Lung Cancer Vaccines

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Introduction

Anecdotal observations of spontaneous regression of tumors in patients with cancer provided the initial evidence of the presence of an inborn anti-tumor immune response. Additionally the observation of paraneoplastic autoimmunity that can accompany occult malignancies indicate the existence immunologic activity (vaccine 2 2). Historically the first reports that of therapeutic immune induced tumor regression came over a century ago when William Coley treated cancer patients by nonspecifically activating the immune system with inoculations of live bacterial cultures. However, little progress was made until the 1980s, when Rosenberg et al. studied the use of high doses of IL-2 in individuals with metastatic kidney cancer or melanoma and achieved objective cancer regressions in 15-20% of treated patients (vaccine.2 1).

Classic prophylactic vaccines that have had great success in the prevention of infectious diseases have relied mainly on generation of high titers of neutralizing antibodies. This chapter discusses therapeutic vaccines that elicit an active specific immune response. These vaccines aim at inducing strong antigen specific T cell responses. The requirements for therapeutic vaccine development are different and far more complex than those of prophylactic vaccines. The first important issue in vaccine design is that of antigen delivery. Therapeutic vaccines are divided into subunit vaccines or cell-based vaccines (Table 1). The subunit vaccine approach is based on the selection of well defined antigens as targets. The term subunit vaccine may include a gene or gene product representing part of or an entire polypeptide fragment carrying an antigen recognized by T cells. These include plasmid DNA, messenger RNA, peptides, recombinant proteins,

or bacterial/viral vectors carrying gene inserts coding for tumor antigens. Cellular vaccines rely on the approach of using whole tumor cells for vaccination. Either irradiated tumor cells or lysates have been used. Tumor cells may be modified by gene transfer to express cytokines that may enhance their overall immunogenicity.

The focus of most lung cancer vaccines has been the generation of a T-cell response against antigens expressed by tumors. Cancer vaccination is based on the premise that an effective antitumor response can be elicited by the induction of major histocompatibility compex (MHC) class I restricted cytotoxic lymphocytes (CTLs), capable of recognizing and lysing tumor cells. Gene modified tumor vaccines (GMTV) and dendritic cell vaccines (DCV), the two main classes of cellular vaccines investigated in lung cancer, utilize this approach.

GMTV use gene transfer technology to transduce tumor cells with genes encoding cytokines or other immunogenic proteins. DCV utilize antigen modification of autologous dendritic cells to elicit a specific T-cell activation against cancer cells. Experimental studies of xenografted animals demonstrated that these vaccines considerably increased the immunogenicity of tumor cells, which in many cases induced tumor rejection and regression (view eg. 5,6,7,8). Several GMT vaccine platforms have been evaluated for cytokine and gene delivery. These include autologous tumor vaccines, allogeneic tumor vaccines, and bystander vaccines. Autologous tumor cell vaccines involve surgically harvested tumor cells that are genetically modified to increase immune recognition. More common, allogeneic vaccines are made up of tumor cell lines that

express tumor associated antigens (TAAs) and are genetically modified to express immunogenic cytokines and proteins. A bystander vaccine is a hybridization of both aforementioned approaches. It utilizes autologous tumor cell antigens, "bystander" cells, in combination with cytokine secreting allogeneic tumor cells to recruit and activate immune effector cells.

The role of Dendritic Cells (DC) in cell-mediated immunity has been extensively investigated [1-5]. DCs have been found to play a central role in the induction of anti-tumor immunity in tumor-bearing host by a process of antigenic cross-presentation and have displayed activity in NSCLC [6]. They efficiently display antigens on major histocompatibility complexes (MHC II) ultimately stimulating proliferation and activation of CD4+ and CD8+ T cells. CD 4+ cells further augment the activity of natural killer cells and macrophages, in addition to amplifying antigen-specific immunity by local secretion of cytokines [7-11]. These attributes make DCs a central component in therapeutic strategies of many current immune based therapies in NSCLC.

Despite progress made in understanding the molecular biology behind carcinogenesis and advancements in our technical proficiency clinical application of immune based cancer vaccines have yielded modest results. There are several hypotheses to explain potential lack of activity, including ineffective priming of tumor-specific T cells, lack of high-avidity of primed tumor-specific T cells, and physical or functional disabling of primed tumor-specific T cells by the primary host and or tumor-related mechanism. For example in NSCLC a high proportion of the tumor-infiltrating lymphocytes are

immunosuppressive T regulatory cells (CD4+ CD25+) that secrete transforming growth factor-β (TFG-β) and express a high level of cytotoxic T lymphocyte (CTL) antigen-4 [12, 13]. These cells have been shown to impede immune activation by facilitating T cell tolerance to tumor associated antigens rather than cross-priming CD 8+ T cells resulting in the nonproliferation of killer T cells that recognize the tumor and will not attack it [12-18]. Elevated levels of IL-10 and TFG-β are found in patients with NSCLC. Animal models have shown immune suppression is mediated by these cytokines serving as a defense for malignant cells against the body's immune system [19-28].

As our understanding of the pathogenesis of cancer steadily evolves researchers are continuously developing novel therapies designed to overcome each new challenge. This chapter will discuss recent vaccine therapeutic strategies in lung cancer focusing on clinical trials that have contributed to our overall understanding of the immune system and its utilization in the treatment of lung cancer.

Non-Small Cell Lung Cancer Cellular Vaccines

Lucanix ™

Lucanix™ is a non-viral gene-based allogeneic vaccine that incorporates the TFG-β2☐ antisense gene into a cocktail of four different NSCLC cell lines [29]. Elevated levels of TFG-β2 are linked to immunosupression in cancer patients [30-35]. Systemic levels of TFG-β is inversely correlated with prognosis in patients in patients with NSCLC [36]. TFG-β2 has an antagonistic effect on natural killer cells, lymphokine-activated killer cells and DCs [21, 25, 26, 37-39]. Using an antisense gene to inhibit TFG-β2☐☐ several

researchers have demonstrated an inhibition of cellular TFG-β2 expression resulting in an increased immunogenicity of gene-modified cancer cells [40-48].

In a recent phase II study involving 75 early-stage (n=14) and late-stage (n=61) patients a dose-related effect of Lucanix ™ was defined [29]. Patients were randomized to one of the three dose cohorts (1.25×10^7, 2.5×10^7, or 5×10^7 cells/injection x 16 injections). Injections were administered one time each month or every other month until progressive disease criteria were fulfilled. Treatment was well tolerated with only 1 grade 3 toxic event attributed to the vaccine (arm swelling). A significant survival advantage at dose levels $\geq 2.5 \times 10^7$ cells/injection compared to the low dose level of 1.25×10^7 cells/injection was demonstrated with an estimated 2-year survival of 47% (Table 2 and 3). This also compared favorably with the historical 2-year survival rate of $< 20\%$ of comparable stage IIIB or IV NSCLC patients [49-54]. Furthermore a correlation of positive outcome with induction of immune enhancement of tumor antigen recognition was observed. Immune function was explored in the 61 advanced stage IIIB or IV patients. Patients who achieved stable disease or better had increased frequency in the production of cytokines (INF-γ , p = 0.006; IL-6, p = 0.004; IL4 p = 0.007) and positive clinical outcomes were correlated with development of HLA-antibody response to the vaccine. Eleven of 20 patients with stable disease or better developed novel HLA-antibody reactivity to one or more allotypes of the vaccinating cell lines compared with two of 16 progressive disease patients (*P*=0.014). It was concluded that further Phase III investigation of Lucanix ™ is justified and warranted.

GVAX® Lung

Given the histological heterogeneity of NSCLC and the relative absence of information on the relevant immunodominant antigens in this disease, in initial trials autologous tumor cells were selected as the source of tumor antigens in NSCLC [55]. The first pilot study of autologous GVAX® Lung conducted by Glenn Dranoff at the Dana-Faber cancer institute using a first generation adenoviral vector and recombinant human GM-CSF [56]. Thirty-five patients underwent tumor harvest and 33 patients received vaccine treatment at 3 different dose levels. The vaccine was administered weekly for 2 weeks then bi-weekly until the supply was exhausted. Vaccines were well tolerated with the most common toxicity being local, self-limited vaccine site reactions and mild flu-like symptoms in a minority of patients. Anti-tumor immunity was demonstrated by induction of delayed-type hypersensitivity (DTH) reaction to injections of irradiated, genetically unmodified autologous tumor cells in 82% of patients as well as the presence of inflammatory infiltrates in metastatic tumor biopsies. In addition, one patient demonstrated evidence of tumor regression (mixed response) and two others have remained recurrence-free for more than five years following resection of isolated metastatic sites for vaccine preparation.

The subsequent study was a multi-center phase I/II trial investigating again an autologous NSCLC tissue vaccine. Manufacturing processes were modified in this trial to enable more rapid commercial development. This study also involved both patients with early stage and advanced stage disease [57]. Patients were enrolled in two cohorts. Cohort A included patients with stage IB or II NSCLC with planned primary surgical resection and

no pre- or postoperative chemotherapy or radiotherapy. Patients in cohort B had surgically nonresectable stage III or IV NSCLC with an accessible tumor to harvest for vaccine processing.

Vaccines were administered subcutaneously every 2 weeks for a total of three to six vaccinations. The vaccine dose was individualized on the basis of yield, and each dose contained $5x10^6$ to $10x10^6$ cells per vaccination, $10x10^6$ to $30x10^6$ cells per vaccination, and $30x10^6$ to $100x10^6$ cells per vaccination (Table 2 and 3).

Eighty-three patients underwent tumor harvest (20 in cohort A, 63 in cohort B) and 43 initiated vaccine treatment (10 in cohort A, 33 in cohort B). All 10 patients in cohort A completed vaccine treatment. The median number of vaccines in cohort B was 5. The median number of days from tumor harvest to vaccine release was 31 and that from harvest to initiation of vaccine treatment was 49 days. Vaccines were successfully manufactured in 80% of patients in cohort A and 81% of patients in cohort B. The majority of manufacturing failures resulted from an insufficient number of tumor cells.

The most common vaccine-related adverse events were local vaccine injection site reactions (93%) followed by fatigue (16%) nausea (12%) and pain, arthralgia, and upper respiratory infection (each at 5%). Two grade 4 (pericardial effusion) and six grade 3 (dyspnea, fatigue, injection site reaction, hypokalemia, malignant ascites, and pulmonary embolism) possibly related events were reported. There was no association between vaccine dose and the total number of adverse events or grade 3 and 4 adverse events.

Vaccine reaction size (skin induration) was positively associated with level GM-CSF secretion from the transfected autologous malignant cells used as the product. Analysis of vaccine site biopsy specimen showed dense infiltration with CD4+ and CD8+ T cells, CD1a+ dendritic cells, and eosinophils.

Three patients in cohort B achieved durable, complete tumor regressions lasting 6 months, 18 months, and 22 months. In addition, there was one minor response (30% decrease in a lung nodule) and two mixed responses; seven patients had stable disease with a mean duration of 7.7 months. Correlation of dose to survival was demonstrated to be significant at a threshold of 40 ng of GM-CSF/per 10^6 cells/24 hours expressed from an aliquot of the vaccine prior to the first injection. Long term follow-up of two of the patients (stage IV refractory disease to prior cytotoxic therapy) achieving complete response reveals continued disease free survival now more than five years after initial GVAX® vaccination (unpublished data).

Salgia *et al.* [56] also conducted the first phase I trial of GVAX® in NSCLC using an autologous vaccine strategy. Thirty-seven patients with stage IIB – IV NSCLC were enrolled and 34 vaccines were successfully manufactured at 3 different dose levels ($1x10^6$, $4x10^6$, $1x10^7$ cells). The vaccines were administered weekly for 2 weeks then biweekly until the supply of vaccine was exhausted. Of these patients 25 received ≥ 6 vaccinations. Toxicities were limited to grade 1 – 2 erythema and induration at the injection site, as well as fatigue and flu-like symptoms (Table 2 and 3).

A total of 18 out of 25 patients who received six vaccinations showed significant local reactions. At the vaccination site, these 18 patients showed infiltration of DCs, macrophages, eosinophils, neutrophils, and lymphocytes. The intensity and frequency of the reaction was related to the dosage administered. Five patients showed stable disease after 33, 19, 12, 10, and 3 months (Table 2 and 3). Based on the outcomes of the study, Salgia and his colleagues concluded that GVAX® enhances anti-tumor immunity in some patients with metastatic NSCLC.

In an effort to remove the requirement for genetic transduction of individual tumors and to optimize GM-CSF transgene expression (given that this correlated with improved survival), a second approach was developed called 'bystander' GVAX®, which is a vaccine composed of autologous tumor cells mixed with an allogeneic GM-CSF secreting cell line (K562 cells) [58] and a phase I/II trial of this vaccine in advanced-stage NSCLC was conducted. Tumors were harvested from 86 patients, tumor cell processing, was successful in 76 patients, and 49 proceeded to vaccination. Serum GM-CSF pharmacokinetics were consistent with secretion of GM-CSF from vaccine cells for ≤ 4 days, with associated transient leukocytosis confirming the bioactivity of vaccine-secreted GM-CSF. Evidence of vaccine-induced immune activation was demonstrated. However, objective tumor responses were not seen despite a 25-fold higher GM-CSF secretion concentration with the bystander GVAX® vaccine (Table 2 and 3). The frequency of vaccine site reactions, tumor response, time to progression and survival

were all less favorable to autologous GVAX®, although results were similar to historical cytotoxic therapy for second line NSCLC.

Overall, these results suggest that autologous malignant tissue transfection with adenovirus-delivered GM-CSF is superior to the bystander approach, despite variability of GM-CSF expression levels and practical limitations inherent to surgically harvested tumor tissue.

L523S Vaccine

L523S is a lung cancer antigen originally identified through screening of genes differentially expressed in cancer cells versus normal tissue [59, 60]. L523S is shown to be expressed in approximately 80% of NSCLC cell lines [59, 60]. In preclinical studies the immunogenicity of L523S in humans was initially shown by detecting the presence of existent antibody and CD4+ T cell responses to L523S in patients with lung cancer. Subsequent studies further validated L523S's immunogenicity demonstrating that human CTLs could specifically recognize and kill cells that express L523S. It has demonstrated preclinical safety when the gene is injected intramuscularly as an expressive plasmid (pVAX/L523S) and when delivered by E1B-deleted adenovirus (Ad/L523S).

A phase I clinical trial of 13 stage IB, IIA, and IIB NSCLC patients was conducted using a prime/boost vaccination strategy first with pVAX/ L523S at a dose of 8 mg on days 0 and 14 then Ad/ L523S at three dosing cohorts of 1, 20, and 400 x10^9 viral particles on days 28 and 56 (Table 2 and 3) [61]. No significant toxic effects related to the

vaccination were reported. Although, all but one patient demonstrated at least a 2-fold increase in anti-adenovirus antibodies, only one patient demonstrated a significant immune response to L523S. The reasons for the minimal detection of immune response are unknown, but suggest that alternate formulations and/or regimens need to be considered in addition to other surrogate immune function parameters. Two patients developed disease recurrence and all patients were alive after the 290 day follow up. The significance of the disease-free survival cannot be assessed due to the small sample size however, one cannot exclude the possibility that the vaccine may induce a T cell response that is below the threshold of detection in peripheral blood. The results of this trial suggest an excellent safety profile, but limited evidence of L523S-directed immune activation.

B7.1 Vaccine (John, could this one be moved up to the cell-based vaccine group)

B7.1 (CD80+) is a costimulator molecule associated with induction of a T and natural killer (NK) cell response [62-65]. Tumor cells transfected with B7.1 and HLA molecules have been shown to stimulate an avid immune response by direct antigen presentation and direct activation of T cells, in addition to allowing cross-presentation [66-68]. In a phase I trial, Raez *et al.*, [69] used an allogenic NSCLC tumor cell line (AD100) transfected with B7.1 and HLA-A1 or A2 to generate CD8+ CTL responses. Patients who were HLA-A1 or –A2 allotype received the corresponding HLA-matched vaccine. A total of 19 patients with stage IIIb or IV NSCLC were treated and most had received prior chemotherapy. Patients who were neither HLA-A1 or –A2 received the HLA-A1 transfected vaccine. Each patient received three intradermal vaccinations of $5x10^7$ cells

every 2 weeks. If the disease remained stable and toxicity was low, treatment was continued.

A total of 18 patients received at least one full course (three vaccinations) of treatment. One patient was removed before the completion of the first course due to a serious adverse event not associated with the vaccine. Three more patients experienced serious adverse events, which were also not associated with the vaccine. Side effects associated with the vaccine included minimal skin erythema in four of the patients (Table 2 and 3).

All but one patient had a measurable CD8+ response after three vaccinations. There was no statistically significant difference in CD8+ response depending in weather or not the patients were HLA matched. One patient showed a partial response for 13 months and 5 patients had stable disease ranging from 1.6 to > 52 months [69, 70]. Based on the six surviving patients, the tumor vaccine appears to elevate immune response for at least 150 weeks. Overall, the Kaplan-Meier estimate for the survival of 19 patients was 18 months. One-year survival was estimated at 52% (Table 2 and 3). The low toxicity and good survival in this study suggested benefit from clinical vaccination. Further clinical investigation is on going.

Non-small Cell Lung Cancer Subunit Vaccines

L-BLP-25 Liposomal Vaccine

Mucin (MUC) 1 is a high molecular weight integral membrane protein on the apical surface of mucin-secreting epithelial cells. The extracellular domain of MUC1 contains a

heavily glycosylated peptide core composed of a tandemly repeating sequence of 20 amino acids [71]. It is expressed in many cancers, including NSCLC [72]. Although the MUC1 glycoprotein is expressed on the cell surface of many normal epithelial tissues and carcinomas, it has been selected as a target due to its high levels of overexpression and aberrant glycosylation patterns on carcinoma cells over normal cells, thereby conferring potentially high immunogenicity [73]. Recent studies have identified that MUC1 is associated with cellular transformation, as demonstrated by tumorigenicity [74], and can confer resistance to genotoxic agents [75]. Both the oligosaccharide portion and the tandem repeat of the MUC extracellular domain have the potential for immunotherapeutic activity.

Clinical testing of a MUC1-directed vaccine called L-BLP-25 (Stimuvax^{TM}) is ongoing. The L-BLP-25 vaccine consists of a synthetic lipopeptide with a sequence matching a part of the peptide core of the mucinous glycoprotein MUC1, immunoadjuvant, monophosphoral lipid-A, and three lipids: cholesterol, dimyristoyl phosphatidylglycerol and dipalmitoyl phosphatidylcholine. Upon reconstitution with saline, lipopeptide and monophosphoryl lipid A associate with the lipid bylayer of liposomes. The vaccine is injected into four anatomical sites in order to stimulate an increased number of lymph nodes to increase the likelihood of an immune response.

Trials of the L-BLP-25 vaccine in stage III and IV NSCLC patients showed the vaccine to be safe but did not demonstrate a statistically significant survival benefit [76, 77]. A phase I dose –comparison trial of 20 and 200 μg vaccine demonstrated the agent could be

administered safely. The relative safety and potential for efficacy found in phase I trials lead to the initiation of a randomized phase IIB study of L-BLP-25 in 171 advanced stage NSCLC patients [78]. Patients with ECOG performance status of 0-2, stable or responding stage IIIB or IV NSCLC following standard first-line chemotherapy were randomized to either L-BLP-25 plus best supportive care (n=88) or best supportive care (n=83) Patients on the L-BLP-25 arm received a single dose of cyclophsphamide 300 mg/m^2 IV followed by eight weekly subcutaneous immunizations with 1000µg of L-BLP-25 (Table 2 and 3). Maintenance immunizations (same dose) were given at 6-week intervals.

The overall survival results indicate a 4.4 month longer median survival for patients on the L-BLP-25 arm (17.4 versus 13 months), however this did not reach statistical significance (Table 2 and 3). In a retrospective analysis searching for a potential subset of patients with greater therapeutic benefit, a closer look was given to the patients with stage IIIB disease without pleural effusions. With a median follow-up of 53 months, patients on the L-BLP-25 arm had a median survival of 30.6 months compared to 13.3 months for the control group p=0.16 (n=75). A favorable toxicity profile was evident in all trials of L-BLP-25 with modest erythema at the injection site and mild flu-like symptoms.

While this study failed to demonstrate a statistically significant survival difference between the L-BLP-25 and control arms in advanced NSCLC, the survival trend in stage III disease may be of clinical benefit in this subgroup that have been downstaged by chemotherapy and thoracic irradiation. A phase III multicenter (North America, Europe,

Australia and Asia) randomized, double-blind, placebo-controlled safety/efficacy study of Stimuvax^{TM} in unresectable stage III NSCLC patients will evaluate patients who have shown stable disease or an objective clinical response after completing first-line chemoradiotherapy, either sequentially or concurrently. The primary endpoint is survival with a planned enrolment of 1322 patients and an expected completion date of 2011.

EP2101

EP2101 is a peptide-based vaccine designed to induce CTLs directed against carcinoembryonic antigen (CEA), p53, HER-2/neu and melanoma antigen E (MAGE)-2/3-tumor associated antigens [79-87]. These are frequently overexpressed in NSCLC. Analogue peptides have been shown to be capable of generating CTLs that are able to recognize wild-type epitopes expressed on tumor cell lines [88-90]. EP2101 has demonstrated immunogenicity in HLA-A2.1/k transgenic mice models [91-93]. It contains 10 lung cancer epitopes (p53, CEA, HER-2/neu and MAGE), of which 9 out of 10 are restricted by HLA-A2.1. This potentially enables vaccination of approximately 45% of the NSCLC population of the US.

Two phase I clinical trials examined the safety and immunogenicity of the EP2101 vaccine in patients with stage III colon cancer or stage IIA or IIIB NSCLC who were rendered disease-free by standard therapy. A total of 24 patients were enrolled, 16 of who completed 6 injections over 18 weeks. No significant toxicity was observed, and analysis of CTL response from 15 out of 16 patients who completes treatment with EP2101 indicated that the vaccine was immunogenic and effective at inducing strong and broad

CTL responses in a high frequency of patients, as measured by the INF-γ enzyme-linked immunosorbent spot assay (presented in IND #10802). A phase II study of EP2101 in 135 patients (64 HLA-A2^{pos} and 71 HLA-A2^{neg}) with stage IIIB or IV disease has recently been completed. EP2101 was administered every 3 weeks for the first 15 weeks of the study, then every 2 months through year 1, then quarterly through year 2, for a total of 13 doses. Each injection contained 5 mg (0.5 mg of each peptide) of peptide. The study compared the survival rate of HLA-A2.1 positive patients when treated with EP2101 versus HLA-A2-negative patients who underwent standard treatment. The vaccine was well tolerated and immune responses were seen in a majority of patients. However, one-year survival of HLA-A2^{pos} patients (55%) versus HLA-A2^{neg} patients (46%) and the median survival of 583 days (HLA-A2^{pos}) and 349 days (HLA-A2^{neg}) did not reach statistical significance (Table 2 and 3).

Epidermal growth factor vaccine

Overexpression of epidermal growth factor receptor (EGFR) and its ligand, epidermal growth factor (EGF), has been linked with the promotion of cell proliferation, survival and mortality. EGF transduces signaling through EGFR following binding to this cell surface receptor, ultimately resulting in cell proliferation. The immunotherapy developed by Ramos *et al.* induces an immune response against self produced EGF [94]. This vaccine is a human protein from *Nisseria Meningitides*. Several pilot studies have been completed [94-96]. Results from these demonstrated that vaccination with EGF is immunogenic and appears to be well tolerated.

In one trial, 43 patients with stage IIIB or IV NSCLC randomly received either a single or double dose [94]. The patients were given four weekly dosed followed by monthly immunizations. Side effects were mild to moderate, including mainly fever (42%), chills (47%), nausea (44%), vomiting (40%), tremors (44%), anorexia (35%) and pain (49%). Slightly higher toxic percentages were seen in the patients who received double dosages of the vaccine (Table 2 and 3).

Tumor responses against EGF were measured in 38 out of 43 patients, 15 achieved a good antibody response (GAR) against EGF following vaccination. Kaplin-Meier analysis separating patients by dose predicted a median estimated life expectancy of 6.4 months for patients who received the single dose and 8.4 months for the patients who received the double dose (Table 2 and 3). Based on immune response, however, patients classified as GARs had an average survival estimated at 12 months, where as those who had a less favorable GAR had an average survival of 7 months, thereby identifying a potentially responsive treatment population of NSCLC patients.

Two other studies conducted by Gonzalez and colleagues compared the effect of different adjuvants on patients' antibody response [95-97]. In the first trial 20 patients with stage IIIB or IV NSCLC were randomly vaccinated with either EGF-p64K absorbed to alum (n=10) or emulsified in montanide ISA 51 (n=10). In the second trial 20 stage IIIB or IV patients were similarly randomized, but all received a single dose of cyclophosphamide three days prior to the first vaccination. The vaccine consisted of EGF conjugated to a P64K *Neisseria meningitides* carrier protein, Patients were vaccinated intramuscularly on

days 0, 7, 14, 21, and 51. The patients were revaccinated when antibody titers decreased to at least 50% of their peak titer at the induction phase.

No patients experienced severe toxicity. Side effects consisted of grade 2 fever, chills, nausea, vomiting, nausea, hypertension, head ache, dizziness, flushing, pain at the injection site, bone pain, mouth dryness and hot flashes (Table 2 and 3).

The combined data of the two Gonzalez trials suggested that higher antibody responses were obtained when the vaccine was emulsified in montanide ISA 51 or when low-dose cyclophosphamide was administered before the vaccination; however, due to the small sample size, the difference was not statistically significant. Percentages of GAR were significantly higher when montanide ISA 51 was used as an adjuvant in both trials compared with alum groups. More than 90% of all vaccinated patients were seroconverted and GAR was achieved by approximately 50% of vaccinated patients. Median survival of GAR patients was 9.1 months, whereas poor antibody responding patients had survival of 4.5 months. The median survival of all vaccinated patients was 8.2 months. In addition, patients with ≥ 60 days response duration showed a significant increase in survival times compared with the corresponding groups with < 60 days response duration (Table 2 and 3). These data as well as the recent approval of EGFR inhibitors gefitinib and erlotinib justify further investigation in targeting EGF by vaccination strategy.

Melanoma-associated Antigen E-3vaccine (MAGE-3)

MAGE-AE is a 361 amino acid protein which belongs to the category of cancer/testis tumor antigens. In normal tissue MAGE-3 is expressed only by testicular germ cells, however, it is aberrantly expressed in a wide variety of tumors, including about 35% of NSCLC [98]. Several CD8+ T cell epitopes of MAGE-3 have been identified *in vitro* [88, 89, 99-105], including HLA-A1-restricted epitope 168-176 [106] and HLA-A2-restricted epitope 271-279 [107]. Based on these findings, synthetic peptides corresponding to these epitopes have been introduced into clinical vaccination studies in which they were associated with regression of melanoma in individual cases [108]. Clinical vaccination studies using full-length recombinant proteins have the advantage that this antigen potentially includes the full range of epitopes for CD4+ and CD8+ T cells. In addition, it is likely that protein vaccination leads to presentation of epitopes in the context of various HLA alleles and therefore, this type of vaccine should be applicable to any patient regardless of HLA restriction [109]. Atanackovic *et al.* [109] used a MAGE-3 protein as a vaccine to induce CD4+ T cells in patients with stage I or II NSCLC. All patients had undergone surgical resection of the primary lung tumor and had no evidence of disease at the onset of the study. A total of 9 of 17 total patients received 300 μg of the MAGE-3 protein alone, and 8 patients received the MAGE-3 protein combined with AS02B (Adjuvant System 2B; GlaxoSmithKline). Patients were given 4 intradermal injections every 3 weeks.

Of the nine patients who received the MAGE-3 protein alone, three developed an increase in antibodies against MAGE-3 protein and one had a CD8+ T cell response. By comparison, of the eight patients who received MAGE-3 antigen combined with the

adjuvant, seven showed an increase in serum concentrations of ant-MAGE-3 and four

had a CD4+ response to HLA-DP4-restricted peptide.

Based on these results, further testing in a larger randomized phase II trial was conducted

(134), involving 182 stage Ib or II completely resected NSCLC MAGE-A3+ patients

(122 vaccine and 60 placebo). Patients received five vaccinations at 3-week intervals. A

total of 1609 vaccinations were administered and no serious toxicities were attributed to

the vaccine after preliminary analysis (Table 2 and 3). After a median follow-up of 28

months 30.6% had recurrence in the vaccine group versus 43.3% in the control group.

The hazard ratio for progression-free survival was 0.73 (95% CI 0.45-1.16, 1 sided log

rank p=0.093). For overall survival the hazard ratio was 0.66 (95% CI 0.36-1.20, 1 sided

log rank p=0.088).

Phase III investigation is underway with the MAGRIT study (MAGE A3 as Adjuvant

Non-small cell lunG canceR ImmunoTherapy).   This ambitious project will screen over

10,000 resected NSCLC patients for 2,270 patients positive for MAGE-A3

immunohistochemistry. Eligible patients may or may not have received adjuvant

chemotherapy and will be randomized between MAGE-A3 immunotherapeutic versus

placebo.

Transcriptase catalytic subunit antigen vaccine

It has been established that human T cells recognize telomerase as a tumor associated

antigen [110-113]. Although telomerase is also expressed in some normal tissue, such as

bone marrow, and in crypts of the gastrointestinal epithelium [114], it is highly expressed in a vast majority of cancer cell lines. GV1001 is a unique peptide corresponding it a sequence of transcriptase catalytic subunit human telomerase reverse transcriptase (hTERT) derived from its active site. It contains the 611 – 626 sequence of hTERT and is capable of binding to molecules encoded by multiple alleles of all three loci of HLA class II [115]. HR2822, is a second peptide corresponding, to sequences 54 – 548 of hTERT. Brunsvig et al. [116] initiated a phase I/II trial of GV1001 (112 mg or 560 μg), HR2822 68 mg, and GM-CSF (30 or 75 μg). A total of 26 patients with stage III or IV NSCLC were given 4 – 21 intradermal injections of the vaccine. No clinically significant toxicities were attributed to the investigational regimen including gastrointestinal or bone marrow toxicities. Side effects were considered mild consisting mainly of flu-like symptoms.

Twenty-four of 26 patients enrolled were considered evaluable having received a minimum of four weeks of treatment. Fourteen patients completed the study while 10 patients were taken off study due to disease progression. Eleven patients demonstrated an immune response against GV1001, and 2 patients demonstrated a response to HR2822. After receiving booster vaccinations, two additional patients converted to immune responders. One patient with stage IIIA NSCLC had a complete response and developed GV1001-specific CTLs that could be cloned from peripheral blood. The median survival time for all 26 patients was 8.5 months (Table 2 and 3). This trial demonstrated GV1001 and HR2882 to a lesser extent are immunogenic targets and warrant further investigation.

Dexosomes

Dexosomes are dendritic cell-derived lipid vesicles that express high levels of a narrow spectrum of cell proteins, which have been shown to play a role in the activation of immune response [117-121]. *In vitro*, dexosomes have the capacity to present antigen to naïve CD8+ cytotoxic T cells and CD4+ T cells [117, 122]. Purified dexosomes were shown to be effective in both suppressing tumor growth and eradicating an established tumor in murine modles [119]. Morse *et al.* [123] developed a vaccine using DC-derived dexosomes loaded with MAGE tumor antigens. The phase I trial enrolled 13 patients with stage IIIB or IV NSCLC demonstrating MAGE-3A or A4 expression. Autologous DCs were harvested to produce dexosomes. They were peptide pulsed with MAGE-3A, 4A, 10A and -3DPG4 antigens. Dexosome vaccinations were administered to 9 patients at a dose of 1.3x1013 MHC II class molecules in a volume of 3 mL via subcutaneous and intradermal injection weekly for 4 weeks.

Patients experienced grade 1 and 2 toxicities including injection site reactions, flu-like symptoms, edema and pain. Three patients exhibited DTH reactions against MAGE peptides. Only one had detectable increases in T cell precursors frequency to MAGE-A10. Disease progression time ranges from 30 – 429 days and survival was in the range of 52 – 665 days (Table 2 and 3). The study concluded that production of dexosome was feasible. The vaccine is well tolerated and produced long-term stable disease in some patients and activation of immune effectors could be induced.

α(1, 3)-Galactosyltransferase

α(1, 3)-Galactosyltransferase (agal) epitopes present on the surface of most non-human mammalian cells are the primary antigen source inductive of hyperactive xenographt rejection. Agal directs the addition of agal to *N*-acetyl glucosamine residues in humans. Expression of agal epitopes after gene transfer of agal (using retroviral vector) in human A375 melanoma cells prevented tumor formation in nude mice [124].

Preliminary results by Morris *et al.* [125] using three irradiated lung cancer cell lines genetically altered to express xenotransplantation antigens by retroviral transfer of the murine agal gene, were recently described in seven patients with stage IV, recurrent or refractory NSCLC. Intradermal injections were given at doses of 3×10^6, 10×10^6, 30×10^6 or 100×10^6 cells/vaccine once every four weeks spanning a total of 4 doses. Only 4 patients received all 4 vaccinations, 2 patients received three vaccinations, and one patient received two vaccinations at the abstract was published. Toxicity involved grade 1 and 2 pain at the injection site, local skin reactions, fatigue, and hypertension. Four patients had stable disease for > 16 months (Table 2 and 3). Morris *et al.* concluded that the agal vaccine was feasible and safe. Full analysis is awaiting completion of this trial.

Dendritic Cell Vaccines

DCs are potent antigen-presenting cells [1-5]. As part of a phase II study Hirshowitz *et al.* [6] recently produced a DC vaccine from CD14+ precursors, which were pulsed with apoptotic antibodies from an allogeneic NSCLC cell line that overexpressed Her2/neu, CEA, WH1, MAGE-2, and survivin. A total of 16 patients with stage IA to IIIB NSCLC were vaccinated. The patients were immunized twice, one month apart.

There were 10 patients who experienced skin reaction at the injection site ad 4 patients experienced minor fatigue. No patients experienced a serious adverse event. Five patients showed no antigen-independent response and six patients showed an antigen-specific response (Table 2 and 3). The study concluded that the vaccine was safe and demonstrated immunologic activity. Further work is ongoing.

Cyclophilin B

Cyclophilin-B (CypB) is a ubiquitous protein playing an important role in protein folding [126, 127], and is expressed in both normal and cancerous cells. CypB-derived peptides are recognized by HLA-A24 restricted cytotoxic lymphocytes (CTL) isolated from lung adenocarcinoma. CypB peptides induce CTLs from leukemic patients, but failed to induce an immune response in cells isolated from patients with epithelial cancer or normal donors. Modification of a single amino acid of the CypB gene increases its immunogenicity and results in CTL activation in both cancer patients and healthy donors [128].

Gohara et al investigated the immune response in advanced-stage lung cancer patients treated with CypB vaccine. 16 HLA-A24+ patients, 15 with NSCLC and 1 with SCLC, were treated with CypB or modified CypB peptide vaccine following completion of chemotherapy [129]. All patients had stable disease at 5-week follow-up. Following vaccination, IFN-γ production by peripheral blood mononuclear cells isolated from patient sera were elevated in 3 of 12 patients. The median time to progression of patients

vaccinated with CypB peptide or modified CypB peptide was 25 or 8 weeks, respectively. Overall survival for NSCLC patients receiving CypB or modified CypB vaccine was 67+ and 28+ weeks, respectively (Table 2 and 3). One patient with SCLC was not evaluable for response.

Small Cell Lung Cancer

Fucosyl GM-1

The ganglioside fucosyl-GM1 is a carbohydrate molecule present in most cases of SCLC [130, 131], but absent in normal lung tissue. Immunostaining has demonstrated the presence of fucosyl-GM1 in culture media from SCLC cell lines, in tumor extracts and in serum of mouse xenografts [132]. Fucosyl-GM1 was detected in the serum of 4 of 20 SCLC patients with extensive-stage disease, but was not present in the serum of 12 patients with non–SCLC or in 20 healthy volunteers [132]. The specificity of fucosyl-GM1 to SCLC makes it a potential target for immunotherapy.

Dickler et al treated 13 patients were with Fuc-GM1 isolated from bovine thyroid tissue; 10 patients completed the study and were evaluable [133]. All 10 patients demonstrated high titers of IgM and IgG antibodies to Fuc-GM1, despite recent chemotherapy and radiation. The most common toxicity was local skin reaction, lasting 2-5 days. Other adverse effects include transient flu-like symptoms, fatigue, diarrhea, and worsening of sensory neuropathy (6 patients). Three of six patients who completed the entire course of vaccinations remained relapse free at 18, 24, and 30 months from diagnosis. Krug et al (2004) administered synthetic fucosyl-GM1 following completion of conventional

therapy to 17 patients [134]. Patients were randomized to receive vaccine doses of 30 μg, 10μg, or 5 μg. Five of six patients at the 30 μg dose demonstrated increased levels of anti-fucosyl GM1 IgM. Three of six patients receiving 10 μg doses showed anti-fucosyl GM1 IgM production, and none of five patients at the 3 μg dose level showed elevated IgM levels. The IgM titers for patients receiving 30μg or 10μg doses were similar to levels reached with patients treated with bovine fucosyl-GM1, while Ig-G levels were lower. Toxicities were minimal and included injection-site reaction, mild flu-like symptoms, myalgias, and sensory neuropathy (19%) (Table 4 and 5).

BEC2

Ganglioside GD3 is a cell surface glycosphingolipid whose expression in normal tissue is limited to cells of neuroectodermal origin and a subset of T lymphocytes [135-137]. High levels of expression have been demonstrated in SCLC tumors and cell lines [138]. Because GD3 is present at low levels in normal tissues, it is poorly immunogenic. BEC2, an anti-idiotypic IgG2b mouse antibody that is structurally similar to GD3, demonstrates strong immunogenic properties in patients with melanoma [139].

Grant et al treated 15 SCLC patients, 8 with extensive-stage and 7 with limited stage disease, with BEC2 vaccination [140]. 13 patients were evaluable for response; all developed IgM antibodies to BEC2, and 3 developed IgG antibodies. Duration of antibody production was variable, with at least one patient demonstrating measurable antibody production one year following treatment. Median survival was 20.5 months from diagnosis, and patients with measurable anti-GD3 antibodies showed the longest

relapse-free intervals (Table 4 and 5). When compared to SCLC patients treated with conventional therapy alone, the authors found patients treated with BEC2 vaccine to have longer than expected survival time, though not statistically significant. Significant toxicity was minimized to local skin irritation. There was no evidence of toxicity related to normal tissue destruction, despite the fact that GD3 is expressed by some normal tissues.

PolySA

Polysialic acid (polySA) is found on the surface of Gram-negative bacteria (such as group B meningococcus), embryonic neural crest cells, and some malignancies of neural crest origin [141, 142]. The large size and negative charge of this molecule inhibit binding of cell adhesion molecules, and it is this property that is believed to contribute to its role in neural crest cell migration and early metastasis of malignant cells [143, 144]. PolySA has been shown to be expressed abundantly by SCLC tissues [145-147], making it a potentially viable target for SCLC vaccine therapy.

Krug et al investigated the immunogenicity of polySA vaccination in 11 SCLC patients following conventional therapy [148]. Two forms of polySA were administered to patients. 5 patients received vaccination with polySA, and 6 patients received polySA manipulated by N-propionylation (NP-polySA), which has been shown to boost the IgG response in mice [149]. One of five patients treated with unmodified polySA demonstrated an IgM response. Of the six patients vaccinated with NP-polySA, all produced measurable IgM antibody responses. In five of the six cases these antibodies

cross reacted with unmodified polySA. Flow cytometry confirmed the presence of IgM antibodies reactive to SCLC cell lines. Despite the demonstrable production of IgM antibodies to polySA, complement-dependent lysis of polySA-positive tumor cells with human complement could not be demonstrated. Common adverse effects were minimal and included injection-site reaction and flu-like symptoms lasting 2-4 days (Table 4 and 5). Four patients reported sensory neuropathy.

p53

The tumor suppressor gene p53 plays a key role in cell cycle regulation, and is mutated in 90% of SCLC [150, 151]. In normal tissue, the p53 protein is present in low levels because of its brief half-life. Mutant p53 in cancer cells has a prolonged half-life and is therefore present at much higher levels in these tissues. When induced, anti-p53 cytotoxic lymphocytes attack tumor tissues while sparing normal tissue in preclinical studies [152-154].

Dendritic cells activated by p53-producing adenovirus were administered to 29 patients with extensive-stage SCLC [155]. 57.1% of patients showed significant p53-specific immune responses (Table 4 and 5). While only one patient showed an objective clinical response following vaccination, 61.9% of the 21 patients treated with second-line chemotherapy demonstrated clinical responses, compared to 2-5% response in nonvaccinated patients.

WT1

The Wilms' tumor gene (*WT1*) is responsible for Wilms' tumor, a pediatric renal cancer, and encodes a protein involved in cell proliferation and differentiation, apoptosis, and organ development [156-158]. WT1 is overexpressed in several hematological malignancies as well as various solid tumors, including lung, breast, thyroid and colorectal cancers [159, 160]. WT1-specific cytotoxic lymphocytes (CTL) lyse WT1 expressing tumor cells *in vitro* without damaging normal tissues that express WT1 physiologically [161, 162].

Oka et al treated 26 patients, including 10 lung cancer patients (histological type not specified), with WT1 vaccine following completion of conventional therapy [163]. Three patients showed decreased serum levels of tumor markers (CEA or SLX) following vaccination; one patient also showed a decrease in tumor size radiographically. One patient had stable disease at followup; four patients developed progressive disease, and 2 were unevaluable. Three patients demonstrated increased activity of WT1-specific CTL activity. A correlation (P=0.0397) between immunological and clinical response was observed for all study patients. Toxicities were limited to injection-site inflammation. Despite the fact that WT1 is expressed in many normal tests, routine laboratory investigates did not reveal damage to these tissues following vaccine administration.

Discussion

The poor overall survival of patients with advanced lung cancer combined with the toxicity associated with many treatment modalities, mandates novel approaches to clinical management of lung cancer. Traditional approaches for management of advanced

stage lung cancer have likely reached a plateau with respect to survival and response advantage to singlet, doublet or triplet cytotoxic therapy combinations. Recent data of combinations of cytotoxic therapies with angiogenesis inhibitors and/or EGFR inhibitors appear encouraging in subsets of patients. Results summarized in this review suggest immune based therapies may also "soon" provide sufficient validation to be considered as part of the therapeutic armetarium for lung cancer. Both "targeted" peptide and gene-transduced cell based vaccines demonstrate the ability to activate and direct adaptive immune effecter cells to recognize and attack cancer. The activity of Lucanix, L-BLP-25 and MAGE-A3 in particular has lead to the conduct of phase III trials and the data generated from these trials will potentially serve to guide direction of these novel therapeutic modalities over the next several years. Yet more remains to be discovered.

This chapter summarizes the current evidence of the clinical activity of immune-based vaccines in lung cancer. Through enhancement of tumor antigen recognition and immune activation these vaccines may one day provide patients with a highly tolerable therapy to use in combination with traditional approaches. However, this treatment strategy is relatively new and will require continued development in order to determine its ultimate role in the treatment of lung cancer.

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