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EXPERT REVIEWS

Therapeutic cancer vaccines in the treatment of non-small-cell lung cancer

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Therapeutic vaccines are different from the well-known prophylactic vaccines in that they are designed to treat patients already suffering from a disease instead of preventing the disease in healthy individuals. Several therapeutic vaccines are today in late-stage clinical development for non-small-cell lung cancer. These vaccines use different approaches including peptides, cell lines and viral vectors, and explore different settings within the pathology. Some are given in monotherapy while others are combined with the classic therapies used with non-small-cell lung cancer. This review gives a summary of the therapeutic vaccines currently in late-stage clinical development for non-small-cell lung cancer.

KEYWORDS: cancer vaccine • immunotherapy • lung cancer • MUC-1 • NSCLC • predictive biomarker

Lung cancer is the most frequent cancer worldwide with a majority of cases corresponding to non-small-cell lung cancer (NSCLC). Unfortunately, less than 40% of cases are resectable at the time of diagnosis and many operated patients will relapse after surgery. In patients with unresectable localized NSCLC, a combination of chemotherapy and radiation therapy is the preferred option. In the case of advanced or metastatic NSCLC, palliative chemotherapy has been proven to improve survival but leaves a clear medical need to be fulfilled by new innovative therapies [1].

Among these new treatments, immunotherapy, with its long-lasting activity, can change the course of the disease by either decreasing the relapse rate after surgery and chemoradiotherapy, or by significantly lengthening the survival of patients with advanced stage NSCLC. Small inhibitory molecules and monoclonal antibodies target tumor growth, angiogenesis or both [2]. Immunotherapy of NSCLC consists of mainly therapeutic vaccination designed to induce or amplify an immune response directed against tumor-associated antigens. These approaches usually have in common stimulation of a cellular immune response and sometimes innate immunity [3,4] (for a review see [5]).

Numerous approaches are being pursued to overcome immune tolerance in cancer patients. TABLE 1 presents lung cancer vaccines in late stage development.

Peptide-based vaccines

L-BLP25: Stimuvax® (emepepimut-S, Merck Serono, Geneva, Switzerland)

Emepepimut-S is a therapeutic vaccine designed to induce an immune response to cancer cells expressing the MUC-1 antigen. MUC-1 is a component of the glycocalyx protecting the epithelia but the molecule also has a regulatory activity through its C-terminal intracellular domain. The MUC-1 protein is a highly glycosylated mucin (molecular weight: >200 kDa), normally found at the apical surface of mucin-secreting epithelial cells in many types of epithelial tissues and frequently overexpressed in cancers of epithelial origin. The tumor marker CA15.3 is the result of the shedding of MUC-1 in the bloodstream and is used as a test for the follow-up of patients with MUC-1-expressing tumors such as breast cancer. In cancer cells MUC-1 usually bears an aberrant glycosylation, revealing new peptide and carbohydrate epitopes [6–9]. Expression of MUC-1 is detected in more than 80% of NSCLC by reverse transcription PCR. The expression of MUC-1 in tumors analyzed by immunohistochemistry often displays a depolarized and sometimes cytoplasmic expression. The expression of MUC-1 in NSCLC with both methods of detection has been associated with a worse prognosis in several studies [10,11]. The L-BLP25 vaccine, a liposomal formulation of a 25-amino acid MUC-1 sequence from the

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Table 1. Selected lung cancer vaccines in advanced development.

Type of vaccine	Company	Product	Composition	Phase
Idiotype	Innogene-Kalbiotech (Singapore)	Racotumomab	Murine anti-idiotypic monoclonal antibody to antiganglioside P3 antibody	III
Vector-based	Transgene SA (Illkirch, France)	TG4010	MVA expressing *MUC-1* and *IL-2* genes	IIb
	GlobeImmune, Inc. (CO, USA)	GI-4000	Yeast-based Tarmogen® products engineered to express the seven most common *KRAS* mutations	II
DNA/RNA	Inovio Pharmaceuticals, Inc. (PA, USA)	VGX-3100	DNA plasmids targeting E6 and E7 proteins of HPV types 16 and 18	II
	CureVac GmbH (Tübingen, Germany)	CV-9201	mRNA-based vaccine, expressing five antigens frequently overexpressed in NSCLC	II
Protein-based	KAEL-Gemvax (Seongnam-si, South Korea)	GV1001	Telomerase peptides vaccine	III
	GlaxoSmithKline plc (London, UK)	GSK1572932A (astuprotimut-R, MAGE-A3)	Melanoma MAGE-A3 antigen, in combination with the AS15 adjuvant, the VaxImmune adjuvant, or the QS-21 adjuvant	III
	Oncothyreon (WA, USA); Merck-Serono (Geneva, Switzerland)	Stimuvax®, BLP 25	Liposome-encapsulated 25-mer peptide of MUC-1, conjugated to immunoadjuvant monophosphoryl lipid A	III
	Vaxon Biotech (Paris, France)	VX-001	Telomerase peptides vaccine	II
	Vaxil BioTherapeutics (Nes-Ziona, Israel)	ImMucin	MUC-1 peptide-based vaccine	II
	Orphan Synergy Europe (Paris, France)	EP2101	Nine CTL epitopes from four tumor-associated antigens plus the PADRE universal helper T-lymphocyte epitope	II
Whole-cell cancer	NovaRx Corp (CA, USA)	Lucanix™ (belagenpumatucel-L)	Allogeneic tumor cells expressing an antisense DNA specific for TGF-β	III
	NewLink Genetics (IO, USA)	Turgenpumatucel-L polyvalent HyperAcute-Lung cancer vaccine	Irradiated allogeneic NSCLC cell lines (HAL-1, -2 and -3) transduced with a MoMLV-based retroviral vector expressing the murine α-1,3-galactosyltransferase (α-Gal) gene	II
	Heat Biologics, Inc. (FL, USA)	Ad100-gp96Ig-HLA A1	Irradiated lung cancer cells transfected with gp96-Ig	II

MoMLV: Moloney murine leukemia virus; MVA: Modified virus of Ankara; NSCLC: Non-small-cell lung cancer.

tandem repeat sequence of MUC-1, is thought to work by stimulating a T-cell-mediated anti-MUC-1 response to cancer cells. The vaccine is in development by Merck-Serono under a license agreement with Oncothyreon (WA, USA). It is administered by eight consecutive weekly subcutaneous vaccinations. In a randomized Phase II trial, 171 patients received subcutaneous vaccinations of L-BLP25 930-µg weekly for 8 weeks, followed by maintenance vaccinations at 6-week intervals plus best supportive care (BSC) or received BSC alone. Median survival time was longer in patients receiving L-BLP25 plus BSC compared with those receiving BSC alone (17.2 vs 13.0 months, respectively). The 3-year survival rate was 31% in patients who received L-BLP25 plus BSC and 17% in those who received BSC alone. In the subset of patients with stage IIIb locoregional disease, median survival time was longer in patients who received L-BLP25 plus BSC than in those who received BSC alone (30.6 vs 13.3 months, respectively) [12]. No major adverse events except some temporary injection site reactions were reported; no autoimmune reactions were noted. Two Phase III trials of Stimuvax versus placebo are ongoing in patients with NSCLC:

- START (ClinicalTrials.gov identifier: NCT00409188 [104]) began in December 2006 and has enrolled more than 1300 patients with unresectable stage IIIA or IIIB tumors who have had a response or stable disease after at least two cycles of platinum-based chemoradiotherapy. The primary end point of this trial is overall survival. In December 2012, Merck KGaA (Darmstadt, Germany) announced that the study did not meet its primary end point, even if notable effect was seen in subgroups of patients [101];
- INSPIRE (ClinicalTrials.gov identifier: NCT01015443 [104]) is similar in design to START; patient enrolment was initiated in December 2009. The primary end point is also overall survival.

GSK1572932A (MAGE-3 ASCI, GlaxoSmithKline plc [London, UK])

MAGE-A3 is a melanoma-associated antigen also expressed in 35% of NSCLCs. The vaccine GSK1572932A contains the entire 360 amino acid-long MAGE-A3 antigen formulated and delivered with proprietary adjuvants (AS02B). Phase II studies had shown that patients with NSCLC who received repeated vaccinations developed a long-lasting T-cell response to this vaccine. The ongoing Phase III trial is designed to evaluate the efficacy of GSK1572932A in preventing recurrence of NSCLC after surgery (MAGRIT; NCT00480025 [104]) [13]; in this placebo-controlled trial, GSK1572932A is administered as an adjuvant to nonmetastatic NSCLC patients following surgical removal of their tumor or following adjuvant chemotherapy, if any. This trial is the largest and most extensive lung cancer study ever conducted; it has enrolled 2270 patients in stages IB, II, or IIIA, and plans to last until September 2022. Patients receive 13 injections over a period of 27 months; the first five injections are given every 3 weeks, then the next eight every 12 weeks. GSK1572932A is the only adjuvant immunotherapy in Phase III development for NSCLC.

EP-2101 (Orphan Synergy Europe-Pharma [Paris, France])

Orphan Synergy Europe-Pharma recently acquired worldwide development rights and European commercialization rights from EU Synergy Epitopes SA (Geneva, Switzerland) to EP-2101. This multiepitope vaccine (initially developed by Epimmune [CA, USA] as EP-2101) includes ten peptides that cover nine HLA-A2-binding epitopes from four tumor-associated antigens commonly overexpressed in NSCLC: CEA, p53, hEGF receptor 2 (HER2/neu), and melanoma antigens MAGE-2 and -3. It also contains CAP1-6D, a heteroclitic CEA analog, and PADRE, a proprietary universal T-cell epitope, which serves to enhance the immunogenicity of the epitopes. In a Phase II trial in 63 patients with stage IIIB or IV NSCLC, the vaccine was administered every 3 weeks for the first 15 weeks, then every 2 months during year 1, then quarterly through year 2, for a total of 13 doses. One-year survival was 60% and median survival was 17.3 months. One complete and one partial response were identified. Interestingly, a correlation between survival and immune response was observed as survival was longer in patients who raised an immune response to epitope peptides [14]. Orphan Synergy Europe-Pharma is preparing an international Phase III trial in NSCLC.

GV1001 (KAEL-Gemvax [Seongnam-si, South Korea])

GV1001 is a peptide-based vaccine against telomerase, an enzyme expressed in approximately 90% of NSCLCs. Results from a Phase II trial (CTN-2006) in stage III NSCLC patients vaccinated after chemoradiotherapy and an 8-year update from a previous Phase I/II trial showed that vaccination with GV1001 was well tolerated, immunizing the majority of NSCLC patients and establishing durable T-cell memory. An 80% immune response rate was observed with immune responders recording a median progression-free survival of 371 days, compared with 182 days for nonresponders. The study included 20 evaluable patients and no treatment-related serious adverse effects were observed [15]. A multicenter multinational clinical trial is planned and is expected to enroll more than 600 patients (NCT01579188) [104].

Cell-based vaccines

Belagenpumatucel-L (Lucanix®, NovaRx Corp. [CA, USA])

Belagenpumatucel-L is an allogeneic therapeutic cancer vaccine in development for metastatic NSCLC following front-line, platinum-based combination chemotherapy. It is derived from four irradiated NSCLC cell lines expressing a TGF-β-specific antisense DNA. Lucanix is given by intradermal injection once a month for 18 months. Two Phase II studies in patients with advanced NSCLC showed promising results (strong correlation was observed between immune responses and survival) with limited toxicity [16,17]. A 700-patient Phase III trial STOP was started in 2008 and is scheduled for completion in October 2012 (NCT00676507 [104]). The vaccine was granted Fast Track status by the US FDA in 2008.

Vector-based vaccines

GI-4000 & GI-6207 (GlobeImmune, CO, USA)

GI-4000 and GI-6207 are probably the only clinical-stage cancer vaccines that use a genetically modified yeast (heat-inactivated *Saccharomyces cerevisiae*), the basis of GlobeImmune's Tarmogen platform. The yeast cells express mutated KRAS (GI-4000) or a modified CEA (GI-6207). GI-4000 is intended to be used as an adjuvant consolidation therapy in patients with stage I–III lung adenocarcinomas that harbor *KRAS* mutations or express CEA. In a Phase IIa trial, 47% of NSCLC patients demonstrated an immune response against KRAS. Five out of nine patients had a treatment-emergent response, and three out of eight improved from their baseline response to the vaccine. Phase I data for GI-6207 presented at the June 2011 American Society of Clinical Oncology meeting showed that monotherapy with the vaccine resulted in stable or lowered CEA levels in 20% of 25 patients with CEA-expressing tumors and good tolerability.

TG4010 (Transgene [Illkirch, France])

TG4010 is a specific immunotherapy product targeting the tumor-associated MUC-1 antigen. It consists of a viral suspension of a modified virus of Ankara (MVA) containing the full-length sequences coding for the human MUC-1 protein and for human IL-2. One difference when compared with L-BLP25 is the theoretically larger epitope repertoire of MUC-1 covered by the vaccine. The MVA is a vaccinia virus that belongs to the poxvirus family. This strain originates from the donkey chorioallantois virus Ankara and has been significantly attenuated by 570 passages on primary chicken embryo fibroblasts. It was specially developed to immunize patients at high risk of complications following vaccination against smallpox with classic strains. TG4010 is supplied as a frozen suspension in glass vials [18]. Two Phase I dose-escalation studies have been completed with TG4010. Tolerance of TG4010 was good and side effects mainly consisted of injection site pain and influenza-like symptoms. Four out of 13 evaluable patients showed stabilization of their disease for 6–9 months. One lung cancer patient,

who was initially progressing after the first injections, later showed a marked decrease in the size of his metastases that lasted for 14 months without any other treatment. Some T-cell proliferative immune responses were seen in five patients. The safety profile and the early signs of clinical and biological activity served as a basis for a Phase II program of TG4010 in several indications, including kidney, breast, prostate and NSCLC [19]. The first Phase II study in NSCLC explored two schedules of the combination of TG4010 with first-line chemotherapy in patients with stage IIIB/IV NSCLC, either combined upfront with cisplatin and vinorelbine, or TG4010 monotherapy until disease progression, followed by TG4010 plus the same chemotherapy as in arm 1. Sixty five patients were enrolled and in arm 1, partial response was observed in 13 patients out of 37 evaluable patients (35.1%); the study met its primary end point. A MUC-1-specific cellular immune response was associated with a significantly improved time to progression and overall survival. Induced ELISpot responses (i.e., responses detected in the post-baseline samples but not in the baseline samples) suggest that concurrent chemotherapy had little, if any, effect on the generation of the cellular immune response against MUC-1. These results raised the hypothesis that TG4010 is able to improve the outcome of patients receiving first-line chemotherapy for the advanced stage of NSCLC [20]. This hypothesis was then tested in a larger randomized, controlled, open-label and multicenter Phase II study evaluating TG4010 as an adjunct to first-line chemotherapy (CT; gemcitabine and cisplatin) in patients with advanced NSCLC. Intent-to-treat analysis showed the achievement of the primary end point with 43% of patients progression free at 6 months in the combination arm compared with 35% in the control arm. Response rate was also higher in the experimental group: 42 against 28%. While overall survival was similar in the two groups (10.7 months with TG4010 and 10.3 months with chemotherapy alone), there was a clear trend for improved long-term survival in patients receiving TG4010 [21]. TG4010 is currently being evaluated in a pivotal Phase IIB/III study in combination with first-line chemotherapy in advanced stage NSCLC (NCT01383148 [104]). The Phase IIB part of the trial is aimed at validating the predictive value of the level of NK cells while the Phase III part is powered to show a significant gain of survival when TG4010 is added to chemotherapy.

The development of therapeutic vaccines in NSCLC

Preclinical models in NSCLC

One important caveat in the development of NSCLC-targeting vaccines is the lack of animal models to evaluate immunologically active products, either by themselves or in combination with standard therapies. Most lung cancer animal models make use of established human lung cancer cell lines or primary tumor explants taken from cancer patients implanted in immunodeficient mouse strains (reviewed in Wang *et al.* [22]). Since xenograft models involve the use of immunodeficient animals, they are suitable to evaluate molecules that act directly on tumor cells but do not allow examination of the interaction of these molecules with the different components of the immune response.

Even if the murine immune system does not fully correspond to the human one and inbred strains have demonstrated biases in the type of immune response they generate, fully immunocompetent mice are the condition *sine qua non* to properly evaluate immunotherapeutics. In this context, syngeneic lung tumor lines may be injected intravenously. Cells (or cell aggregates) get trapped in the lung's capillaries and will generate a multifoci, pseudo-metastasis model. This type of system may be manipulated such that the immune response against cancer-specific antigens may be estimated. One drawback, however, is that the fast growth rate of most of these models is incompatible with time required to generate a full-blown specific immune response. Moreover, unless the mouse ortholog gene is highly homologous to the human gene to be used in the clinic, the antigen will be seen as 'foreign'. This bias precludes a proper evaluation of immune response generated by the immunotherapeutic since in the human setting, patients will be presumed immunologically tolerant. This can be addressed by using animals that are transgenic for the human version of the cancer-specific antigen, rendering them immunologically tolerant [23].

Whole-genome disequilibrium studies, gene inactivation experiments [24,25] and chemically induced carcinogenesis [26] in immunocompetent mice were used to identify lung tumorigenesis susceptible genes [27] and reviewed in [28] (a list of lung cancer models is also provided in [102]). Manipulating these genes and/or these animals generates strains of mice with a higher frequency of lung cancers than in untreated/unmanipulated animals and offers models that are somewhat closer to the human situation. While particularly interesting to study oncogenesis and anticancer approaches with direct effects on tumor cells, these systems are cumbersome with limited penetrance, making it difficult to evaluate an immunotherapeutic in large animal cohorts. Moreover, cancer immunoediting makes it difficult to predict which antigen will be expressed, even when the oncogenic processes are identical [29].

Significant efforts are still required to establish robust preclinical models that allow proper evaluation of immunotherapeutics to treat advanced lung cancer. Some of the difficulties described above are currently being addressed by including preclinical imaging modalities, HLA-transgenic mice and animals reconstituted with human immune cells.

Preclinical safety of therapeutic vaccines; the example of TG4010

Preclinical safety studies on therapeutic cancer vaccines are intended to define a safe and immunogenic starting dose for clinical trials and to identify target organs for toxicity to identify safety parameters for clinical monitoring (Center for Biologics Evaluation and Research guidance, 2009 [103]). An indication of the studies that are required is given in the regulatory guidelines. However, there is no standard approach and the nonclinical toxicity strategy needs to be tailored to the particular vaccine product and will depend on the product features, specific safety concerns and available test systems. The toxicity profile of TG4010 was investigated in mice, rats and rabbits following single and/or repeated administrations under good laboratory practice conditions as summarized in Table 2.

A single intravenous and repeat intramuscular dose toxicity study was carried out in rats to support the initial Phase I study. TG4010 was well tolerated and anatomopathology did not evidence target organs for potential toxicity. Further clinical development was continued by subcutaneous route and a study in rabbits was performed to assess the local tolerance at the injection site. The frequency and duration of administration to rabbits was determined by the proposed conditions in clinical use. Minor local reactions were observed at the injection sites frequently observed following administration of vaccines. An additional subcutaneous toxicity study over a 3-month period did not reveal any additional toxicity. As MUC-1 is a self-antigen expressed by some normal cells, TG4010 immunization could possibly trigger an autoimmune response. The long-term 3-month toxicity study in mice did not reveal any signs of potential immunotoxicity, hematological changes or alteration in immune system organ weights and histology (e.g., changes in thymus, spleen, lymph nodes and bone marrow). However, the mouse model used in this study was not fully relevant to address the autoimmunity risk.

Table 2. Toxicity studies performed on TG4010.

Study, type and duration	Species	Route	Dose (PFU/kg)
Single-dose toxicity	Rat	iv.	3.6×10^7
Repeat-dose toxicity. Twice a week for 4 weeks (D1, D4, D8, D11, D15, D18, D22 and D24)	Rat	im.	0.216×10^7 2.16×10^7
Local tolerance. Twice a week for 3 weeks, then once 3 weeks after (D0, D3, D7, D10, D14, D17, D21 and D42)	Rabbit	sc.	1.0×10^8 per injection
Repeat-dose toxicity. Once a week for 6 weeks then once every 3 weeks (D0, D7, D14, D21, D28, D35, D42, D63 and D84)	C57BL/6 mice	sc.	4.0×10^7 PFU/animal

D: Day; im.: Intramuscular; iv.: Intravenous; sc.: Subcutaneous.

The potential induction of autoimmunity could be theoretically performed in MUC-1 transgenic mice [30]. However, so far TG4010 administration could not break immune tolerance against MUC-1, which impedes investigation of immunotoxicity and autoimmunity in these mice.

From the current clinical experience with TG4010, autoimmunity parameters were measured in patients: anti-DNA antibodies, thyroid stimulating hormone, anti-thyroperoxidase antibodies, and anti-nuclear antibodies. There was no clinical evidence of autoimmunity signs.

Assembling preclinical and clinical data with careful monitoring of autoimmunity reactions in the clinical trials might compensate for the lack of long-term immunotoxicity studies.

The issue of potential induction of autoimmunity was also questioned for other therapeutic cancer vaccines after multiple vaccination protocols with different poxvirus-based vectors encoding self-antigens such as CEA [31], human oncofetal antigen 5T4 [32,33] or MUC-1 for which no vaccine-induced autoimmunity was neither reported in animals [34] nor in patients.

Nonclinical toxicology evaluation has evolved during the last decade through the development of many different types of vaccines (e.g., therapeutic vaccines, DNA vaccines, novel routes of administration, novel adjuvants and immune system modulation). This poses challenges regarding approaches to preclinical safety evaluation of these products.

Due to the lack of a relevant animal model, preclinical safety fails to address immune issues relating to immune tolerance breaking or immune responses. Consequently, immune responses should be extensively measured in clinical studies and a strong safety database is needed. So far, no significant autoimmune reactions have been reported in clinical trials using therapeutic cancer vaccines in NSCLC despite the fact that the majority of the candidate vaccines target self-antigens.

Therapeutic cancer vaccine & biomarkers

Biomarkers are of great use in preclinical and clinical research as well as in therapeutic vaccine development. They can be used in order to discover new vaccine targets, monitor or predict treatment response during clinical trials and aid in patient selection by allowing stratification and risk assessment.

Rapid advances in technologies such as genomics and proteomics allow a deeper understanding of cancer biology and have made the field one of the most fertile for personalized medicine. To date, 27 drugs, including small molecules and biologics, have been approved by the FDA for the treatment of cancer with the use of companion diagnostics for patient selection (Table 3).

Many types of genetic mutations may occur in NSCLC patients. Some are associated with a response to small inhibitory molecules; this is the case for the activating mutations of EGF receptor (EGFR), the translocations of the anaplastic lymphoma kinase gene (*ALK*) [35] and the rearrangement of the gene *ROS1* [36]. Identifying them will help to decide the most suitable targeted therapy for a given patient.

EGFR mutations are found in 10–15% of Caucasian and 40% of Asian NSCLC patients and are associated with a response to Iressa® (AstraZeneca, London, UK) and Tarceva® (Genentech, CA, USA). Pfizer's (NY, USA) recently approved Xalkori® (crizotinib) is appropriate for less than 7% of the total NSCLC cancer patient population, those with anomalies of *ALK* or *ROS1*. These examples indicate a shift in focus to more personalized cancer treatments.

Cancer vaccines are no exception as the choice of immunotherapies available for clinical trials has vastly increased in recent years, ranging from single peptide and recombinant viral vector vaccinations to whole-cell therapies. These different strategies are not necessarily appropriate to every individual and the use of biomarkers may help in prescribing particular therapies to a given patient. Cancer vaccine developers thus often include biomarker programs in clinical trials in order to discover biomarkers that

could be useful to select patients who would benefit from the immunotherapy. These biomarkers can be based on the mechanism of action of the vaccine and thus used as inclusion criteria from the Phase I clinical studies, or have been discovered retrospectively in extensive biomarker programs during more advanced stages of clinical vaccine development.

Biomarkers based on the mechanism of action for patient screening

Depending on the mechanism of action of the vaccine, biomarkers can be used as an inclusion criterion for patient screening. For instance, the expression level of the MAGE-A3 tumor-associated antigen mRNA is assessed by qPCR in tumors for GSK1572932A antigen-specific vaccine treatment [37], and the percentage of positive cells for MUC-1 in tumors is evaluated by immunohistochemistry for treatment with TG4010 [21]. Other examples include HLA genotyping for NSCLC patients treated with VX-001, a *HLA-A*0201* restricted telomerase-specific antitumor vaccine [38], and Ras sequencing for the treatment of patients with GI-4000, a heat-inactivated *S. cerevisiae* yeast expressing a unique combination of three Ras mutations [39]. These biomarker assays are an integral part of the clinical development process and are usually codeveloped as companion tests with their vaccine counterpart.

Table 3. FDA cancer-approved drugs with pharmacogenomic biomarkers.

Drug	Biomarker
Arsenic trioxide	PML/RARα
Brentuximab vedotin	CD30
Busulfan	Ph chromosome
Capecitabine	DPD
Cetuximab	EGF receptor, KRAS
Cisplatin	TPMT
Crizotinib	ALK
Dasatinib	Ph chromosome
Denileukin diftitox	CD25
Erlotinib	EGF receptor
Exemestane	ER and PgR
Fulvestrant	ER
Gefitinib	EGF receptor
Imatinib	C-Kit, Ph chromosome, PDGFR, FIP1L1-PDGFRα
Irinotecan	UGT1A1
Lapatinib	HER2/neu
Letrozole	ER and PgR
Mercaptopurine	TPMT
Nilotinib	Ph chromosome,UGT1A1
Panitumumab	EGFR, KRAS
Pertuzumab	HER2/neu
Rasburicase	G6PD
Tamoxifen	ER
Thioguanine	TPMT
Tositumomab	CD20 antigen
Trastuzumab	HER2/neu
Vemurafenib	BRAF

ER: Estrogen receptor; HER2: HEGF receptor 2; PDGFR: PDGF receptor; PgR: Progesterone receptor.
Data taken from [104].

Prognostic & predictive biomarkers

Exploratory biomarker programs can be set-up in clinical trials in order to discover prognostic (that can predict the outcome regardless of the treatment) and predictive biomarkers (that can predict the outcome of a specific therapy). These latter biomarkers are of particular interest as they may help select patients who would benefit from the therapeutic vaccine, or exclude patients that could experience safety issues following therapy. In order to be efficient, these approaches require well-controlled sample collection and preanalytical steps as both are well known to be important sources of variability. Different types of samples and properly validated technologies can be used for this purpose, such as tumoral and/or whole blood transcriptome or proteome analysis, cytokine profiling as well as immunophenotyping. One example of such an approach is the identification of a tumoral gene signature at baseline (before treatment), possibly predictive of benefit, from GSK Biologicals' (London, UK) MAGE-A3 therapeutic vaccine treatment in a Phase II study with 182 patients with completely resected stage IB or II NSCLC. In MAGE-A3-positive patients with this gene signature, the vaccine achieved a 43% reduction in the relative risk of relapse compared with placebo. This signature is being prospectively validated as a secondary objective of the MAGRIT Phase III clinical trial [13]. An extensive biomarker program including transcriptome and proteome analyses on whole blood, multiplex cytokine profiling and immunophenotyping was conducted during the TG4010 Phase II study in 148 patients with late stage (IIIB/IV) NSCLC. It was retrospectively found that at baseline (pretreatment), normal levels of $CD16^+$ $CD56^+$ $CD69^+$ lymphocytes, a phenotype of activated NK cells (aNK), were associated with a better outcome including a longer survival only in patients treated with TG4010 and CT (median survival of 17.1 vs 11.3 months in the control arm). Moreover, pretreatment (day 1), normal levels of soluble CD54 (sCD54), IL-6 and M-CSF were also associated with longer survival especially in patients treated with TG4010 and CT [21]. Finally, at day 43 (after six injections of TG4010), elevated levels of $CD3^+$ $CD69^+$ lymphocytes, as well as increased levels of IFN-γ, were also associated with longer survival in the combination arm [40]. The aNK signature is being prospectively validated as the primary objective of the IIb part of a Phase IIb/III trial of TG4010 combined with best supportive care in stage IV NSCLC patients. In this part of the study, both patients with normal and high aNK levels are being included, whereas only patients with normal levels are expected to be included in the Phase III part of the study.

Expert commentary & five-year view

After the first therapeutic monoclonal antibody for cancer nearly two decades ago, the standard of care for cancer is evolving to become more complex, involving the combination of surgery, radiotherapy, chemotherapy, small molecules, biotherapies and molecular or cellular drugs. The development of therapeutic vaccines, coupled with the identification of efficacy-based biomarkers, will certainly be a part of the future of combined multi-modality therapies of NSCLC with the aim of either decreasing the risk of relapse after surgery or increasing the overall survival in more advanced settings. The first therapeutic vaccines should be approved and part of multi-therapeutic approaches within 5 years. The next step will be to combine targeted immunotherapies with drugs increasing the ability of the immune system to respond to targeted immunotherapy. Manufacturing issues need to be taken into consideration as well. Vector-based approaches and peptides have the advantage of being easier to produce and to scale-up, along with a lower cost than cell-based immunotherapies. Therefore, the next generation of therapeutic vaccines will more likely be 'off-the-shelf'.

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Key issues

- Therapeutic cancer vaccines engaged in non-small-cell lung cancer use different platforms: peptides, cellular vaccines and microbial vectors; they have shown clinical and immunological activity.
- For some of these vaccines, potential biomarkers predictive of activity have been identified.
- The vaccines are used either in monotherapy or in combination with chemotherapy.
- The most advanced products in development today target different nonoverlapping subpopulations of non-small-cell lung cancer patients.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Reck M. Current approaches in chemotherapy of advanced and metastatic non-small cell lung cancer (NSCLC). *Anticancer Res.* 25(3A), 1501–1506 (2005).

2 Auberger J, Loeffler-Ragg J, Wurzer W, Hilbe W. Targeted therapies in non-small cell lung cancer: proven concepts and unfulfilled promises. *Curr. Cancer Drug Targets* 6(4), 271–294 (2006).

3 Hirschowitz EA, Yannelli JR. Immunotherapy for lung cancer. *Proc. Am. Thorac. Soc.* 6(2), 224–232 (2009).

4 Gérard C, Debruyne C. Immunotherapy in the landscape of new targeted treatments for non-small cell lung cancer. *Mol. Oncol.* 3(5–6), 409–424 (2009).

5 O'Mahony D, Kummar S, Gutierrez ME. Non-small-cell lung cancer vaccine therapy: a concise review. *J. Clin. Oncol.* 23(35), 9022–9028 (2005).

6 Acres B, Limacher JM. MUC1 as a target antigen for cancer immunotherapy. *Expert Rev. Vaccines* 4(4), 493–502 (2005).

7 Hareuveni M, Tsarfaty I, Zaretsky J *et al.* A transcribed gene, containing a variable number of tandem repeats, codes for a human epithelial tumor antigen. cDNA cloning, expression of the transfected gene and over-expression in breast cancer tissue. *Eur. J. Biochem.* 189(3), 475–486 (1990).

• **Outlines the discovery of the MUC-1 antigen along with its polymorphic structure containing a number of tandem repeats and its overexpression in breast cancer tissue.**

8 Burchell J, Gendler S, Taylor-Papadimitriou J *et al.* Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. *Cancer Res.* 47(20), 5476–5482 (1987).

9 Kufe DW. Mucins in cancer: function, prognosis and therapy. *Nat. Rev. Cancer* 9(12), 874–885 (2009).

10 Guddo F, Giatromanolaki A, Koukourakis MI *et al.* MUC1 (episialin) expression in non-small cell lung cancer is independent of EGFR and c-erbB-2 expression and correlates with poor survival in node positive patients. *J. Clin. Pathol.* 51(9), 667–671 (1998).

11 Ohgami A, Tsuda T, Osaki T *et al.* MUC1 mucin mRNA expression in stage I lung adenocarcinoma and its association with early recurrence. *Ann. Thorac. Surg.* 67(3), 810–814 (1999).

12 Butts C, Maksymiuk A, Goss G *et al.* Updated survival analysis in patients with stage IIIB or IV non-small-cell lung cancer receiving BLP25 liposome vaccine (L-BLP25): phase IIB randomized, multicenter, open-label trial. *J. Cancer Res. Clin. Oncol.* 137(9), 1337–1342 (2011).

•• **Phase IIB results which have justified entry of Stimuvax® (Oncothyreon, WA, USA) into Phase III trials.**

13 Tyagi P, Mirakhur B. MAGRIT: the largest-ever Phase III lung cancer trial aims to establish a novel tumor-specific approach to therapy. *Clin. Lung Cancer* 10(5), 371–374 (2009).

14 Barve M, Bender J, Senzer N, *et al.* Induction of immune responses and clinical efficacy in a phase II trial of IDM-2101, a 10-epitope cytotoxic T-lymphocyte vaccine, in metastatic non-small-cell lung cancer. *J. Clin. Oncol.* 26(27), 4418–4425 (2008).

• **Clinical and immunological activity of EP2101.**

15 Brunsvig PF, Kyte JA, Kersten C *et al.* Telomerase peptide vaccination in NSCLC: a Phase II trial in stage III patients vaccinated after chemoradiotherapy and an 8-year update on a Phase I/II trial. *Clin. Cancer Res.* 17(21), 6847–6857 (2011).

• **Clinical activity of hTERT therapeutic vaccine.**

16 Nemunaitis J, Dillman RO, Schwarzenberger PO *et al.* Phase II study of belagenpumatucel-L, a transforming growth factor β-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-

cell lung cancer. *J. Clin. Oncol.* 24(29), 4721–4730 (2006).

17 Nemunaitis J, Nemunaitis M, Senzer N *et al.* Phase II trial of Belagenpumatucel-L, a TGF-β2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther.* 16(8), 620–624 (2009).

•• **Clinical activity of Lucanix® (NovaRx, CA, USA).**

18 Cohen S, Kaufman HL. TG-4010 Transgene. *Curr. Opin. Investig. Drugs* 5(12), 1319–1328 (2004).

19 Rochlitz C, Figlin R, Squiban P *et al.* Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. *J. Gene Med.* 5(8), 690–699 (2003).

20 Ramlau R, Quoix E, Rolski J *et al.* A Phase II study of TG4010 (MVA-MUC1-Il2) in association with chemotherapy in patients with stage III/IV non-small cell lung cancer. *J. Thorac. Oncol.* 3(7), 735–744 (2008).

21 Quoix E, Ramlau R, Westeel V, *et al.* Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small-cell lung cancer: a controlled Phase 2B trial. *Lancet Oncol.* 12(12), 1125–1133 (2011).

•• **Outlines the findings of a phenotype of activated NK cells that were a potential predictor of outcome in patients who received TG4010.**

22 Wang Y, Rouggly L, You M, Lubet R. Animal models of lung cancer characterization and use for chemoprevention research. *Prog. Mol. Biol. Transl. Sci.* 105, 211–226 (2012).

23 Acres B, Apostolopoulos V, Balloul JM *et al.* MUC1-specific immune responses in human MUC1 transgenic mice immunized with various human MUC1 vaccines. *Cancer Immunol. Immunother.* 48(10), 588–594 (2000).

24 Berndt A, Cario CL, Silva KA *et al.* Identification of *fat4* and *tsc22d1* as novel candidate genes for spontaneous pulmonary adenomas. *Cancer Res.* 71(17), 5779–5791 (2011).

25 Wang D, You M. Five loci, *SLT1* to *SLT5*, controlling the susceptibility to spontaneously occurring lung cancer in mice. *Cancer Res.* 65(18), 8158–8165 (2005).

26 Kreisel D, Gelman AE, Higashikubo R *et al.* Strain-specific variation in murine natural killer gene complex contributes to differences in immunosurveillance for urethane-induced lung cancer. *Cancer Res.* 72(17), 4311–4317 (2012).

27 Tanaka-Okamoto M, Hori K, Ishizaki H *et al.* Increased susceptibility to spontaneous lung cancer in mice lacking LIM-domain only 7. *Cancer Sci.* 100(4), 608–616 (2009).

28 Meuwissen R, Berns A. Mouse models for human lung cancer. *Genes Dev.* 19(6), 643–664 (2005).

29 DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. *Nature* 482(7385), 405–409 (2012).

30 Turner MS, Cohen PA, Finn OJ. Lack of effective MUC1 tumor antigen-specific immunity in MUC1-transgenic mice results from a Th/T regulatory cell imbalance that can be corrected by adoptive transfer of wild-type Th cells. *J. Immunol.* 178(5), 2787–2793 (2007).

31 Chakraborty M, Schlom J, Hodge JW. The combined activation of positive costimulatory signals with modulation of a negative costimulatory signal for the enhancement of vaccine-mediated T-cell responses. *Cancer Immunol. Immunother.* 56(9), 1471–1484 (2007).

32 Mulryan K, Ryan MG, Myers KA *et al.* Attenuated recombinant vaccinia virus expressing oncofetal antigen (tumor-associated antigen) 5T4 induces active therapy of established tumors. *Mol. Cancer Ther.* 1(12), 1129–1137 (2002).

33 Harrop R, Connolly N, Redchenko I *et al.* Vaccination of colorectal cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) induces immune responses which correlate with disease control: a Phase I/II trial. *Clin. Cancer Res.* 12(11 Pt 1), 3416–3424 (2006).

34 Ryan SO, Turner MS, Gariépy J, Finn OJ. Tumor antigen epitopes interpreted by the immune system as self or abnormal-self differentially affect cancer vaccine responses. *Cancer Res.* 70(14), 5788–5796 (2010).

35 Kwak EL, Bang YJ, Camidge DR *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* 363(18), 1693–1703 (2010).

36 Bergethon K, Shaw AT, Ou SH *et al. ROS1* rearrangements define a unique molecular class of lung cancers. *J. Clin. Oncol.* 30(8), 863–870 (2012).

37 Brichard VG, Lejeune D. GSK's antigen-specific cancer immunotherapy programme: pilot results leading to Phase III clinical development. *Vaccine* 25(Suppl. 2), B61–B71 (2007).

•• **Clinical activity of MAGE-A3 vaccine in the non-small-cell lung cancer adjuvant setting.**

38 Vetsika EK, Konsolakis G, Aggouraki D *et al.* Immunological responses in cancer patients after vaccination with the therapeutic telomerase-specific vaccine Vx-001. *Cancer Immunol. Immunother.* 61(2), 157–168 (2012).

39 Lu Y, Bellgrau D, Dwyer-Nield LD *et al.* Mutation-selective tumor remission with Ras-targeted, whole yeast-based immunotherapy. *Cancer Res.* 64(15), 5084–5088 (2004).

40 Acres B, Marie-Bastien B, Grellier B, *et al.* A signature of circulating biomarkers correlates with clinical outcome in a randomized phase IIb trial evaluating the therapeutic vaccine TG4010 in NSCLC patients. *Proc. Am. Assoc. Cancer Res. Denver CO. AACR* Abstract 733, 718–722 (2009).

Websites

101 Merck Serono press release. www.merckserono.com/corp.merckserono_2011/en/images/Stimuvax%20START_20121219_FINAL_1_tcm1494_104186.pdf?Version=

102 National Cancer Institute, Cancer Models database. http://cancermodels.nci.nih.gov/camod/searchResults.do

103 Center for Biologics Evaluation and Research (CBER). Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines. www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/UCM182826.pdf

104 ClinicalTrials.gov. http://clinicaltrials.gov