Progenics, Somatix in phase III cancer vaccine trials

In 1996, therapeutic tumor vaccines are once again back in phase III trials. Progenics Pharmaceuticals (Tarrytown, NY) and Somatix Therapy (Alameda, CA) both have advanced-stage products to treat melanoma. Progenics plans to begin two phase III trials in the first quarter of 1996 involving the GMK vaccine recently licensed from Memorial Sloan-Kettering Cancer Center (New York). The vaccine contains GM2 ganglioside, a glycolipid present on most melanoma cells. Later in the year, it will follow with an investigational new drug (IND) application for MGV, a multiganglioside vaccine containing GM2 and GD2 and targeting other types of cancer, including colorectal, gastric, and small-cell lung cancer. In essence, the Somatix product consists of the patient's own tumor cells, expanded *ex vivo*, engineered to make an immunostimulant, then reinjected. Many other innovative vaccine strategies will enter clinical trials later this year. But why should we expect success now when earlier cancer vaccines have failed?

Two fundamental problems have plagued the tumor vaccine field: a lack of tumor antigens of a sufficient specificity, and an inadequate understanding of the molecular mechanisms behind an effective immune response. Researchers did not know why the immune system tolerated tumor antigens. They could not reliably select antigens or delivery vehicles to ensure that the antigens are processed by antigen-presenting cells and elicit a cytotoxic T-lymphocyte (CTL) response. The recognition of the importance of a T-cell-mediated immune response against cancer, and the means to manipulate antigen presentation, have only come about in the last several years.

But researchers have now identified tumor antigens that both stimulate a CTL response and are unique to cancer cells such as the MAGE family of antigens found on melanoma cells and in breast and lung tumors. Recognition of tissue-specific antigens has opened the door to vaccines that target a tissue type, rather than a tumor—useful for cancers of the prostate, pancreas, breast, and skin, for example.

Furthermore, researchers have overcome the immune system's tolerance of normal proteins, such as carcinoembryonic antigen (CEA), which can induce a T-cell mediated response when overexpressed in tumor tissue. Proteins that are present in different forms in normal and cancer cells, such as a mucin polypeptide (MUC-1) expressed on epithelial cells throughout the body, are also being exploited as tumor antigens. On the surface of normal cells, MUC-1 has a carbohydrate coating that shields its protein core from the immune system. Transformed cells, however, glycosylate incorrectly, exposing patches of MUC-1 that can serve as targets for immunotherapeutic assault. Another approach being used in prophylactic cancer vaccines is to target viral antigens associated with malignant transformation, such as the E6 and E7 gene products of human papilloma virus (HPV), a major cause of cervical cancer. Other viral targets include Epstein-Barr, hepatitis B virus.

The race to discover new tumor antigens has captured the attention of several biotechnology companies, including Corixa (Seattle, WA), which has filed patents on more than 40 tumor-specific genes for breast and prostate cancer, and Therion Biologics (Cambridge, MA), which plans to file INDs on nine different recombinant antigens in 1996, including MART-1 and gp100, both found on melanoma cells.

Yet finding immunogenic tumor proteins is only part of the challenge. "Even if you have antigens, you still have to change the immune system from a state of tolerance to one of activation," explains Drew M. Pardoll of Johns Hopkins University School of Medicine (Baltimore, MD). Injecting a soluble protein into a person will not generate a CTL response against the protein. That is why many early tumor vaccines failed. The antigen must find its way inside an antigen-presenting cell, a macrophage or dendritic cell, which breaks down the antigen and presents a component peptide in the antigen-binding cleft of cell surface major histocompatibility complex (MHC) molecules. Several possible solutions to this problem are in development.

By engineering the gene for a tumor antigen into a recombinant virus, such as vaccinia or pox viruses, researchers can trick the body into seeing the antigen as part of the virus and mounting an immune response. Jenner Technologies (Danville, CA) uses a virus-free system—recombinant prostate-specific antigen (PSA) in liposomes—to target the antigen to the reticuloendothelial system. Corixa packages its tumor antigens in a polymer-based microsphere of a specific size to trick the immune system into generating a CTL response. "It's a size phenomenon—antigen-presenting cells just like eating these things," says Steven Gillis, president and CEO.

Companies involved in tumor vaccine development

Aphton	(Woodland, CA)
Apollon	(Malvern, PA)
Biomira	(Edmonton, Alberta, Canada)
Boehinger Ingelheim Pharmaceuticals	(Ingelheim, Germany)
Cambridge Biotech	(Worcester, MA)
Cantab Pharmaceuticals	(Cambridge, U.K.)
Centocor	(Malvern, PA)
Chiron/Viagene	(San Diego, CA)
Corixa	(Seattle, WA)
Cytel	(San Diego, CA)
Idec Pharmaceuticals	(Mountain View, CA)
ImClone Systems	(New York, NY)
Jenner Technologies	(Danville, CA)
MedImmune	(Gathersburg, MD)
Merck	(Rahway, NJ)
Oncotech	(Irvine, CA)
Progenics Pharmaceuticals	(Tarrytown, NY)
Ribi ImmunoChem	(Hamilton, MT)
SmithKline Beecham	(London, U.K.)
Somatix Therapy	(Alameda, CA)
Therion Biologics	(Cambridge, MA)
Vaxcel	(Atlanta, GA)

Pardoll and colleagues have engineered tumor antigens into *Listeria monocytogenes*, which are taken up by macrophages. The same group recently described a molecular tag used to direct HPV E7 antigen into the endosomal and lysosomal compartments of antigen-presenting cells, and into the MHC class II pathway for presentation to cytotoxic T cells (Lin et al. 1996. *Cancer Research* 56:21-26). In preclinical studies, the group's HPV vaccine protected tumor-free mice from challenge with a tumorigenic cell line, and cured mice that had small E7-expressing tumors.

In January, Hsu et al. (1996. *Nature Med-*

icine 2:52-53) described antitumor cellular immune responses in a pilot study in which all patients were infused with autologous dendritic cells that had been cocultured *ex vivo* with tumor-specific idiotype protein. Clinical responses included complete tumor regression in one patient.

Proper presentation of a T-cell activating tumor antigen, however, still does not ensure a therapeutic immune response against an established tumor. The third piece of the cancer vaccine puzzle is immunostimulation. One hot area of cancer vaccine research focuses on replacing nonspecific adjuvants, such as bacillus Calmette-Guerin (BCG) and alum, with "designer" adjuvants.

Studies in infectious disease, which researchers are now trying to extend to cancer, demonstrate that the type of immune response induced by a vaccine is crucial to the outcome. "It is important to generate a cytokine pathway, as opposed to a T_H2 response," said Gillis. Corixa's proprietary adjuvant is the cloned Leishmania eukaryotic initiation factor (LeIF).

Progenics has licensed Cambridge Biotech's (Worcester, MA) QS-21 adjuvant for use in its ganglioside conjugate vaccines. QS-21, purified from the bark of the South American *Quillaja saponaria* Molina tree, induces a T_H1 response and enhanced T-cell-mediated cytotoxicity.

Pardoll has devised a collagen microsphere to encapsulate irradiated tumor cells mixed with the immunostimulant, granulocyte macrophage-colony stimulating factor (GM-CSF). Injection of the particle directly into the tumor generates a cytokine-induced immune response to the tumor antigens. GM-CSF drives the differentiation of hematopoietic stem cells toward antigen-presenting dendritic cells.

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Somatix Therapy also uses GM-CSF, creating its patient-specific vaccines by expanding the number of tumor cells from an excised tumor *ex vivo*, inserting the GM-CSF gene, and returning the cells to the patient. Oncotech's (Irvine, CA) technology involves mixing a patient's tumor cells with heterologous lymphocytes that have been transfected with IL-2 or IL-4 genes. Having boosted the lymphocytic response to the tumor antigens, the activated lymphocytes are irradiated and introduced subcutaneously to the patient. Oncotech has filed an IND to test the approach in women with ovarian cancer.

Clinical data from phase II trials involving 147 patients given Biomira's (Edmonton, Albera, Canada) synthetic carbohydrate cancer vaccine, Theratope, show enhanced survival of treated patients with metastatic ovarian, colon, and breast cancer. Treatment extended survival consistently across the disease types by 7 to 9 months. The trials also demonstrated that raised antibody levels were a good predictor of survival time. Biomira is looking for a clinical partner to take Theratope to phase III studies.

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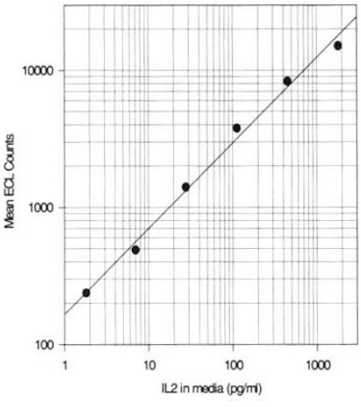
prepared in AIM V medium (Irvine Scientific, Irvine, CA) containing 10% Fetal Bovine Serum. The IL2 calibrator concentrations ranged from 1.8 to 1800 pg/ml.

Replicate samples were loaded with 50 µl of each calibrator in the above tissue culture medium, 50 µl of TAG-goat anti-IL2 IgG (2 µg/ml) diluted in PBS, pH 7.8, containing 1% (v/v) normal goat serum (Life Technologies Inc., Gaithersburg, MD) and 1% (v/v) Tween-20, and 50 µl of biotinylated mouse anti-IL-2 (2 µg/ml) in the same diluent. The assay was shaken for 90 minutes at room temperature and then 50 µl of streptavidin M-280 beads (1.0 mg/mL) (Dynal Corp., Lake Success, NY) in ORIGEN Assay Buffer (IGEN, Inc.) was added to each sample. The assay was shaken for an additional 10 minutes before adding 100 µl of ORIGEN Assay Buffer. The amount of IL-2 captured in immune complexes on the paramagnetic beads was quantitated on the ORIGEN Analyzer.

Figure 3



RESULTS AND DISCUSSION

A log-log plot and linear regression (r^2 =0.993) of the IL2 in tissue culture media data is presented in Figure 3. A sensitivity of approximately 1 pg/ml of IL-2 has been achieved in this medium. This assay has both a wide dynamic range and greater sensitivity, while using both smaller sample volumes and shorter incubation times, than currently available kits. The assay is also easily performed, eliminating all manual wash steps and radioactivity.

The cost per tube (including the antibodies, TAG, biotin, beads, Cell Cleaner and Assay Buffer) is approximately $0.56. This represents a significant savings over the costs associated with pre-packaged ELISA kits, which typically run between $4.00 and $6.00 per well. The reagent cost savings coupled with the reduced labor costs associated with processing this assay will substantially reduce the total operational expenses of assessing IL2 levels in a variety of matrices.

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