Gene-Modified Cellular Vaccines: Technologic Aspects and Clinical Problems

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ABSTRACT

Activity in the cancer vaccine sector has quadrupled in the last decade. A number of therapeutic cancer vaccines are reaching the market. The huge number of clinical trials in progress is expected to undergo evaluation shortly. Whole cell tumor vaccines or gene-modified whole cells are being intensively tested in clinical trials. However, the specificity of the product makes the drug development process, including clinical trials, a considerable challenge. Their complex nature, standardization of manufacturing, and characterization often pose problems. Accordingly, to develop a well characterized controlled vaccine, more than a few factors need to be established. The final cell vaccine formulation must be characterized for product identity, purity, impurities, sterility, potency, cell viability, and total cell number. Therapeutic cancer vaccines show different clinical characteristics than cytotoxic anticancer agents. Unfortunately, the rules of clinical trial design for active immunotherapy have been adapted from the designs for examination of cancer chemotherapy. Accordingly, many research groups and clinical consortia have postulated modifications and unifications of existing clinical trial designs. A clinical development model has suggested that cancer vaccines be investigated in 2 categories of clinical trials: proof-of-principle and efficacy. Moreover, it is becoming clear that no drug demonstrates anticancer activity in all patients. Thus, intensive studies have been performed to seek specific biomarkers which could help stratify patients who are likely to respond to a particular treatment. This presents a big challenge beyond the analysis of the immune system status necessary to assess the effects of active immunotherapy.

Somatic cell–based pharmaceuticals have been used to prepare active immunotherapeutic products, such as vaccines for infectious diseases. For >2 decades, so-called cellular therapeutic cancer vaccines have been tested in humans. Due to the progress in gene engineering, a next-generation of cellular vaccines have been developed as gene-modified products. However, owing to the complexity of human anticancer immune responses and poorly understood mechanisms governing tumor growth and cancer immunologic escape processes, progress has been inconsistent in this field. The major problems relate to the manufacture of medicinal products fulfilling regulatory requirements and to the conduct of properly designed and executed clinical trials. Errors in these processes which may occur during drug development may result in failure to obtain approval of the drug to authorize marketing. Herein we have discussed technologic and clinical problems based on our 20 years of experience in the development of gene-modified cellular vaccines for melanoma.

CELL-BASED VACCINES

A number of cellular cancer vaccines have been evaluated in clinical trials (Table 1). Cell-based vaccines include cancer cell lysates; whole cell vaccines with adjuvants; gene-modified tumor cells; dendritic cells (DC) pulsed with antigens in the form of RNA, peptides, proteins or cell lysates; DC modified with genes encoding immune stimulators; or cancer cells fused with DC or B lymphocytes.1

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The first generation of whole tumor cell vaccines was based on autologous or allogeneic irradiated live cells or tumor cell lysates admixed with adjuvants. Vaccines of the second generation are composed of genetically modified autologous or allogeneic cancer cells with gene(s) encoding cytokines, designer cytokines, growth factors, HLA, or costimulatory molecules. A variant option includes vaccines consisting of autologous cells mixed with genetically modified allogeneic tumor cells or modified autologous noncancer cells, such as fibroblasts. Modification of allogeneic cancer vaccines secreting cytokines or other factors is expected to costimulate immune cells. Moreover, ternsgen proteins acting in an autocrine manner may autostimulate vaccine cells, thus changing their properties. Such stimulation may change the phenotype of the vaccine making it more immunogenic and/or induce secretion of immunomodulatory cytokines, such as interleukin-2 or other factors.2

Melaccine is an allogeneic cancer vaccine composed of tumor lysates from 2 melanoma cell lines (MSM-M-1 and MSM-M-2) together with Detox adjuvant.3 After encouraging early phase studies, the product failed a phase III trial. However, a retrospective analysis indicated that patients treated with Melaccine who expressed at least 2 of 5 HLA antigens present on the vaccine cells showed longer relapse-free and overall survival (OS).4–6 Accordingly, the HLA pattern of the patient served here as a biomarker allowing stratification of patients who would respond to the treatment. Thus Melaccine could be considered to be precursor of a personalized cancer vaccine.

OncoVax, an autologous whole cell vaccine, was evaluated in a phase III adjuvant study enrolling patients with stage II and III colon cancers. The study demonstrated that treatment with OncoVax was most beneficial for patients in stage II, but not stage III. The 5-year recurrence-free survival among patients with stage II colon cancer treated with OncoVax was 79% versus 62% in the control group (P = .009); the OS rate was 82.5% versus 72.7%, respectively (P = .01).7 A phase III trial recruiting patients with stage II colon cancer is planned in the near future.

Retionale, an autologous kidney cancer cell lysate vaccine examined in a randomized phase III study, demonstrated a significant increase in 5-year progression-free survival (PFs) among patients with stage III nonmetastatic renal cancer after nephrectomy.8 A genetically modified prostate cancer vaccine (GVAX) consists of 2 allogeneic cell lines secreting granulocyte-macrophage colony-stimulating factor. A phase II trial of GVAX in patients with hormone-refractory prostate cancer showed a decline in serum prostate-specific antigen levels. Two phase III trials were terminated in late 2008: the VITAL-1 study comparing GVAX with docetaxel plus prednisone, due to a futility analysis showing <30% chance of meeting the primary endpoint9; and the VITAL-2 trial using GVAX plus docetaxel versus docetaxel plus prednisone, due to IDMC (Independent Data Monitoring Committee) recommendation.10 A cancer vaccine awaiting marketing authorization by the U.S. Food and Drug Administration is Sipuleucel-T (Provenge), which consists of DC modified with a fusion protein composed of prostatic acid phosphate and GM-CSF. The phase III study demonstrated that patients with hormone-refractory prostate cancer who were treated with Sipuleucel-T showed extended OS for 4.1 months compared with a placebo control group. Three-year survival was 31.7% in Provenge-versus 23% in placebo-treated patients.11

Characterization and Quality Control of Cellular-Based Cancer Vaccines

The complex nature, standardization of the manufacture, and characterization of the product pose considerable challenges for the development process of therapeutic cancer cell vaccines. Accordingly, to develop a well characterized controlled vaccine requires establishing more than a few factors. The final formulation must be characterized for product identity, purity, impurities, sterility, potency, cell viability, and total cell number according to the European Medicines Agency’s (EMEA) “Guideline on human cell-based medicinal products.”12 The identity of the cellular vaccine during the manufacturing process and product release should be characterized in terms of phenotypic and
genotypic profiles, because long-term cell culture and extensive cell passages may influence the product’s quality. The ratio between nonviable and viable cells needs to be established, because cell viability, an important parameter for product integrity, directly correlates with biologic activity. To ensure that no adventitious microbial agents (viruses, mycoplasma, bacteria, fungi), contaminants, or impurities are introduced during the manufacturing process, the choice of starting raw materials and the use of suitable in-process control are crucial for drug quality and safety. Laborious but important steps of buffer washes of the harvested cells seek to reduce in-process impurities.

Developing a suitable testing system for biological potency of vaccines presents a significant challenge. According to the International Conference on Harmonization guideline Q6B, biologic potency assay is the “quantitative measure of biologic activity based on the attribute of the product, which is linked to the relevant biologic properties.”13 The World Health Organization, at a November 2003 meeting of the Expert Committee on Biological Standardization, commented, “Potency tests measure biologic activity of a vaccine but do not necessarily reflect the mechanism of protection in humans.”14 After pivotal clinical trials a validated potency assay is obligatory in the process of drug registration before product release; it should be developed through the early clinical phases. A properly validated potency assay demonstrating the activity should be based on the intended biologic effect as close as possible to the mechanism of action/clinical response. The potency assay should demonstrate that the given batch displays minimal potential biologic activity and shows batch-to-batch consistency using techniques that in some way depend on or are a sign of biologic activity. It is known that cellular associated markers and in vivo biologic responses that correlate with efficacy have not been identified to date. Serologic/immunologic correlations of clinical protection are not available; they have not been established in clinical trials.

Petricciani et al15 suggested potential pathways for development of a biologic potency assay for whole cell cancer vaccines. When efficacy is established, the following outcomes are possible: 1) immune response to antigen correlates with clinical efficacy, i.e., quantification of antigen as a potency assay; 2) immune response to the antigens is demonstrated, but there is a lack of clinical correlation between antigen-quantitation potency assay development in larger trials; and 3) lack of an antigen-specific immune response, but demonstrated clinical efficacy. To reduce the risk of having an unacceptable potency assay at the end of a pivotal phase III study, an additional bioassay should be developed parallel to a quantitative antigen expression assay. Petricciani et al have suggested cell viability count measurements using trypan blue in addition to other measures of potency, such as quantitative antigen expression.15 In mid-2008 the EMEA’s “Guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer” came into effect. They cover some of the issues raised by Petricciani et al. The guidelines present the possibility that in vivo assays using transgenic animals for major histocompatibility antigens can be used to present human antigens to the immune system of these animals or of immune-compromised animals repopulated with human T cells. In vitro assays may document biochemical and physiologic responses measured at the cellular level by cytotoxic T-cell activity or cytokine production by specific elements. In cases when direct measurement of potency is impossible, surrogates for potency may be developed if they show a correlation with the biologic activity of the vaccine. Surrogate analyses include determination of cell surface markers, activation markers, factor secretion, and expression of a single gene product or protein expression pattern.16

For proper quality control, the final product should undergo stability testing according to the EMEA “Guidelines on human cell-based medicinal products.” Cancer vaccines are usually stored in liquid nitrogen, which is effective to maintain cell quality for a cold chain–transport network. The effects of temperature change on the cells should be determined and the cold chain validated to provide evidence of maintained operating ranges. More advanced technology (formulation) that do not require a cold chain would be beneficial for whole cell vaccines.10 In addition, special quality requirements have been directed to genetically modified cell-based vaccines: characterization of the vector for cell modification; description of the process; and quality control tests on the product that address issues pertaining to the transfected gene(s) of interest, such as integrity, expression (constitutive or regulated), genetic stability, and copy number. The occurrence of replication competent virus, residual vectors, or plasmid nucleic acids and associated polymers should be controlled where applicable. For the measurement of biologic activity after cell transfection, a suitable assay should be established to assure maintained biologic activity during storage.17

The draft EMEA “Guideline on human cell-based medicinal products” is not cancer vaccine directed; however, it includes several steps which are common to all cell-based medicinal products, namely, control of the raw materials, process development, and manufacturing validation. It offers an excellent guide for vaccine development.12 In addition, “Guideline on potency testing of cell-based immunotherapy medicinal products for the treatment of cancer” by the Committee for Medicinal Products for Human Use of the EMEA, which came into effect in mid-2008, set clear standards for cancer vaccines.18

CLINICAL TRIALS

A number of immunotherapeutics have shown high efficacy in early-phase clinical trials but have failed in randomized phase III trials.19 Badly planned and performed clinical trials may lead to a failure in authorization for drug marketing, risking the chance to license a preparation which could eventually give clinical benefit. Therapeutic cancer
vaccines form a wide group of compound biology with clinical characteristics distinct from cytotoxic anticancer agents. The recent clinical trial design for cancer vaccine development is based on criteria developed for cytotoxic drugs. Biological therapies vary from cytotoxic agents in terms of mechanism of action and toxicity.

Development of medicinal products using advanced technologies for cancer treatment brings new challenges for the design and execution of clinical trials. Many research groups and clinical consortia have postulated modifications and unifications of existing clinical trial designs to adjust them to clinical testing of medicinal products of advanced technologies.

NEW CLINICAL DEVELOPMENT PARADIGM

Clinical development paradigms were recently proposed by the Cancer Vaccine Clinical Trial Working Group (CVCTWG) consisting of 50 American and European experts from academia, regulatory bodies, and the biotechnology and pharmaceutical industries. The authors proposed a clinical development model in which cancer vaccines are investigated in 2 categories of clinical trials: proof-of-principle and efficacy.

Proof-of-principle exploratory trials join some characteristics of conventional phase I and II clinical trials. Their purpose is to generate data to plan efficacy trials. The objectives are safety (initiation of database), dose selection and vaccination schedule, and demonstration of proof-of-principle (biologic or clinical activity). The authors highlighted issues that should be considered in the design of an early-phase trial: 1) a minimum of 20 enrolled patients as a homogeneous well-defined population to evaluate safety; 2) eligible patients should not have rapidly progressive disease, thereby allowing time for the immune system to display clinical activity; 3) patients with a nonclinically significant progression should continue treatment, allowing for evidence of a late clinical response; and 4) patient withdrawal from the study should be based on toxicity or clinically significant disease progression after a minimum period and number of vaccinations sufficient for a clinical response. Evidence of biologic activity should be observed in the proof-of-principle trials, allowing conduct of further trials. Activity should include the demonstration of an impact on the target disease or on the patient’s immune system using study endpoints of biologic markers: clinical, molecular, or immune response. It is proposed to not place emphasis on clinical activity but rather to focus on biologic activity in proof-of-principle trials. After the success of proof-of-principle trials, the efficacy trial may be conducted.

Efficacy trials are randomized clinical trials which demonstrate clinical benefits either directly or through a surrogate. This is different from single-arm phase II clinical trials that evaluate cytotoxic drugs, which usually use response rate as the primary endpoint versus historical control groups as comparators. If well-defined trigger-point criteria are met, efficacy trials may use prospective adaptive designs to expand from randomized phase II to a phase III trial. These trials should be direct follow-ups of proof-of-principle trials to confirm the data and demonstrate efficacy. They may be designed as conventional phase III studies or comparative randomized phase II trials with an adaptive component that can be expanded into phase III. Phase II studies include noncomparative randomized trials, comparative randomized studies with possibility of full phase III, and comparative randomized trials with an adaptive component. Also, there is a possibility of designing tandem proof-of-principle and efficacy trials. The concept of efficacy trials is to yield the possibility of an early assessment of vaccine efficacy and more flexible, rapid, and informed development of cancer vaccines.

The new clinical development paradigm proposed by CVCTWG is a big step forward toward developing a more feasible regulatory pathway for cancer vaccines. However, it is uncertain how the proposed development paradigm can be applied effectively in the near future to quicken cancer vaccine development in the setting of patients with a better prognosis. The shortage of early markers of efficacy and the timeline required to develop disease recurrence remain obstacles to accelerated decision making inherent in the CVCTWG model.

PATIENT SELECTION

Historically, patients enrolled in cancer vaccine trials displayed late-stage disease having undergone first- or second-line chemotherapy and/or radiotherapy. However, therapeutic cancer vaccines are likely to be most beneficial for patients with low tumor burdens or who are treated in an adjuvant setting. Furthermore, in contrast to subjects treated with cytotoxic agents, patients undergoing cancer vaccine therapy need more time to build responses to the treatment. Also patients with high tumor burden may be less likely to survive long enough to develop an immune response after vaccination. Accordingly, the inclusion criteria should reflect a population that has the potential to benefit from the vaccination. The use of biomarkers and other surrogates to evaluate prognosis could be considered, even if these markers have not been validated as surrogates for efficacy. Moreover, patients with resected metastases may progress rapidly, so more attention should be paid to the dynamics of the course of the disease before and during screening for enrollment.

Endpoints

Clinical trial endpoints for cytotoxic agents may not be sufficient for cancer vaccine trials. Clinical tumor response as a classic endpoint for early chemotherapy trials may not reflect the benefits of cancer vaccine treatment. After immunotherapy, the patient’s immune system needs time to develop clinical activity. Patients with metastatic melanoma treated with AGI-101H vaccine (a genetically modified melanoma vaccine secreting hyper-interleukin-6) usually require 3–4 months to display a response. However, in a
number of cases, tumor responses were observed after a number of months or even years after stabilization of the disease. As has been observed in several immunotherapy trials, clinically responsive tumors tend to enlarge first and then shrink. Furthermore, the immunotherapy may be unsuccessful to induce a decreased size, although it is still effective to slow the rate of progression. The CVCTWG proposes that patients after primary clinically in significant progression should continue treatment; after regression, the response rate could be scored based on the largest progression should continue treatment; after regression, the response rate could be scored based on the largest tumor volume measured after the start of treatment rather than from the baseline tumor volume. Wolchok et al recently published guidelines to evaluate immune-related response criteria (irRC) based on the Ipilimumab antibody against cytotoxic T-lymphocyte antigen 4 phase II clinical trials in patients with advanced melanoma. In some patients, Ipilimumab monotherapy resulted in responses after an increase in total tumor burden and despite the presence of new lesions. Those patterns were associated with favorable survival. According to irRC: 1) new, nonmeasurable lesions do not define progression; 2) new measurable lesions are not defined as progression but are incorporated into tumor burden; and 3) disease progression has to be confirmed by a repeat consecutive assessment ≥4 weeks from the first documented date.

Overall survival is the “gold standard” to evaluate the clinical benefit of a product for the treatment of cancer. OS is the primary endpoint of choice in phase III clinical trials. For early-phase studies, OS may be affected by subsequent therapies, is time consuming, and may require larger groups of patients. It may be obligatory for randomized phase II efficacy trials with an adaptive component. Disease-free survival (DFS) in adjuvant and PFS in metastatic disease settings are acceptable surrogate primary endpoints that may shorten the time for randomized efficacy trials. As discussed earlier, patients with nonclinically significant progression may benefit from a cancer vaccine. Using classic definitions of DFS or PFS, patients with progression can be excluded earlier from a the trial as not experiencing benefit from vaccine therapy. These definitions could be modified: 1) requirement for confirmation of progression on ≥2 observations, and 2) lack of early progression within a defined time interval (eg, 3 months from therapy start). In patients responding rapidly after an early period of progression, the onset date of DFS or PFS calculation should remain the start of treatment. Furthermore, for some tumors, such as melanoma, where there is no alternative effective treatment, cancer vaccines should be continued. Disease progression may require changes in vaccine schedule (induction phase) or performance of surgical resection. In our own studies, patients entering the trial were vaccinated with AGI-101H 8 times at 2-week intervals (induction phase) and then once a month in the maintenance phase. We observed that this dose schedule was clinically beneficial as regression of metastatic tumors was found. Furthermore, to maintain clinical effectiveness, vaccination must be continued as long as the patients live.

BIOMARKERS

There are 2 types of biomarkers: first, tumor/host-related factors that correlate with tumor biologic behavior and patient prognosis, eg, Breslow tumor thickness and presence of ulceration at the primary melanoma site; and second, biomarkers that serve as reliable indicators of a treatment response, eg, tests for hormone receptor status in breast or prostate cancer or evaluated human epidermal growth factor receptor 2 status in breast cancer. Immune parameters must be established to predict clinical outcomes in cancer vaccine treatments in order to reduce the development time and length of clinical trials. Definition and validation of immunologic biomarkers outline factors that during screening identify patients who could respond to vaccine therapy, which provides immunologic (laboratory) endpoints and monitoring tools for clinical trials. However, to date there are no validated biomarkers for immunotherapy or vaccines. Cancer patients, especially with high tumor burdens, are immunosuppressed; therefore, effectiveness of active immunotherapy is linked to their immune system status. Furthermore, the lack of correlation between immunologic and clinical responses may relate to the complexity of responses necessary for an antitumor response. Perhaps in the future, in vitro tests to evaluate TH1 cellular responses, eg, cytokine production by T cells stimulated with specific antigen, might be replaced by multiplex cytokine assays combined with an artificial neural network. This development could allow for a more sophisticated interpretation of cytokine data, giving perhaps the possibility of developing immunologic profiles which could serve as biomarkers for clinical responses.

CONCLUSIONS

The field of therapeutic and prophylactic cancer vaccines for active immunotherapies is rapidly emerging as a promising area. The use of passive immunotherapy (ie, antibody) products for this disease widespread. There has been enormous activity in the vaccine sector over the past 10 years. However, no specific active immunotherapy product has yet reached the market in the U.S., but it may be getting close. “A recent analysis indicated that the vaccine market will be the fastest-growing therapeutic area in the pharmaceutical industry, with an annual growth rate of 14% over the next 5 years. That surpasses oncology, currently the largest therapeutic pharmaceutical area, which is projected to grow at 11% annually.”

REFERENCES

therapy of metastatic melanoma with allogeneic melanoma
4. Mitchell MS, Harel W, Groshen S: Association of HLA
phenotype with response to active specific immunotherapy of
HLA class I allele expression on outcome in melanoma patients
treated with an allogeneic melanoma cell lysate vaccine. Final
6. Sondak V, Sosman J, Unger JM, et al: Adjuvant immunother-
apy of resected, intermediate-thickness, node-negative melanoma
with an allogeneic tumor vaccine: impact of HLA I antigen
Immunotherapy with autologous tumor cell–BCG vaccine in pa-
tients with colon cancer: a prospective study of medicinal and
economic benefits. Vaccine 23:2379, 2005
8. Bringing therapeutic cancer vaccines and immunotherapies
through development to licensure, February 8–9, 2007. Available
Videocast available at: http://videocast.nih.gov/
10. Available at: http://www.clinicaltrial.gov/ct2/show/NCT00133224
ized, double-blind, placebo-controlled, multi-center, phase III trial
of Sipuleucel-T in men with metastatic, androgen independent
prostatic adenocarcinoma (AIPC). Presented at the American Uro-
logical Association 104th Annual Scientific Meeting (Abstract 9)
12. Committee for Human Medicinal Products, European Me-
dicinal Agency: Draft guideline on human cell-based medicinal
emea.europa.eu/pdfs/cpwp/410869en.pdf
13. International Conference on Harmonization: Guideline
Q6B, specifications: test procedures and acceptance criteria for
biotechnological/biological products, March 10, 1999
14. WHO Expert Committee on Biological Standardization:
WHO technical report series: guidelines for the production and
quality control of synthetic peptide vaccines. Annex I. Technical
Report Series 889:38, 1999
16. Committee For Medicinal Products for Human Use, Euro-
pean Medicines Agency: Guideline for potency testing of cell-
based immunotherapy medicinal products for treatment of cancer:
cfds/human/bwp/27147506.pdf
therapeutic cancer vaccines. Curr Opin Drug Discov Devel 11:168
2008
18. EMEA/CHMP: Note for guidance on the quality, preclinical
and clinical aspects of gene transfer medicinal products (CPMP/
BWP/3008/99)
randomized phase III studies with active cancer immunotherapies—
outcomes from the 2006 Meeting of the Cancer Vaccine Consortium
20. Copier J, Ward S, Dalgleish A: Cell based cancer vaccines:
21. Michaelis LC, Ratain M: Measuring response in a post-
RECIST world: from black and white to shades of grey. Nat Rev
Cancer 6:409, 2006
22. Nawrocki S, Mackiewicz A: Clinical trials of active cancer
designs for the early clinical development of therapeutic cancer
vaccines, J Clin Oncol 19:1848, 2001
clinical development challenges and proposed regulatory ap-
proaches for patient access to promising treatments. Cancer 112:
955, 2008
25. Copier J, Dalgleish A, Britten CM: Improving the efficacy of
paradigm for cancer vaccines and related biologics. J Immunother
30:1, 2007
tumour vaccines (GMTV) in melanoma clinical trials. Immunol
Lett 15:81, 2000
28. Wolchok JD, Hoos A, O’Day S: Guidelines for evaluation of
immune therapy activity in solid tumors: immune-related response
29. Gutman S, Kessler: The Food and Drug Administration
perspective on cancer biomarker development. Nat Rev Cancer
6:565, 2006
30. Copier J, Dalgleish A, Britten: Improving the efficacy of
31. Sannes LJ: Immunotherapies and vaccines for cancer and
infectious diseases. Insight Pharma Reports. Cambridge Health
Institute, 2008. Available at: http://www.insightpharmareports.com