing endogenous ligand binding to the receptor, thus blocking its activation. Antibody binding causes internalization of the receptor, inhibition of cell proliferation, enhanced apoptosis, reduced angiogenesis, invasiveness and metastasis (Silvestri and Rivera, 2005). It may also trigger antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity (Kim et al., 2009). This agent has been shown to enhance sensitivity to chemotherapy *in vitro* and in humans (Kim et al., 2009). Several clinical trials combining cetuximab with chemotherapy in the first- or second-line setting of NSCLC treatment have been carried out (Rosell et al., 2008). The FLEX phase III study indicated that the addition of cetuximab to chemotherapy (cisplatin/vinorelbine) in the first-line setting led to a modest overall survival (OS) benefit and increased tumor response rates in patients with EGFR-expressing advanced NSCLC (Table 1) (Pirker et al., 2009; Stinchcombe and Socinski, 2009). A marginal progression-free survival (PFS) benefit was seen in another phase III study (BMS-099) testing the addition

of cetuximab to another chemotherapy doublet (carboplatin/taxane) in unselected advanced NSCLC patients. However, this study was not powered to detect a significant difference in OS (Stinchcombe and Socinski, 2009) (Table 1).

Several other phase III trials are currently evaluating cetuximab as first- or second-line treatment in distinct NSCLC disease settings, and in combination with several other treatment modalities (Table 3). The main adverse effect from cetuximab was grade 3–4 acne-like skin rash.

2.1.2.2. Bevacizumab. Bevacizumab (AvastinTM) is an anti-VEGF humanized recombinant monoclonal antibody that binds and neutralizes all human VEGF-A isoforms, thus preventing them to interact with their receptors. The antibody blocks the growth of new blood vessels, can induce the regression of immature, VEGF-reliant tumor vasculature, and is thought to transiently normalize the tumor abnormal leaky blood vessels. This observation strongly suggests that combination of bevacizumab with chemotherapy could further improve the drug delivery and efficacy (Horn and Sandler, 2009; Manegold, 2008; Stinchcombe and Socinski, 2009). This antibody is particularly promising. It has recently been approved as first-line treatment for metastatic HER2-negative breast cancer, in combination with paclitaxel and as first- or second-line treatment of metastatic colorectal cancer.

An improved time to disease progression (TTP) and overall survival (OS) have indeed been shown in phase II clinical trials in advanced recurrent NSCLC patient receiving bevacizumab and platinum-based chemotherapy. However, severe side effects, including pulmonary hemorrhage, were recorded in patients with lung cancer of squamous histology. Based on this observation, the phase III trial (ECOG 4599) combined

Figure 1 – Overview of passive and active specific immunotherapeutic approaches for NSCLC. Passive immunotherapy approaches include administration of monoclonal antibodies targeting the extracellular domains of tyrosine kinase receptors (EGFR and IGF1R) or ligands of VEGFR (VEGF-A), thus affecting signaling required for tumor formation and progression. Active specific immunotherapeutic approaches entail ways of activating the immune system to recognize known or unknown tumor antigens to attack cancer cells. These approaches are based on the delivery of tumor antigens by different means initiating the uptake, processing and presentation of these antigens by antigen presenting cells (APC). This leads to activation and expansion of antigen-specific T cells which recognize and attack cancer cells presenting tumor antigen-derived peptides on their cell surface.

bevacizumab in the same settings but only in non-squamous NSCLC patients. This study confirmed the benefit in OS (12.3 versus 10.3 months, HR = 0.079 (0.67–0.92); $p = 0.003$), progression-free survival (PFS) (median 6.2 versus 4.5 months; HR = 0.66 (0.57–0.77); $p < 0.001$) and response rates (RR) (35 versus 15%; $p < 0.001$) for patients treated with the combination (Table 1).

These data represented a significant achievement in improving clinical outcomes in first-line advanced NSCLC. Bevacizumab-based therapy until progression is indeed the first treatment to achieve overall survival beyond 1 year in the first-line NSCLC setting and is a new standard of care for all eligible patients (Manegold, 2008). Unfortunately, this is associated with important side effects, such as neutropenia, hypertension, proteinuria, bleeding events and treatment-related deaths.

Other phase II trials (AVA11) showed that the benefits of adding bevacizumab also extend to the combination with other chemotherapy doublets (cisplatin/gemcitabine) in the same population (Table 1).

Currently, bevacizumab is also being evaluated in phase III in adjuvant settings, e.g. the ECOG E1505 trial assessing overall survival in patients with completely resected stage IB to stage IIIA disease, receiving cisplatin-based chemotherapy with or without bevacizumab (Supplementary Table S1).

2.1.2.3. Figitumumab. Figitumumab (CP-751871), is a potent human monoclonal antibody which selectively binds to IGF1R, a tyrosine kinase receptor expressed on most tumor cells that is involved in mitogenesis, angiogenesis, and tumor cell survival. Figitumumab prevents the binding of insulin-like growth factor type 1 (IGF1) to the IGF1R and as a consequence, the anti-apoptotic activity of IGF is reduced, and tumor growth is inhibited (Cohen et al., 2005). Results from a phase II trial which tested figitumumab as first-line treatment in advanced NSCLC showed an increased response rate in patients receiving the combination of figitumumab and chemotherapy (54%),

compared to chemotherapy alone (42%) (Karp et al., 2009). Figitumumab is currently being evaluated in a large program (ADViGO) of phase III trials to evaluate the antibody in combination with chemotherapy in first-line treatment for advanced NSCLC (Supplementary Table S1).

2.1.3. Active anticancer immunotherapy

Active cancer immunotherapy encompasses two main strategies to stimulate the immune system, one consisting in a non-specific stimulation with no tumor antigen specifically targeted, which will not be discussed in this paper. In contrast, the second strategy consists in mounting immune responses specifically targeting antigens expressed by tumor cells. This last approach can be based on the use of (i) whole tumor cells or tumor lysates prepared from autologous or allogeneic tumor cells, genetically modified to be rendered more visible by the immune system; (ii) dendritic cells loaded with tumor-derived products (peptides, lysates, RNA) or directly fused to tumor cells; (iii) recombinant DNA encoding tumor antigens delivered either naked or as recombinant live vectors (adenovirus, bacteria or yeast); (iv) plain or adjuvanted peptides derived from tumor antigens; or (v) tumor antigens delivered as adjuvanted recombinant whole proteins (Figure 1). Among the active immunotherapies under evaluation for NSCLC treatment, some have now reached evaluation in randomized phase III clinical trials (Bradbury et al., 2008).

Historically, the first attempts to treat NSCLC using immunotherapy have mainly used vaccines made of autologous or allogeneic whole tumor cells rendered more antigenic by engineering them to express immunostimulatory molecules like granulocyte macrophage colony-stimulating factor (GM-CSF) or interleukin 2 (IL2). This approach is attractive as the tumor cell is used as a source of antigens and it does not require any knowledge of the specific antigens that are targeted. The use of whole tumor cell vaccines has led to limited success in the treatment of other indications, such as melanoma and prostate cancer, and some have led to worse outcomes in

vaccinated patients, compared to controls (Goldman and DeFrancesco, 2009). However, at least one product of this kind has reached phase III development for NSCLC treatment: the allogeneic vaccine belagenpumatucel-L (Lucanix™).

With the identification of tumor antigens expressed by NSCLC cells, better characterized and targeted active immunotherapies are being developed. Three products, targeting two different tumor antigens, namely mucin-1 (MUC1), or MAGE-A3 have also reached late-stage clinical development. Another active immunotherapy target in NSCLC is EGF. The EGF-based vaccine, which is also in phase III clinical development, aims to mount an immune response to the ligand, thereby indirectly blocking oncogenic EGFR signaling. Results of phase II trials evaluating these immunotherapies are summarized below and in Table 2. It is important to note that some active immunotherapies have been evaluated in non-controlled phase II trials or in trials involving a limited numbers of patients, and efficacy was seen only in subset of patients. This is especially the case for trials with the VX-001 vaccine targeting yet another tumor antigen, hTERT, which is involved in preventing cellular senescence. The VX-001 vaccine consists of a cryptic peptide derived from the telomerase antigen hTERT, emulsified in Montanide™ ISA51. VX-001 has been tested in a small phase I/II trial involving 22 advanced NSCLC patients who had received at least one prior chemotherapy regimen. No objective response was observed in this study, but eight patients showed disease stabilization from 6.8 to more than 20 months (Bolonaki et al., 2007). Based on this proof of principle, a multicenter randomized phase III trial is now planned in locally advanced and metastatic NSCLC patients.

2.1.3.1. Belagenpumatucel-L. This vaccine, named Lucanix™, is composed of multiple lung cancer cell lines transfected with plasmid carrying a transforming growth factor $\beta 2$ (TGF $\beta 2$) antisense gene which inhibits the cellular TGF $\beta 2$ expression and results in increased immunogenicity of the cancer cells. The belagenpumatucel-L vaccine was tested in an open-label randomized dose-variable phase II trials involving 75 stage II, III and IV NSCLC, after chemotherapy, and some clinical activity was seen (Nemunaitis et al., 2006). Each patient received intradermally a maximum of 16 monthly injections of either 1.25, 2.5 or 5×10^7 cells/injection. The data suggested that at doses above to 2.5×10^7 cells/injection there was a trend for benefit in 1- and 2-year survival (68–52% for the highest doses of cells versus 39–20% for the lowest dose) for advanced patients ($n = 61$). The vaccine was well tolerated and no significant adverse events were reported. Based on this survival advantage, a placebo-controlled phase III evaluation is ongoing in advanced NSCLC patients.

GVAX is another product of this family of tumor cell vaccines to be evaluated for the treatment of NSCLC. It is an autologous tumor cell vaccine engineered to express granulocyte macrophage colony-stimulating factor (GM-CSF), but this product has just entered phase II.

2.1.3.2. EGF vaccine. The particularity of this vaccine is that it does not target the EGF receptor itself, but its main ligand EGF. Indeed, it is composed of the human recombinant EGF protein produced in yeast, chemically conjugated to a recombinant bacterial protein, P64K of *Neisseria meningitidis*, produced in

Escherichia coli (*E. coli*). The EGF-p64K conjugate is adjuvanted with a water-in-oil emulsion, Montanide™ ISA51. This adjuvant has been widely used and has been shown to induce T cell responses to different antigens. It provides a slow release of the antigen, which favours the induction of high antibody responses. A randomized, open, controlled phase II study evaluated the vaccine in eighty locally advanced or metastatic NSCLC patients (stage IIIB–IV). Patients were randomly assigned to the control group or to the group receiving the vaccine (containing 50 μ g of EGF-p64K protein given at 4 injection sites) once a week for 4 weeks and monthly thereafter. Vaccination started one month after first-line chemotherapy, irrespective of their response to the latter (Neninger Vinageras et al., 2008). Three days before the first injection, patients in the vaccine group also received a low dose of cyclophosphamide (200 mg/m²).

The EGF vaccine was shown to be well tolerated. Mostly grade 1–2 fever, headache and asthenia, and no grade 3 or 4 treatment-related adverse events were observed. In addition, there was a trend towards a survival advantage for the vaccine group. The production of EGF-specific antibodies led to a decrease in circulating levels of EGF, and a direct correlation was seen between antibody response, decrease in serum EGF, and survival. In patients younger than 60, vaccination was associated with more pronounced increased survival (Table 2), which may be linked with a better immune status of this patient subpopulation. Additional data were recently published showing that an increased dose of this EGF vaccine can be combined with a chemotherapy regimen without deleterious effect on the antibody response. Currently, phase III studies are ongoing with this EGF vaccine in Cuba and Malaysia.

2.1.3.3. L-BLP25. The vaccine named L-BLP25 or Stimuvax™, targets the exposed core peptide of MUC1. Indeed, aberrant glycosylation of the extracellular domain of MUC1 in cancer cells generates new carbohydrate epitopes and causes exposure of peptide epitopes. Both types of epitopes are acknowledged targets for active immunotherapy. L-BLP25 is a liposome-encapsulated 25-aminoacid-long peptide derived from the MUC1 protein formulated with monophosphoryl lipid A (MPL), a TLR4 agonist. The vaccine (subcutaneously injected weekly at 4 sites for 8 weeks followed by injection every 6 weeks) has been evaluated in an open, randomized phase IIb study in advanced stage IIIB/IV NSCLC patients who presented with stable disease or response to first-line chemotherapy. The observed survival difference of 4.4 months in favor of the vaccine did not reach statistical significance, but a post-hoc analysis by stage indicated that any potential benefit of vaccine treatment seems to be confined to patients with loco-regional stage IIIB disease. The L-BLP25 vaccine was well tolerated, grade 1 flu-like symptoms being the most common adverse events related to the treatment.

Although limited, these data prompted the decision to start a multicenter randomized, double-blind placebo-controlled phase III study (START) in unresectable stage III NSCLC following a response or stable disease after first-line chemoradiotherapy.

2.1.3.4. TG4010. The vaccine named TG4010 also targets the MUC1 antigen, but in this case the antigen is delivered by

Table 2 – Overview of phase II trials in NSCLC evaluating active immunotherapy providing data supporting the launch of phase III trials.

Therapy name	Antigen	Adjuvant	Cyclo treatment	Schedule of administration	NSCLC population targeted	Results
Belagenpumatucel-L (Nemunaitis et al., 2006)	4 allogeneic NSCLC cell lines transfected with a TGFβ2 antisense gene	No	No	Q1MX16 max One site ID 3 cohorts 1.25, 2.5 or 5 × 10 ⁷ cells/injection	Stage II, III, IV NSCLC Randomized open-label Uncontrolled phase II (n = 75) After first-line chemotherapy or not (patient refusal) Phase III ongoing	1–2 Y survival >2.5 × 10 ⁷ , 68–52% (CI 95%: 55–80% to 35–68%) <2.5 × 10 ⁷ , 39–20% (CI 95%: 22–563% to 4–36%)
EGF vaccine (Neninger Vinageras et al., 2008)	Recombinant Conjugated protein (50 μg) EGF-p64K Yeast-E. coli	Montanide™ ISA51	Yes 1 injection 200 mg/m ²	Q1WX4 Q1M 4 injection site IM	Advanced NSCLC Randomized (1:1) controlled phase II (n = 80) After first-line chemotherapy Irrespective of response Phase III ongoing	Median survival CTRL: 5.33 M GAR: 11.7 M PAR: 3.6 M 1 Y survival rate Vac: 67% (75% < 60 Y) CTRL: 33% (25% < 60 Y) In < 60 Y
EGF vaccine + chemotherapy (Neninger et al., 2009)	Recombinant Conjugated protein EGF-p64K Yeast-E. coli	Montanide™ ISA51	Yes 1 injection 200 mg/m ² , before vaccination	Q2W X2 vaccine, 4–6 cycles chemotherapy C/V Q1M vaccine 4 injection sites IM	Advanced NSCLC (n = 20), single arm study First-line treatment Phase III ongoing	Median survival Overall: 12.8 M sGAR: 19.3 M GAR: 8.4 M TTP: 8.4 M 1 Y survival rate: 70%
L-BLP25 (1000 μg) (Butts et al., 2005)	Muc1 lipopeptide Synthetic	Liposome -MPL	Yes 1 injection 300 mg/m ²	Q1W X8 Q6W 4 injection sites SC	Advanced NSCLC Randomized (1:1)- controlled Phase II (n = 171) Pts who respond (OR or SD) to first-line- chemotherapy/radio Phase III ongoing	Median survival Vac: 15.1 M CTRL: 12.9 M 2 Y survival rate Vac: 43% (St IIIB 60%) CTRL: 28.9%(St IIIB 36.7%)
TG4010 (10e8 pfu) (Acres et al., 2009)	Recombinant modified Ankara virus (MVA) MUC-1-IL2	No	No	TG4010 (10 ⁸ plaque forming unit) Q1W X 6 then Q3W 1 injection site SC TG4010 + C/G versus C/G alone	Advanced NSCLC Randomized (1:1)- controlled phase II (n = 148) First-line treatment MUC1 positive tumor by IHC (≥25% of cells) Phase III ongoing	6 M PFS TG4010 + C/G: 43% C/G: 35% Response rate TG4010 + C/G: 42% C/G: 28% TTP TG4010 + C/G: 5.9 M C/G: 5.2 M Overall survival TG4010 + C/G: 10.7 M C/G: 10.3 M

MAGE-A3 immuno therapeutic (300 µg) (Manuscript in preparation)	Recombinant MAGE-A3 protein <i>E. coli</i>	AS15 Liposomal MPL-QS21-CpG	No	Q3W X 5 Q3M X 8 1 injection site IM	Early stage IB/II NSCLC Double blind, randomized placebo-controlled (2:1) phase II (n = 182) First-line adjuvant therapy Phase III recruiting	27% relative improvement of disease-free survival compared to placebo
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Abbreviations: NSCLC: non-small cell lung cancer; Cyclo: cyclophosphamide; TGFβ2: transforming growth factor 2; EGF: epidermal growth factor; Y: year; M: month; W: week; ID: intradermal; IM: intramuscular; CI 95%: confidence interval at 95%; OR: objective response; SD: stable disease; CTRL: control; Vac: vaccine; GAR: vaccinated patients with good antibody response; PAR: vaccinated patients with poor antibody response; sGAR: super GAR; referring to patients with a high antibody titer; OS: overall survival; TTP: time to progression; PFS: progression-free survival; C: cisplatin; V: vinorelbine; G: gemcitabine.

a recombinant viral vector. It consists of a recombinant modified vaccinia virus strain Ankara (MVA) expressing both MUC1 and IL2 and it is designed to induce and amplify cellular immune responses against MUC1, IL2 playing the role of an adjuvant. This vaccine was evaluated in combination with standard chemotherapy (cisplatin/vinorelbine) in a first open phase II trial involving 65 patients with advanced NSCLC. The 2 arms of the study compared 2 treatment regimens consisting of a combination of vaccine with chemotherapy. TG4010 (10^8 plaque-forming units injected subcutaneously weekly for 6 weeks, then every 3 weeks) was either given upfront with chemotherapy ($n = 44$) or patients were first treated with TG4010 as monotherapy until disease progression, followed by TG4010 plus chemotherapy ($n = 21$). For the first treatment regimen, partial response was observed in 35.1% of the evaluable patients ($n = 37$), the median time to progression being 4.8 months, the overall survival (OS) 12.7 months and the survival at one year 53%. In contrast, in the second arm, no objective response was observed with the vaccine alone. Only 2 patients had disease stabilization for at least 6 months, which was not considered sufficient to meet the efficacy criteria. In both arms, the immune responses to the vaccine were measured, indicating that concomitant chemotherapy had no deleterious effect (Ramlau et al., 2008). Based on these data a second randomized, controlled, open-label phase II study further evaluated TG4010 in combination with first-line chemotherapy (cisplatin/gemcitabine) compared to chemotherapy alone. Although not statistically significant, this study again showed a benefit for the combination over chemotherapy alone in term of progression-free survival at 6 months (43% versus 35%), response rate (42% versus 28%), time to progression (5.9 versus 5.2 months) and overall survival (10.7 months versus 10.3 months) (Acres et al., 2009). In the 2 studies, the vaccine was well tolerated with mild to moderate injection site reactions, and the most frequent adverse events were flu-like symptoms and fatigue.

2.1.3.5. MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI). The MAGE-A3 antigen-specific cancer immunotherapeutic (ASCI) is made of a purified recombinant full-length MAGE-A3 protein produced in *E. coli*. (Vantomme et al., 2004) and formulated in an immunological Adjuvant System named AS02_B. AS02_B is an oil-in-water emulsion-based Adjuvant System which combines a vehicle to deliver the antigen and immunomodulatory molecules, such as MPL (a TLR4 agonist) and QS21 that have been shown to synergize to increase both humoral and cellular immune responses. (Garçon et al., 2007). The MAGE-A3 ASCI aims at stimulating an immune response towards the tumor antigen MAGE-A3 as illustrated in Figure 2. Using a full-length protein rather than a peptide has the advantage of presenting multiple potential epitopes, able to bind to all HLA molecules, which avoids the need to select the patient for the expression of one particular HLA. MAGE-A3 is an interesting antigen. It is a tumor-specific shared antigen and it has been previously validated as target for cancer immunotherapy. Indeed, T cells specific for the MAGE-A3 antigen have been isolated from melanoma patients and these cells were shown to recognize and kill MAGE-A3 expressing cancer cells (van der Bruggen et al., 1994). Early trials using the MAGE-A3 recombinant protein conducted in

Figure 2 – Theoretical mechanism of action of antigen-specific cancer immunotherapeutics (ASCI). ASCIs are designed to stimulate a comprehensive immune response against a tumor antigen, including both the cellular and humoral compartments. The immunostimulants contained in the Adjuvant System quickly activate the innate immunity and trigger the activation of antigen-presenting cells (APC), such as dendritic cells. These cells internalize and process the recombinant tumor antigen and display on their surface short antigen-derived peptides associated with human leucocyte antigen (HLA) molecules. The resulting matured APCs migrate from the periphery to the draining lymph nodes, where they enter into contact with naïve CD4⁺ and CD8⁺ T-lymphocytes. This triggers the activation and expansion of antigen-specific T-lymphocytes that acquire cytotoxic capacity, circulate, recognize and kill tumor cells that present the antigen-derived peptides on their cell surface.

either melanoma (Marchand et al., 2003; Vantomme et al., 2004) or NSCLC patients provided the evidence that this protein is poorly immunogenic by itself, and that an Adjuvant System is needed in order to induce strong and sustained immune responses (Atanackovic et al., 2004). The MAGE-A3 + AS02_B ASCI was evaluated in a double-blind, randomized, placebo-controlled phase II study involving 182 early (stage IB and II) NSCLC patients with MAGE-A3 expressing tumors as an adjuvant treatment after complete resection. Patients were treated with either a placebo ($n = 60$) or the MAGE-A3 protein in combination with the Adjuvant System ($n = 122$). The schedule of product administration consisted of 5 intramuscular injections at three-week intervals followed by 8 injections at three-month intervals. After 28 months of follow-up, a 27% relative improvement in disease-free interval was observed in the group receiving the MAGE-A3 ASCI when compared to the placebo (hazard ratio: 0.73; confidence interval: 0.44–1.20) (Vansteenkiste et al., 2007). Although the majority of patients reported some grade I and II side effects, such as injection-site related symptoms (pain, redness and swelling being the most common) and general symptoms such as flu-like symptoms, fatigue, myalgia and headaches as a result of the use of a strong Adjuvant System in this ASCI approach, only very few actually led to a treatment discontinuation. Therefore, it is considered as a well tolerated treatment, especially in the oncology setting. These encouraging data prompted the initiation of a multicenter worldwide double-blind randomized phase III trial, called MAGRIT (acronym for MAGE-A3 as Adjuvant non-small cell lung cancer Immunotherapy). The study is designed to evaluate the efficacy of

MAGE-A3 ASCI in patients with MAGE-A3-positive, completely resected stage IB, II, or IIIA tumors with or without prior chemotherapy. Based on preclinical and clinical data from a proof-of-concept phase II study in metastatic melanoma (Brichard and Lejeune, 2008; Kruit et al., 2008), an optimized Adjuvant System (AS15) is being used in this trial. It is a liposome-based Adjuvant System containing not only MPL and QS21 but also a low dose of CpG. (Krieg, 2002, 2007; Speiser et al., 2005). The trial is now recruiting, with a total of 2270 patients to be enrolled. The primary endpoint is disease-free survival.

2.1.4. Factors predictive of response to immunotherapy

From the above-mentioned data it is clear that, as for all other treatments, only a subset of patients respond to the immunotherapeutic treatments. Genetic expression profiling of the tumors is progressively used to help to predict the toxicity of new products, to better classify heterogeneous cancer patient populations and to define sub-populations with a higher chance of benefit from treatment. As of today, however, there are no established gene expression profiles able to predict response of NSCLC patients to cancer immunotherapy. The characterization of such putative predictive biomarkers has been investigated for the response to the MAGE-A3 ASCI in the above mentioned randomized study involving 182 stage IB/II NSCLC patients (Vansteenkiste et al., 2007). Patients were first selected based on the fact that their tumor expresses the gene coding for the MAGE-A3 targeted antigen. Next, the gene expression profiling was carried out using micro-array technology on tumor biopsies taken before

immunization. A specific gene profile was identified that defines a patient population in which MAGE-A3 ASCI reduce the relative risk of recurrence by 43%, instead of 27% when considering the total population of the trial (Vansteenkiste et al., 2008). Interestingly, this predictive gene signature identifies essentially immune-related genes, and half of them are also found among the predictive gene signature associated with clinical benefit in response to the MAGE-A3 ASCI in metastatic melanoma patients (Louahed et al., 2008). These findings might allow in the near future the determination of a few key genes in the microenvironment of the tumor that could be predictive of clinical benefit from the treatment with MAGE-A3 ASCI, and maybe with immunotherapy in general.

2.2. Targeting tyrosine kinase receptors with small molecule inhibitors

Inhibition of tyrosine kinase receptor (TKR) activity using small-molecule inhibitors as anticancer treatment is also a field of investigation that has exploded in the last years (Krause and Van Etten, 2005). Amongst the most studied TK receptors are those belonging to the erb-B family, which includes EGFR (also named erb-B-1). In human cancer cells, the EGFR pathway can become constitutively activated by EGFR gene mutation, amplification/over-expression (Charpidou et al., 2008) or autocrine production of ligand. In NSCLC, overexpression of EGFR, as assessed by IHC, is reported to occur in 62% of NSCLC (Hirsch et al., 2003). Aberrant EGFR signaling has been associated with increased tumor proliferation, poor differentiation, enhanced metastatic potential, chemoradio resistance and a bad prognosis (Charpidou et al., 2008).

Lung cancer cells also produce EGF, and detectable levels of circulating EGF are found in the serum of lung cancer patients (Neninger Vinageras et al., 2008). Moreover, cross-talk between components of different TK receptor signaling cascades exists. For example, activation of EGFR can increase the production of vascular endothelial growth factor (VEGF) in human cancer cells (De Luca et al., 2008). VEGF, which is also secreted by tumor cells under conditions of hypoxia, triggers the formation of new vessels, hence increasing the supply of nutrients and oxygen. This does not only permit further growth of the primary tumor, but also provides a pathway for migrating tumor cells to gain access to systemic circulation and to establish distant metastases. Its importance in the course of NSCLC has been demonstrated by studies showing that VEGF mRNA levels correlate with tumor angiogenesis, patient survival and post-operative relapse (Yuan et al., 2001).

Insulin-like growth factor type I receptor (IGF1R) and platelet-derived growth factor receptor (PDGFR) are two other possible targets. IGF1R is another TK receptor expressed on most tumor cells that is involved in mitogenesis, angiogenesis, and tumor cell survival. IGF1R is a target of particular interest in NSCLC as it was recently observed that high co-expression of both IGF1R and EGFR is associated with shorter disease-free survival in early NSCLC patients (Ludovini et al., 2009). The conceptual basis for targeting PDGFR along with other TK receptors is based on its possible function in causing tumoral interstitial hypertension, thereby blocking efficient delivery of chemotherapy, and its function in the homeostasis

of pericytes, key endothelial support cells. Cytoplasmic expression of PDGF occurs in 60% of the NSCLC specimens and has been reported as a negative prognostic indicator (Bauman et al., 2007).

Small molecules blocking the activity of one or more TK receptors have been developed, acting at the catalytic site of the kinase and interfering with the activation of natural substrates or with ATP binding.

In the context of NSCLC, a number of agents that inhibit the activity of TK receptors have reached late-stage clinical development and are progressively integrated as standard of care, mostly as second or third line treatment for advanced stages of the disease and usually in combination with other therapies. Currently, phase III trials are ongoing to evaluate some of these molecules in adjuvant settings as well. Importantly, subset or pooled analyses of data from clinical trials have contributed to the identification of predictive markers for toxicity and clinical benefit, which paves the way towards individualized, tumor-tailored therapy.

2.2.1. Single targeted tyrosine kinase inhibitors: erlotinib and gefitinib

Besides the monoclonal antibody-based approaches, two EGFR TK inhibitors (EGFR TKI) are being investigated to treat NSCLC, namely gefitinib (*Iressa*TM) and erlotinib (*Tarceva*TM). Both agents bind competitively with ATP at the catalytic cleft of EGFR, suppressing receptor autophosphorylation and subsequent downstream signaling. Both agents have been tested as monotherapy and in combination with other treatments. So far, the outcome of phase III trials combining gefitinib or erlotinib with chemotherapy has been disappointing. These EGFR TKIs did not improve survival when added to chemotherapy in first-line treatment. Indeed, four randomized phase III trials (INTACT 1–2 trials, TRIBUTE and TALENT) failed to demonstrate any improved efficacy of chemotherapy plus gefitinib or erlotinib, as compared with chemotherapy alone, possibly due to mechanistic interaction between the TKIs and chemotherapy (Table 3); TKIs, by blocking the cells in the G1 phase of the cell cycle, potentially alleviate the effect of chemotherapy. The negative results obtained with gefitinib and erlotinib are in sharp contrast with the clinical results seen with cetuximab. This difference could be related to the different mechanisms of action of these agents, e.g. the ability of cetuximab to induce internalization of the antibody receptor complex and to induce antibody-dependent cell-mediated toxicity as well as complement-dependent cytotoxicity. The results of the trials with gefitinib and erlotinib suggested that sequential rather than concomitant administration of these agents could be a better therapeutic approach. This was tested in a number of phase III trials. The SWOG0023 trial, which compared the effects of gefitinib to placebo as maintenance therapy in unresectable stage III NSCLC, failed to show a survival advantage for the gefitinib-treated patients. Similarly to gefitinib, erlotinib is being tested as maintenance therapy in advanced NSCLC (SATURN trial, Supplementary Table S1). The first data released indicate a statistically significant improvement in progression-free survival for erlotinib compared to the placebo control in the overall patient population, including patients with squamous carcinoma (Cappuzzo et al., 2009).

Table 3 – Overview of reported phase III trials in NSCLC with small molecules blocking tyrosine kinases (www.clinicaltrials.gov).

Agent	Study	Disease setting	n	Treatment	mPFS HR (95% CI)	mTTP	mOS (months)	RR (%)
Erlotinib	BR.21 ⁽¹⁾	2nd and 3rd line Second-line Advanced NSCLC	691	Erlotinib versus placebo	2.2 versus 1.8 HR = 0.70 (0.58–0.87); $p < 0.001$		6.7 versus 4.7; HR = 0.61 (0.51–0.74); $p = 0.001$	9 versus < 1 ($p < 0.0001$)
	BETA ⁽¹⁾	Second-line Advanced NSCLC	626	Erlotinib monotherapy versus erlotinib + bevacizumab	1.7 versus 3.4; HR = 0.62 (0.52–0.75); $p < 0.0001$		9.2 versus 9.3; HR = 0.97 (0.80–1.18); ($p = \text{NS}$)	6.2 versus 12.6 ($p = 0.006$)
	TRIBUTE ⁽¹⁾	First-line Advanced NSCLC	1079	CP + erlotinib versus CP + placebo		5.1 versus 4.9 ($p = \text{NS}$)	10.6 versus 10.5 ($p = \text{NS}$)	21.5 versus 19.3 ($p = \text{NS}$)
	TALENT ⁽¹⁾	First-line Advanced NSCLC	1069	CG + erlotinib versus CG + placebo		5.1 versus 4.9 ($p = \text{NS}$)	10.0 versus 10.3 ($p = \text{NS}$)	31.5 versus 29.9 ($p = \text{NS}$)
Gefitinib	ISEL ⁽²⁾	Second and 3rd Advanced NSCLC platinum-refractory disease	1692	Gefitinib monotherapy versus placebo			5.6 versus 5.1; HR 0.89 (0.77–1.02) ($p = \text{NS}$)	8 versus 1 ($p < 0.0001$)
	INTEREST ⁽²⁾	Second-line platinum refractory	1466	Gefitinib monotherapy versus docetaxel	2.2 versus 2.7; HR = 1.04 (0.93–1.18)		7.6 versus 8; HR 1.02 (0.90–1.15) ($p = \text{NS}$)	9.1 versus 7.6 ($p = \text{NS}$)
	INTACT-1 ⁽²⁾	First-line Advanced NSCLC	1093	CG + gefitinib versus CG + gefitinib versus CG + placebo	5.8 versus 5.5 versus 6.0 ($p = \text{NS}$)		9.9 versus 9.9 versus 10.9 ($p = \text{NS}$)	51.2 versus 50.3 versus 47.2 ($p = \text{NS}$)
	INTACT-2 ⁽²⁾	First-line Advanced NSCLC	1037	CP + gefitinib (250 mg/day) versus CP + gefitinib (500 mg/day) versus CP + placebo	5.3 versus 4.6 versus 5.0 ($p = \text{NS}$)	NR	9.8 versus 8.7 versus 9.9 ($p = \text{NS}$)	30.4 versus 30 versus 28.7 ($p = \text{NS}$)
	V-15-32 ⁽³⁾	Second-line Advanced NSCLC	489	Gefitinib versus docetaxel	2.0 versus 2.0 ($p = 0.34$)		11.5 versus 14 ($p = 0.33$)	22.5 versus 12.8 ($p = 0.009$)
	SWOG0023	Maintenance therapy after chemoradiation stage III NSCLC	243	Gefitinib versus placebo	8.3 versus 11.7 ($p = 0.17$)		23 versus 35 ($p = 0.01$)	
	WJTOG0203	following platinum-doublet chemotherapy Advanced NSCLC	600	Chemotherapy with gefitinib versus chemotherapy	4.6 versus 4.2 ($p < 0.001$)		13.68 versus 12.89 ($p = 0.10$)	34.2 versus 29.3

Abbreviations: CV: cisplatin/vinorelbine; C + P or D: carboplatin + paclitaxel or doxorubicin; CG: cisplatin/gemcitabine; CP: carboplatin + paclitaxel; n: number of patients; mPFS: median progression-free survival; mTTP: median time to progression; mOS: median overall survival; RR: response rate; NS: not significant. (1) Adapted from (Katzel et al, 2009). (2) Adapted from (Hida et al, 2009).

Results from another similar trial (WJTOG0203) demonstrate a possible clinical benefit for sequential therapy of gefitinib, especially in patients with tumors of adenocarcinoma histology (Hida et al., 2009). The INTEREST trial, in addition, demonstrated non-inferiority of gefitinib as monotherapy, compared with docetaxel in the previously treated-patients, with improved quality of life and less toxicity for gefitinib. Dose-dependent and reversible diarrhea and acne-like rashes are the most frequently reported side effects for these EGFR TKIs.

2.2.2. Multi-targeted tyrosine kinase inhibitors

2.2.2.1. Targeting VEGF and EGFR signaling: vandetanib. The development and progression of lung cancer results from complex interplay between different signaling pathways. Therefore, targeting multiple pathways simultaneously appears to be a rational approach. In addition, such combined strategies might help to combat possible mechanisms of resistance to one of the anticancer agents. This idea is pursued further with the clinical development of multi-targeted tyrosine kinase inhibitors acting on several tyrosine kinase receptors at once. One such molecule is vandetanib (Zactima™), a dual TK inhibitor that targets both VEGFR and EGFR receptors. A series of phase II trials in patients with advanced NSCLC have demonstrated the anti-tumor activity of vandetanib, both as monotherapy and in combination with certain chemotherapies. This product is currently being evaluated in several phase III studies as second-line treatment for advanced NSCLC patients who failed first-line chemotherapy or failed treatment with an EGFR TKI (ZEAL, ZODIAC, ZEST and ZEPHIR trials) (Supplementary Table S1). Overall, the most commonly reported adverse events in these studies were rash, diarrhea and hypertension.

2.2.2.2. Targeting VEGFR and PDGFR: pazopanib, sorafenib and sunitinib. A number of other agents are able to inhibit the activity of the VEGFR and PDGFR receptor families along with some other tyrosine kinases. By inhibiting both VEGFR and PDGFR function, these agents target both endothelial cells and pericytes.

2.2.2.2.1. Pazopanib. This is a TKI that targets VEGFR, PDGFR and the stem cell factor receptor c-KIT. In one phase II study, 86% of patients with stage I–II NSCLC who received neo-adjuvant pazopanib monotherapy experienced a reduction in tumor volume (Altorki et al., 2008). Based on these promising data, another phase II trial is ongoing in advanced NSCLC patients (stage IIIB–IV), and a randomized, double-blind, placebo-controlled phase II–III study is planned to evaluate pazopanib versus placebo as adjuvant treatment in patients with stage I NSCLC (tumor ≤ 5 cm).

2.2.2.2.2. Sorafenib and sunitinib. Sorafenib (Nexavar™) inhibits the activity of Raf-kinases, VEGFR-2, PDGFR-B and the stem cell factor receptor c-KIT, whereas sunitinib (Sutent™) targets the PDGFR family, c-KIT, fms-like tyrosine kinase3 (FLT-3), VEGFR and RET. Both sorafenib and sunitinib have been approved by the US food and drug administration (FDA) for the treatment of renal cell carcinoma but have also

shown promising activity in patients with advanced NSCLC (Katzel et al., 2009). Some interesting objective responses and stable diseases were observed in a phase II trial (Blumenschein et al., 2008), which prompted the design of three ongoing randomized phase III trials investigating sorafenib with different doublets of chemotherapy. Future clinical development of sunitinib in the treatment of advanced NSCLC includes the combination with chemotherapy or with other targeted therapies. Sunitinib is also evaluated in a phase III trial with erlotinib in second-line advanced metastatic NSCLC (Supplementary Table S1).

2.2.3. Factors predictive of response to tyrosine kinase inhibitors

Interestingly, retrospective as well as prospective analyses of a number of trials involving tyrosine kinase inhibitors especially those targeting the EGFR, indicated that clinical benefit is considerably increased in patients with specific clinical characteristics, i.e. patients of female gender, Asian origin, non-smokers and patients with adenocarcinoma histology of the tumor. These characteristics were later correlated with the presence of somatic mutations in the EGFR (most commonly small in-frame deletions in exon 19 or an L858R amino-acid substitution) that result in increased EGF-induced activation and gefitinib-induced TK inhibition (Heist and Christiani, 2009; Neninger Vinageras et al., 2008; Sequist et al., 2007; Silvestri and Rivera, 2005). These findings were extended to erlotinib-responsive cases (Sequist et al., 2007). Increased EGFR gene copy number and EGFR protein overexpression were also reported to correlate with improved response and survival following gefitinib treatment of advanced NSCLC (Sequist et al., 2007). The importance of EGFR mutations was assessed further in the IPASS phase III trial. Subset analysis in patients with activating EGFR mutations demonstrated a significantly longer PFS with gefitinib than with carboplatin and paclitaxel (HR 2.85, 95% CI 2.05–3.98, $p < 0.0001$). In contrast, in patients without activating EGFR mutations, the PFS was significantly shorter with gefitinib than with carboplatin and paclitaxel. Overall, these data could change the treatment paradigm in advanced NSCLC (Fukuoka et al., 2009; Stinchcombe and Socinski, 2009). Altogether, however, it remains unclear whether patients with EGFR mutation gain a survival benefit when treated with EGFR inhibitors or whether the mutations are positive prognostic indicators outside the setting of TKI inhibition (Charpidou et al., 2008; Hida et al., 2009). Moreover, despite the clinical benefit, a large number of patients with EGFR mutations eventually experience disease progression associated with the occurrence of secondary mutations in the receptor (Heist and Christiani, 2009). Another biomarker predicting response to TKI treatment is the presence or absence of mutations in the K-ras oncogene which result in its constitutive activation. In this case mutations have been associated with primary resistance to gefitinib and erlotinib (Linardou et al., 2008). Nevertheless, reported SATURN trial data suggested that patients whose tumors present K-ras mutation should not be excluded from treatment with erlotinib (Brugger et al., 2009). Recent studies using pre-treatment serum from patients report the identification of proteomic classifiers predicting the response to erlotinib (Salmon et al., 2009) or both

erlotinib and gefitinib (Taguchi et al., 2007). In addition, germline polymorphisms in the EGFR gene or EGFR pathway-related genes are also being assessed as predictive factors for response or toxicity to EGFR TKI treatment (Heist and Christiani, 2009).

Today it is not fully clear who should or should not receive TKIs. Similar searches for clinical or molecular markers predicting response or resistance to molecules targeting TK receptors other than EGFR are probably underway and should provide useful information to better select the patient population that has the highest chance of benefiting from these treatments.

3. Combination of new treatments in NSCLC

With the development of the tumor-targeted approaches to treat lung cancer, new treatment combinations might be envisaged, some being already under clinical evaluation. Currently, several of the above-discussed single targeted agents to attack both angiogenic and proliferative signaling are being evaluated as combinations e.g. cetuximab and bevacizumab together with chemotherapy in patients with incurable NSCLC, or bevacizumab combined with erlotinib (Pennell and Lynch, 2009).

Recent results demonstrate longer progression-free survival (PFS) or response rate (RR) for the combination of bevacizumab with erlotinib compared to bevacizumab plus placebo (ATLAS – BeTA studies – Supplementary Table S1 and Table 3). The possibility to combine immunotherapy with other anti-tumor strategies is also a relevant option to be explored. As previously mentioned, multiple tumor antigens have been found to be expressed by NSCLC cells. Combination of immunotherapies targeting more than one antigen would allow the treatment of patients whose tumor expresses multiple antigens, and compensate for the fact that not all cells inside a tumor express a given targeted antigen. In addition, it can be envisaged to combine cancer immunotherapy with other targeted biological therapies, including TKIs. Indeed, as these approaches are based on very different modes of action, it might be expected that their respective effect be at least additive or even synergistic. There is increasing evidence of cross-talk of TK signaling with the immune system. For example, VEGF is emerging as a significant agent of immune tolerance in the tumor microenvironment. Consequently, anti-VEGF therapies might enhance the response of tumors to immunotherapy (Johnson et al., 2007). Sunitinib has recently been described to positively impact the immune system in mice and human and it has been shown to act in a synergistic manner with an immune therapy in large tumor-bearing mice (Ozao-Choy et al., 2009).

Primary tumors are currently treated by a combination of surgery, local radiotherapy and chemotherapy. Combining cancer immunotherapy with such standard oncology treatments is also a relevant approach. Although the combination of chemotherapy with vaccines has generally been considered as implausible because of toxic effects on the immune system, there is now evidence that some of the chemotherapeutic agents commonly used in the treatment of NSCLC have immunostimulatory activities both in mouse and human (Zitvogel et al., 2008). A recent trial reports that the administration of

the EGF vaccine before, as well as after first-line chemotherapy in advanced NSCLC patients is feasible and a well tolerated induction of high anti-EGF antibody titers, correlating with survival, was seen (Neninger et al., 2009). In addition, although high doses of cyclophosphamide have direct tumoricidal effects and cause immunosuppression by lympho-ablation, low doses of the same compound have been clearly shown to break tolerance and enhance active specific immunotherapy. The mechanisms underlying these beneficial effects are not yet fully understood but could be due to enhanced homeostatic expansion of T cells, stimulation of the innate immunity, induction of interferons, and/or elimination of proliferating T cells, including regulatory T cells (Salem et al., 2007). Patients in the two phase II studies with the EGF vaccine and L-BLP25 vaccine received a single injection of low-dose cyclophosphamide (CY) before starting the vaccine regimen. From these studies, it is difficult to draw a conclusion on the added value of the single injection of a low dose of CY on vaccine efficacy. To date, no randomized human trial has been designed to show the impact of this pre-treatment. Nevertheless, the administration of metronomic low dose CY in advanced cancer patients confirmed the reduction of regulatory T cells in blood. This led to restoration of immune responses and reduction of tumor angiogenesis (Ghiringhelli et al., 2007). In summary, depending on the chemotherapeutic agent and administration schedule, windows may exist in which combinations might be beneficial.

4. Discussion

NSCLC is the leading cause of cancer-related death worldwide and today, late diagnosis of this disease is common with more than 60% of patients presenting with advanced or metastatic stage IIIB/IV. The outcome of these advanced patients remains poor despite optimized chemotherapy regimens, explaining why most of the trials evaluating potential new treatments were conducted in that patient population. Currently, only a few trials evaluate emerging therapeutics in the adjuvant setting, such as the RADIANT trial with erlotinib (Supplementary Table S1), the ECOG E1505 (NCT00324805) trial with bevacizumab and the MAGRIT trial with the MAGE-A3 ASCI (see Supplementary Table S1 and Table 2). New screening methods are under development to detect earlier stages of NSCLC and even pre-neoplastic lesions (Bauwens and Ninane, 2007) where new agents with better tolerability and good safety profile could play a role.

It is interesting to note that some immunotherapeutic approaches have now demonstrated sufficient activity in phase II trials to justify the start of large confirmatory phase III trials. This delay in clinical development compared to the TK-targeted treatments, for which phase III data are already available, may be indicative of the complexity of these approaches. In contrast to small molecules or antibodies used to target the TKR, immunotherapies can not be tested in *in vitro* assays and their efficacy can not be quickly evaluated through bioavailability studies, pharmacokinetics or tumor xenograft models. Past experience, has shown that immune responses induced by immunotherapeutic approaches take time to reach their maximum amplitude and require an immune system which has not been impaired by the disease

or by multiple previous treatments. The fact that most of the early trials were conducted in advanced NSCLC patients has certainly reduced the chances of immunotherapies to show their efficacy and has probably slowed down their clinical development. This confirms the need to propose these new approaches as adjuvant treatment to NSCLC patients in early stage of the disease, in patients who are at high risk of relapse after surgery.

If we compare the phase II data obtained with all the immunotherapeutic approaches in order to try to extract common features that could explain their respective success, it is striking to note that they have little in common. Each of the targeted tumor antigens has particularities. For example, MAGE-A3 is an intracellular protein that belongs to the tumor-specific shared antigens. The EGF vaccine directly targets soluble EGF to avoid its binding to EGFR, expressed by normal cells. Likewise, MUC1 is also expressed by normal cells but is a surface antigen. Today there is no firm evidence supporting that one family of antigen would be a better target for immunotherapy than another in term of immunogenicity, but some kind of tolerance to antigens constantly exposed to the immune system would not be surprising (Lucas and Coulie, 2008).

The identification of predictive biomarkers is key to selecting the best population that is likely to benefit from a given treatment. For classical treatments like chemotherapy, low expression levels by individual tumors of molecular markers such as the DNA repair genes ERCC1 (excision repair cross complementation) and RRM1 (the regulatory subunit of ribonucleotide reductase) have been associated with better response to gemcitabine and platinum-based chemotherapy treatment respectively. These markers are now used to customize chemotherapy treatments (Bepler et al., 2008). The identification of biomarkers which could predict response to either the TKR-targeted treatments and immunotherapy will also be key. For some of the treatments, not only the presence of the targeted molecule should be demonstrated (i.e. EGFR) but the molecular status (i.e. EGFR mutation) of the target may be critical and could predict response to treatment. Moreover, the histology of the tumor might be important in the choice of treatment; for example gefitinib is more active in squamous NSCLC, whereas bevacizumab is more efficient and less toxic in adenocarcinoma NSCLC.

The importance of using biomarkers to better select the patient population and customize treatments has been highlighted. Not only biomarkers able to predict response to treatment, but also markers able to predict potential treatment associated toxicity, should be used (Gettinger, 2008). This is of particular importance before combining different treatments.

The efficacy of these new treatments should be put in balance with their toxicity. Indeed, adverse events obtained with monoclonal antibodies or TKI are ranging from acne-like rash (cetuximab) to bleeding, neutropenia or hypertension (bevacizumab). In contrast to standard treatment or to the TKR targeted approaches, all the immunotherapies tested in phase II were reported to be well tolerated. Only few severe adverse events (grade 3–4) were considered to be treatment-related and the majority of the adverse events were grade 1 or 2 fatigues and flu-like symptoms.

In conclusion, future effective treatments for NSCLC will probably rely on the combination of multiple new and/or standard therapies with different mechanisms of action. Within the choice, immunotherapeutic agents which are well tolerated treatments are now becoming available for testing. All the ongoing phase III studies should provide data leading to a better understanding of the mode of action of each of these new agents and also will help to optimize combined treatment regimens. These new treatments should be given to patients who are the most likely to respond because they have been carefully selected, based on their history, the histology or the genetic profile of their tumor.

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Conflict of interest statement

Authors are either employees of GlaxoSmithKline Biologicals or work under contract for GlaxoSmithKline Biologicals.

Appendix. Supplementary information

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