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STATE-OF THE-ART IN THE MANAGEMENT OF CANCER

PANCREATIC CANCER — PART VI
IMMUNOTHERAPY/VACCINES

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This issue is the last of a 6-part series covering pancreatic cancer. Previous issues provided information on the etiology, global epidemiology, staging, and state-of-the-art in the treatment of pancreatic cancer. In addition, molecular markers associated with disease were described as well as screening, diagnostic, prognostic, and disease monitoring approaches. Finally, the status of novel drugs in clinical or preclinical development, including conventional cytotoxics, targeted drugs, novel formulations, etc., was discussed in detail. The table of contents of previous issues may be found at www.nmok.net.

IMMUNOTHERAPY CHALLENGES IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

Immunotherapy has not been widely pursued in pancreatic cancer, as the disease is not particularly immunogenic in nature. However, several immunotherapies are in early stages of development, and a few have advanced to later stages (Exhibit 1). Vaccines targeting pancreatic cancer are based on many of the same immunization approaches currently in development for many other malignancies.

All of the immunization approaches described in this article aim to intervene in established disease rather than prevent it from occurring in the first place. Conventional prophylactic vaccines such as those used in the infectious disease area, prevent the pathogen from establishing a foothold in the host; the immune system, bolstered by the vaccine, destroys the invader, sparing the host. In the cancer area, immunization attempts to reverse the effect of an ongoing and established process to prevent disease progression or recurrence. The aim of immunotherapy in

cancer is to direct the immune system against an already established tumor rather than protect the individual from future disease, although if efficacious, therapeutic vaccines can be produced that may also be useful in a prophylactic setting.

In general, immunization targets humoral (antibody-based) and/or cellular (T-cell-based) mechanisms of response, employing active and/or passive/adoptive immunotherapy approaches. Pancreatic vaccines are commonly either delivered in combination with adjuvants, or are transfected/conjugated/fused with such adjuvants that enhance the immune response.

There are many different ways of using the human immune system's resources to coax a response against a malignancy. Immunotherapy approaches being currently investigated in pancreatic cancer include, among others:

- autologous/allogeneic whole cell vaccines, modified to express antigens/immunologic adjuvants
- vaccines based on naturally occurring or synthetic peptides
- monoclonal antibodies (MAb)
- DNA vaccines
- Immunomodulators such as adjuvants and cytokines, among others

Despite the possible straightforward application of immunotherapy in cancer, similar to the successful use of prophylactic vaccines against infection, the process has proven nothing but daunting with nary one commercialized approach to date after several decades of painstaking research and clinical evaluation. One of the major obstacles in this area is the poorly understood function of the immune system in dealing with malignancy. In contrast to the immune system's bold single-minded definitive response to an invading foreign pathogen, its response to an innate cancer cell is nuanced by a multitude of conflicting signals.

Defects in the Cellular Immune Response in PDAC

Cellular immunity, also known as 'cell-mediated immunity', is the branch of the immune system that involves a T-cell rather than an antibody response. Cellular immunity is based on the activation of macrophages and natural killer (NK) cells, the production of cytotoxic T cells (CTL), and the release of cytokines. Successful cancer immunotherapy interventions involve priming T cells specific for tumor antigens, and aiding in their transformation into effector cells able to destroy tumor targets. Priming and directing T-cell responses is the principal function of dendritic cells (DC), the major class of antigen-presenting cells (APC) in the body.

Cell-mediated immunity is being evaluated in the clinic in cancer, and T-cell targeting immunotherapy is also being investigated in patients with PDAC. Until recently, this therapeutic strategy has been used as a single specific treatment, and clinical trials have demonstrated that tumor-specific T-cell responses can be induced in a subset of patients, even in those with advanced disease. However, it remains to be demonstrated whether these T-cell responses can mediate clinically relevant antitumor reactivity. Furthermore, *in vitro* studies of cancer cell susceptibility to antitumor T-cell reactivity are often limited to cancer cell lines, because native tumor cells are frequently not available from patients. It is, therefore, still a hypothesis that T-cell targeting immunotherapy can be developed into an efficient therapeutic strategy in patients with PDAC. Future investigations of this therapeutic strategy should probably focus on its incorporation into treatment regimens that also include other newer treatment strategies, for example, tumor-reactive antibodies coupled with toxins or cytotoxic drugs. Also, use of DC and enhancement of antigenic presentation in the tumor microenvironment will probably improve vaccination procedures in cancer (Glenjen N and Bruserud Ø, Expert Opinion on Biological Therapy, 1 October 2002;2(7):693-701).

CTL and NK cells are main effector cells in cellular immunity against tumor cells. T-cell immunotherapy is based on the assumption that tumor-associated antigen (TAA) peptides are correctly presented by HLA class I molecules on target tumor cells. Furthermore, NK-cell immunotherapy is based on the hypothesis that cell-surface TAA or ligands for NK receptors are widely expressed by tumor cells. However, human tumor cells often lose HLA class I molecules, and target cell ligands for NK receptors are not always expressed in human tumor cells. These altered HLA class I phenotypes and nonubiquitous expression of NK receptor ligands constitute the major tumor escape mechanism hampering tumor-specific CTL- and/or NK cell-mediated responses. These unique attributes of cancer cells indicate that it would not be easy to eliminate the target tumors only by activating tumor-specific CTL or NK cells with cancer vaccine treatments. On the other hand, it is easily confirmed by immunohistochemistry whether or not antibody-recognized TAA exist on the cell surface of target tumor cells. Therefore, endowing CTL or NK cells with antigen-binding specificity with anti-TAA monoclonal antibodies (MAb) is a promising approach for retargeting these effector cells to tumor cells in an HLA-independent manner.

At the Annual Meeting of the Association for Immunotherapy of Cancer, in Mainz, Germany, on May 6–7, 2004, investigators from Julius-Maximilians University (Wuerzburg, Germany) reported findings regarding the role of immune-mediated pathways that elucidate the role of regulatory T cells in the impairment of antitumor immune responses in invasive PDAC. Generally, invasive PDAC is associated with relatively low levels of expression of genes associated with immune activation and regulation, which may lead to insufficient antitumor immune responses Expression, distribution, and protein products of gene classes of regulatory T cells (CD4, CD25, CD8, IDO, Foxp3), co-stimulatory molecules (CD28,

Exhibit 1
Immunotherapies in Clinical or Preclinical Development for the Treatment of Pancreatic Cancer

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Development Status ❑ Indication(s)
Active Biotech ❑ Pfizer	Anatumomab mafenatox ❑ ABR-214936, TTS-CD2 (formerly PNU-214936)	Tumor-targeted superantigen (TTS)-based immunotherapy, involving a monoclonal antibody (MAb) coupled to a superantigen, that identifies tumor cells and activates the body´s immune defense to attack and destroy them ❑ IV	Phase IIa (begin 2/02, completed 3/04)➢Europe (UK) ❑ pancreatic cancer
Antigenics ❑ Fordham U, Perseptive BioSystems, Sigma-Tau, Mount Sinai School of Medicine, Medison Pharma	HSPPC-96 ❑ Oncophage	Individualized heat-shock protein (hsp) cancer vaccine that elicits an immune response without requiring adjuvants ❑ IV, intradermal (ID), *ex vivo*	Phase I/II (begin 97, closed 6/02) ➢USA ❑ PDAC
Aphton ❑ U Nottingham, aventis pasteur	Anti-gastrin-17 (G17DT) ❑ Insegia (formerly Gastrimmune)	Oil-based vehicle incorporating a synthetic peptide fragment of hormone G17 which is targeted to be neutralized, a carrier (diphtheria toxoid) conjugated to the synthetic peptide, and an adjuvant ❑ intramuscular (IM)	Phase III (begin 11/98, completed 1/00)➢Europe (UK), phase III (begin 2/01, completed 3/03) ➢Europe (UK, Russia), phase III (begin 4/00, closed 6/04)➢USA, Europe (combination); NDA (6/04) Europe (Switzerland), NDA (8/04) Canada ❑ inoperable or metastatic pancreatic cancer
AVI BioPharma ❑ Ohio State U, SuperGen	CTP-37, CTP37 ❑ Avicine	Active specific immunization using a synthetic peptide conjugate vaccine designed to elicit an antihuman chorionic gonadotropin (hCG) immune response targeting hCG-producing cancer cells ❑ injection, intramuscular (IM)	Phase II (begin 4/99, completed 12/01)➢USA (combination); phase III (planned 8/04)➢USA ❑ advanced pancreatic cancer
BioVex	OncoVEX GM-CSF	Oncolytic virus based on a novel modified herpes simplex virus (HSV) type 1 vaccine platform carrying the gene encoding human granulocyte macrophage-colony stimulating factor (GM-CSF) ❑ intratumoral, intralesional	Phase I/II (begin 6/02, closed 05) ➢Europe (UK) ❑ solid tumors
CancerVax ❑ Centre of Molecular Immunology	SAI-TGF-α	Specific active immunotherapy (SAI) approach targeting the epidermal growth factor receptor (EGFr) signaling pathway ❑ intradermal (ID)	Preclin (ongoing 1/05)➢Cuba, Canada, USA ❑ advanced solid tumor
Celgene ❑ Cornell U, EntreMed, Children's Hospital Boston, National Institutes of Health (NIH)	Lenalidomide ❑ CDC501, CDC-501, CDC-5013, CC5013, CC-5013 ❑ Revlimid (formerly Revimid)	Small molecule compound belonging to the family of immunomodulatory drugs (IMiD) that are structurally and mechanistically similar to thalidomide ❑ PO	Phase I (begin 6/02, closed 12/04) ➢USA ❑ advanced or metastatic solid tumors
Cell Genesys ❑ Fletcher International, Whitehead Institute, Johns Hopkins U	GVAX	Tumor cells irradiated and genetically modified to secrete (GM-CSF) ❑ intradermal (ID), subcutaneous (SC)	Phase II (begin 10/01, ongoing 8/04) ➢USA ❑ resectable pancreatic cancer, phase II (completed 6/04) ➢USA (combination) ❑ inoperable, metastatic pancreatic cancer
Cerus ❑ Chugai Pharmaceutical, Johns Hopkins University		Listeria-mesothelin cancer vaccine comprising recombinant Listeria DeltaactA/DeltainlB expressing mesothelin ❑ injection	Preclin (ongoing 2/05)➢USA ❑ pancreatic cancer

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Dendreon	APC8024, BA7072	Vaccine against tumors overexpressing HEr2 protein on their surface, constructed from autologous antigen-presenting cells (APC) loaded with BA7072, an antigen construct consisting of recombinant sequences from both the extracellular and intracellular domains of HEr2 (HER500) fused to human GM-CSF ❑ IV	Phase I (ongoing 7/03, completed 6/04)➢USA ❑ metastatic, advanced solid tumors expressing HER2
Epeius Biotechnologies ❑ BioFocus	Reximmune	Retroviral expression vectors that combine Rexin-G with a targeted vector bearing the GM-CSF (Reximmune-C) and/or IL-2 (Reximmune-L) genes ❑ IV, intra-arterial, intrahepatic	Preclin (ongoing 3/05)➢USA ❑ solid tumors
Fresenius Biotech ❑ Trion Pharma	Removab	Trifunctional anti-EpCAM and anti-CD3 MAb that induces a polyclonal humoral and cellular antitumor immunity ❑ intraperitoneal (IP)	Phase I/II (begin 03, completed 1/04)➢Europe; phase II/III (ongoing 2/05)➢Europe ❑ malignant ascites; phase I (ongoing 2/05)➢Europe ❑ peritoneal carcinomatosis
GemVax ❑ Norwegian Radium Hospital, Norsk Hydro	GV1001, HR2802	Dual specific peptide vaccine derived from the telomerase (hTERT) active site that induces both T helper and cytototoxic T lymphocyte (CTL) responses ❑ ID	Phase I/II (begin 9/00, ongoing 7/03) ➢Europe (Norway) ❑ inoperable pancreatic cancer, first line
GemVax ❑ Norwegian Radium Hospital, Norsk Hydro	GV1002	Vaccine based on a combination of telomerase vaccine GV1001 and p21 Ras peptides ❑ intradermal (ID)	Preclin (ongoing 3/05)➢Europe (Norway) ❑ pancreatic cancer
Genzyme Oncology (Ilex Oncology) ❑ Dana-Farber Cancer Institute, Abgenix		Drug discovery and development program to identify MAb that target the MUC1 protein at the extracellular domain level closest to the cell surface ❑ IV	Research (ongoing 3/05)➢USA ❑ solid tumors
igeneon ❑ Protein Design Labs	HuABL-364, IGN311 (previously SMART ABL-364)	Humanized IgG1 MAb directed against Lewis y carbohydrate antigen, a hexasaccharide selectively expressed on tumors of epithelial cell origin ❑ IV	Phase I (begin 12/02, ongoing 9/04) ➢Europe (Germany) ❑ solid tumors
Immuno-Designed Molecules (IDM) ❑ Eli Lilly, Walter Reed Army Institute of Research (WRAIR)		Vaccine consisiting of recombinant antigen KSA (Ep-CAM) formulated into liposomes ❑ injection	Phase I/II (begin 8/97, completed 03)➢USA ❑ metastatic colorectal cancer
Nemod Biotherapeutics	PankoMab	PankoMab recognizes a tumor-specific carbohydrate-induced MUC1 epitope ❑ IV	Preclin (ongoing 12/04)➢Europe (Germany) ❑ solid tumors
Nemod Biotherapeutics	PankoVac	PankoVac is an allogeneic whole cell vaccine against cancer ❑ IV	Preclin (ongoing 12/04)➢Europe (Germany) ❑ solid tumors
Pepscan Systems ❑ Proteomika, AlgoNomics, Medical Center (UMC), National Cancer Research Center, U Louis Pasteur	Binding Bodies	Fully synthetic antibodies against gastrin that represent a novel approach for the production of protein-based molecules that are highly specific for their targets ❑ IV	Research (begin 2/05)➢Europe (Belgium, France, Spain, the Netherlands) ❑ pancreatic cancer

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Procyon BioPharma ❑ Harvard Medical School, Massachusetts General Hospital	2C5	Novel chimeric MAb derived from the ANA platform ❑ injection	Preclin (ongoing 7/04)➢Canada ❑ solid tumors
Therion Biologics ❑ sanofi pasteur	Fowlpox-CEA (6D)-TRICOM, rF-TRICOM, rF-B7.1/ICAM-1/LFA-3	Live recombinant fowlpox (avipox) virus expressing CEA, and a TRIad of COstimulatory Molecules (TRICOM), including B7.1, LFA-3, and ICAM-1 ❑ IV, intratumoral, intravesical	Phase I (begin 2/01, closed 7/02)➢USA, phase I (begin 1/02, closed 8/03), phase I (begin 1/02, ongoing 3/05)➢USA (combination) ❑ solid tumors expressing CEA; phase I (begin 2/02, ongoing 3/05)➢USA (intratumoral) ❑ inoperable, metastatic solid tumors
Therion Biologics	Panvac-VF	Recombinant vaccine targeting CEA antigen and mucin-1 (MUC-1), two proteins found in pancreatic tumor cells, incorporating TRICOM ❑ - subcutaneous (SC)	Phase I (ongoing 6/04)➢USA, phase III (begin 6/04)➢USA ❑ refractory, metastatic, pancreatic cancer
Triton BioSystems ❑ Xoma	ING-1 antibody (heMAb)	High affinity human-engineered IgG1 MAb to the tumor-associated Ep-CAM antigen, that destroys target cells by recruiting host immune system cells to induce apoptosis ❑ IV, SC	Phase I (completed 3/03)➢USA, phase I (begin 7/02, completed 10/04)➢USA, phase I (begin 11/00, completed 8/01)➢USA ❑ advanced, refractory adenocarcinoma
ViRexx Medical ❑ United Therapeutics	GivaRex	Murine MAb binding with high affinity to CA19.9 tumor-associated antigen (TAA) ❑ injection	Preclin (ongoing 3/05)➢Canada ❑ gastrointestinal (GI) cancer

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), April 2005

CTLA-4), transcription factors (GITR, GATA-3), apoptotic markers (Bcl-2, Fas, FasL), cytokines (IFN-α, IL-6/10), tumor suppressors (p53, APC), and the tumor marker carcinoembryonic antigen (CEA), were analyzed in blood and tumor samples of 14 patients undergoing surgery for Stage II/IV PDAC. A highly dissimilar gene expression pattern was associated with disease stage. A significantly lower expression of most genes, 14 out of 19, was seen in Stage II samples compared to Stage III or IV samples. The other characteristic difference in the gene expression pattern of the individual sample was attributed to the location of the analyzed tissue within the tumor. Expression of immune response related genes was significantly lower in samples from the tumor center than in those from the tumor borders. Gene expression related to immune activation and regulation was low (CD69<Foxp3 and CTLA-4<CD28 and IFN-α and IL-10 and CD4<IDO and CD25 and GATA-3<Fas and IL-6) in all samples obtained from the tumor center. Expression of the antiapoptotic genes Bcl-2 and GITR was higher than the proapoptotic genes Fas and FasL. The highest expression was seen for APC>CEA>Bcl-2>CD8 (Gasser M, etal, Cancer Cell International, July 2004, 4(Suppl 1):S27).

Investigators at Washington University School of Medicine (Saint Louis, MO), report that regulatory T cells that prevent autoimmune diseases by suppression of self-reactive T cells, may also suppress the immune response against cancer. Regulatory T-cell-mediated suppression of a tumor-specific immune response may account for poor clinical response to vaccine-based immunotherapy against human cancer. In mice carrying chemically induced fibrosarcoma, a class of CD4+ suppressor cells abolishes CTL activity against tumor cells. Recent experiments in mice have demonstrated that depletion of CD4+CD25+ regulatory cells from host circulation leads to more efficient tumor rejection. Prevalence of regulatory T cells is increased in the peripheral blood as well as in the tumor microenvironment of patients with invasive breast or pancreatic cancer. This may explain the poor immune response against tumor antigens even when tumor-specific CTL can be generated using vaccine strategies. Prevalence of regulatory cells that coexpress CD4 and CD25 markers in the peripheral blood, tumor infiltrating lymphocytes (TIL) and regional lymph node lymphocytes (LNL), was measured in 60 patients with either PDAC (n=25) or breast ductal adenocarcinoma (n=35), and prevalence of CD4+CD25+ regulatory T cells as a percentage of total CD4+ cells were also measured in peripheral blood lymphocytes (PBL) of these patients and normal donors (n=35). Regulatory T cells accounted for 16.6% of the total CD4+ cells in patients with breast cancer, 13.2% in those with PDAC, and 8.6% in normal donors. Prevalence of regulatory T cells was significantly higher in patients with breast and pancreatic cancer compared to

normal donors. In TIL and LNL, prevalence of regulatory T cells was 20.2% in patients with breast cancer, and 20.1% in PDAC. Regulatory T cells constitutively coexpressed CTLA-4 and CD45RO markers and, in culture, secreted TGF-α and IL-10, but not IFN-α. When co-cultured with CD8+ cells or CD4+25- cells, regulatory T cells potently suppressed proliferation as well as secretion of IFN-α from CD8+ and CD4+25- cells. These findings combined with the results of animal studies, suggest that regulatory T cells may play a role in immune tolerance to cancer (Liyanage UK, etal, AACR02, Abs. 4160).

Dendritic cells (DC) play a pivotal role in T-cell-mediated immunity and have been shown to induce strong antitumor immune responses *in vitro* and *in vivo*. Various approaches using different vaccine cell formats, cell numbers, vaccination schedules, site of vaccination, and maturation stages of DC have been investigated worldwide. While clinical trials have demonstrated the safety of such strategies, outcomes were disappointing in most cases. This may in part be attributed to immunodeficiencies in the host imposed by tumors and immunoediting of tumor cells.

To overcome these obstacles, new approaches to improve DC-mediated immunotherapeutic strategies are under investigation. First, functional enhancement of monocyte-derived DC can be generated using flt3-ligand. Second, diverse antigenic determinants from heat shock-treated tumor cells may improve the immunogenicity of DC-based vaccines. Third, inclusion of *ex vivo* expanded NK/NKT cells in DC-based vaccines may be beneficial because the bidirectional interaction of these two cell types is known to enhance NK cell effector function and to induce DC maturation. Application of these approaches may broaden the antitumor immune response and, thereby, promote elimination of tumor antigen-negative variant clones that escape immunosurveillance or undergo immunoediting. Feasibility of such immunotherapeutic approaches is being investigated using a murine PDAC model system (Song SY and Kim HS, Yonsei Med J, 30 Jun 2004;45 Suppl:48-52).

According to investigators at Tohoku University Graduate School of Medicine (Sendai, Japan), PDAC suppresses the function of DC through the downregulation of costimulatory molecules. Delayed type hypersensitivity (DTH) to the tuberculosis antigen is frequently suppressed in patients with advanced PDAC. Because DC play a critical role in cellular immunity, the effect of PDAC on the function of DC was examined to elucidate the mechanism of cancer-induced immunosuppression. DC were induced from the purified monocytes from healthy donors, and the supernatant of pancreatic cancer cell line PK-1, or LPS was added to the DC culture media. After incubation, the total RNA was extracted, and expression of 10,000 genes was assessed using a microarray assay. DC were cultured with autologous naïve CD4+ T cells under the presence of superantigen staphylococcal enterotoxin B (SEB), or tuberculosis antigen to ascertain the antigen's presentation ability and the production of Th1 cytokine, IL-12 p70. Expression of IL-12β and IFN-α was increased, while that of IL-3, IL-5, and IL-17 was decreased, and that of IL1α , IL-1β, IL-2, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-18, IL-19, and IL-20 remained unchanged. Expression of CD40 and CD80 was decreased, while that of CD14, CD86, and CD83 was unchanged. There was no change in the expression of chemokine receptors, toll-like receptors, tumor necrosis factor (TNF) and its receptors, caspase, Fas receptors, or vascular endothelial growth factor (VEGF) and its receptors. Proliferation of naïve T cells against the presented antigen and production of IL-12 p70 by the DC exposed to the tumor-supernatant were partly suppressed up to 30% compared to controls. Exposure to the supernatant of a pancreatic cancer cell line downregulated expression of costimulatory molecules while expression of the cytokines important in evoking cellular immunity, was preserved. Partial suppression of naïve CD4 T-cell proliferation and IL-12 p70 production still suggests the possibility of *ex vivo* isolation of DC and their usefulness as APC for PDAC immunotherapy (Egawa S, etal, AACR04, Abs. 681).

Cancer treatment may also impede the immune process. Chemotherapy and immunotherapy induce apoptosis in tumor cells. Apoptotic cells are known to activate homologous complement and to be opsonized with iC3b. For instance, release of iC3b from apoptotic pancreatic tumor cells induces tolerance by binding to immature DC. Because DC maturation may be inhibited by binding of iC3b to the complement receptor 3 (CR3, CD11b/CD18), and because immature DC induce tolerance, researchers at the University of Heidelberg in Germany investigated the induction of tolerance after pulsing DC with apoptotic cells in the presence or absence of native serum. Apoptosis in pancreatic carcinoma cells was induced either by heat stress, chemotherapy or anti-HEr2 antibody. All of these strategies for apoptosis induction resulted in iC3b release. In monocyte-derived DC, pulsed with apoptotic cells with or without native serum, tolerance was prevented by addition of substances such as anti CD11b or N-acetyl-D-glucosamine (NADG), which block iC3b binding to CR3. Pulsing DC with apoptotic cells in the presence of serum prevented maturation of DC and induced tolerance, which could be prevented almost completely by blocking the interaction of iC3b with the CR3 receptor (J Schmidt, etal, Cancer Cell Int, July 2004, 4(Suppl 1):S24).

Animal Models

Investigators from Medizinische Hochschule Hannover, in Germany, and the University of California San Diego (La Jolla, CA) used a novel spontaneous PDAC tumor model to investigate immunotherapeutic approaches against pancreatic cancer. Crossbreeding of p53 knockout mice with TGF-α transgenic mice that overexpress TGF-α in the pancreas and thus develop fibrosis and PDAC at the age of one

year, dramatically accelerated tumor development Also this is the first model of PDAC with genetic alterations as well as characteristics similar to the human disease. A total of 28 murine adenopancreatic cell lines (mPAC) were established, derived from 6 different TGF-α p53-/- mice. Analysis of growth kinetics in normal syngeneic mice *in vivo* showed that some cell lines progress after injection to form lethal tumors, while others grow during the first 10 days and then regress. Next, these tumors were injected into SCID beige and in nude mice. In these mice both progressors and regressors grew progressively indicating that the regression in normal euthymic mice is an immune-mediated response. CTL responses against MHC I positive mPAC are induced after immunization with irradiated mPAC in mice with spontaneous PDAC. Injection of mPAC leads to the induction of IFN-α secreting CD8 T cells *in vivo*, which is also the case in tumor-bearing mice. This new model makes it possible to investigate spontaneous immune responses against PDAC in a genetically well defined tumor model mimicking human PDAC (Greten TF, etal, Cancer Cell Int, July 2004, 4(Suppl 1):S54).

ACTIVE-SPECIFIC IMMUNOTHERAPY

In order to develop tumor-specific immunotherapies, it is imperative to establish unique characteristics that distinguish tumors from healthy tissue. Tumor markers present in PDAC were described in Part I of this series (FO, pp 1648-1659). Several of these and other molecular markers are being targeted by immunotherapies in clinical or preclinical development for the treatment of PDAC (Exhibit 2).

Autologous Cell Vaccines

An autologous cancer vaccine is constructed from tumor cells removed from a patient during surgery. The patient's tumor cells are treated to render them nontumorigenic, enhanced in some fashion, and then either shortly after surgery, or after they were grown in the lab or preserved by freezing, are reinjected into the patient from whom they had been removed.

In August 2000, Aastrom Biosciences was awarded a Phase II, Small Business Innovation and Research (SBIR) grant from the National Cancer Institute (NCI) to support the development of the AastromReplicell System for the clinical production of human DC. Aastrom's DCV-II DC vaccine production kit and the AastromReplicell System produce sufficient, clinical amounts of patient-derived DC to allow each patient to be immunized multiple times.

APC8024, under development by Dendreon (Seattle, WA) as Neuvenge, is a vaccine against tumors overexpressing HEr2 protein on their surface. APC8024 is prepared from autologous DC collected by leukapheresis and loaded *ex vivo* with BA7072, an antigen construct with sequences from both the intracellular and extracellular domains of HEr2 (HER500) fused to human granulocyte-macrophage colony stimulating factor (GM-CSF). APC8024 uses Dendreon's proprietary Antigen Delivery Cassette technology to genetically engineer antigens such as HEr2 to bind to APC and stimulate T-cell immunity. APC8024 has been evaluated in phase I clinical trial in breast cancer and other solid tumors, including colorectal cancer, and may be applicable to HEr2 expressing PDAC.

Fowlpox-CEA-TRICOM, under development by Therion Biologics (Cambridge, MA), is an active-specific immunotherapy approach in clinical evaluation in patients with advanced or metastatic malignancies expressing CEA, including gastrointestinal (GI) malignancies. The vaccine comprises autologous DC infected with Fowlpox-CEA-TRICOM, CMV pp65 peptide, and tetanus toxoid.

In order to activate T cells two signals are required by APC, a peptide/MHC complex and costimulatory molecules. In this vaccine, a poxvirus vector that is capable of expressing multiple genes has been engineered to express CEA, and three costimulatory molecules, B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3). This costimulatory complex was designated TRICOM, for TRIad of COstimulatory Molecules.

A dose-escalation, phase I clinical trial (protocol IDs: DUMC-2840-02-6R1; NCI-1864; NCT00027534), initiated in December 2001, is ongoing at Duke University (Durham, NC), sponsored by the NCI, to study the effectiveness of vaccine therapy in CEA-expressing malignancies. Trial objectives are to determine the safety and feasibility of this active immunotherapy approach, and assess any CEA-specific immune response. According to the protocol, autologous DC are harvested and infected with fowlpox-CEA-TRICOM vaccine. Patients are administered the infected DC intradermally (ID) and subcutaneously (SC), followed by DC mixed with CMV pp65 peptide, and DC mixed with tetanus toxoid SC and ID on day 1. Treatment repeats every 3 weeks for a total of 4, 8, or 12 immunizations in the absence of unacceptable toxicity. Cohorts of 6 patients are treated with an escalating number of immunizations until maximum tolerated dose (MTD) is determined. Patients are followed every 3 months for 1 year. A total of 6-18 patients will be accrued for this trial. Herbert Kim Lyerly, MD, is Study Chair.

Human chorionic gonadotropin β (hCGβ) vaccine, is a novel antibody-based DC-targeted cancer vaccine, being evaluated by investigators at Medarex (Princeton, NJ) and Dartmouth Medical School (Lebanon, NH), that is capable of eliciting cellular immune responses directed against hCGb, an oncofetal antigen expressed by a number of tumors, and a prognostic indicator in renal, colorectal, bladder, and pancreatic cancer. In this fusion protein, hCGb was coupled genetically to human anti-DC antibody B11. The resulting fusion protein, B11-hCGb, was evaluated for its ability to promote tumor antigen-specific cellular immune responses in a human *in vitro* model.

Monocyte-derived human DC from normal donors were exposed to purified B11-hCGb, activated with CD40 ligand, mixed with autologous lymphocytes, and tested for their ability to promote hCGb-specific proliferative and CTL responses. B11-hCGb was a soluble, well defined, and readily purified product that specifically recognized the human mannose receptor via the B11 antibody portion of the fusion protein. B11-hCGb functionally promoted the uptake and processing of tumor antigen by DC, which led to the generation of tumor-specific HLA class I and class II-restricted T-cell responses, including CTL capable of killing human cancer cell lines expressing hCGb (He LZ, etal, Human Clin Cancer Res, 15 Mar 2004;10(6):1920-7).

Mutant ras peptide vaccine was clinically evaluated by the NCI in a pilot phase II clinical trial as adjuvant therapy in patients with pancreatic and colorectal cancer. Because mutant ras protein is processed and displayed through HLA molecules on the tumor cell surface, it is an attractive target for vaccine therapy. In such an approach, autologous APC are loaded with synthetic ras peptides, carrying identical mutations to those found in the patient.

In a phase I clinical trial, vaccination with a 13-mer peptide reflecting the patient's mutant ras, produced specific immunologic responses; a dose up to 5000 µg was well tolerated. In the follow-up phase II clinical trial, this vaccine is being investigated in the adjuvant setting in patients with fully resected pancreatic and Dukes C or D colorectal cancer. According to the protocol, patients are vaccinated SC with 5000 µg of the vaccine, corresponding to their tumors' ras mutations, admixed with Detox (Corixa) adjuvant. Vaccinations are administered every four weeks, for up to a total of 6 cycles. According to interim results involving the first 11 patients treated (9 had also completed adjuvant chemotherapy or radiotherapy prior to vaccination), minor local reactions such as erythema and soreness were common, but there were no serious systemic side effects or delayed toxicities. A few patients developed mild fatigue, and one patient had a Grade 2 rash. Regarding vaccine efficacy, 3 patients with Stage III PDAC remained disease free for 14 to 30 months, and 3 patients with Dukes C colorectal cancer remained disease-free for 14 to 42 months; 2 patients with Stage III PDAC progressed at 11 and 21 months, and disease progressed while on therapy, or within a month of completion, in 3/11 patients (Behrens RJ, etal, ASCO02, Abs. 112). Currently, the NCI is investigating this immunotherapy approach in a phase I clinical trial (protocol ID: 990023; 99-C-0023) in colorectal cancer.

Oncophage, under development by Antigenics (New York, NY), is an individualized autologous heat-shock protein (HSP) cancer vaccine that elicits an immune response without requiring adjuvants. The vaccine is constructed using patients' cells and HSP glycoprotein 96 (HSPPC-96). Patient's tumors are sent to an Antigenics facility where individualized vaccines are produced by a standardized, cost-effective process. Oncophage contains the 'antigenic fingerprint' of the patient's particular tumor, and is designed to reprogram the body's immune system to target and destroy only cancer cells bearing this fingerprint. Oncophage is intended to leave healthy tissue unaffected and limit the debilitating side effects associated with traditional cancer treatments such as chemotherapy and radiation therapy.

HSP exist ubiquitously across all species, and function as chaperones stabilizing and delivering peptides. They keep other proteins in the right place and in the correct conformation in cells. As a result, they interact with a large number of cellular proteins, and are involved in the process of antigen presentation. Purification of HSP results in the copurification of various peptides. If these HSP-peptide complexes are purified from tumor cells, they carry tumor-specific peptides, and thus, can be used as a tumor-specific vaccine. Complexes formed from the binding of HSP to antigen peptides promote antigen presentation. Tumor-derived HSP-peptide complex has been known to induce immunity against the original tumor in preclinical studies. HSP-based vaccines work across tumor types and bypass the need for identifying the peptide(s) responsible for inducing immunity. These vaccines are tumor- and patient-specific in that they capture the tumor cells' fingerprints.

HSP-based vaccines are a novel vaccine preparation with a promising role in the management of cancer. HSP-based vaccines have been studied in early phase clinical trials, mostly using HSPPC-96, in various types of malignancies including melanoma, renal cell carcinoma (RCC), gastric cancer, pancreatic cancer, low grade lymphoma, colorectal cancer, and chronic myelogenous leukemia (CML). In all cases, toxicity was minimal, and the vaccines demonstrated potential efficacy. Phase III trials in malignant melanoma and RCC are ongoing (Oki Y and Younes A, Expert Rev Vaccines, Aug 2004;3(4):403-11).

Phase I/II clinical trials with Oncophage were initiated in May 1997 at Memorial Sloan-Kettering Cancer Center, under PI Robert G. Maki, MD, PhD and Murray Brennan, MD, involving 10 patients with completely resected PDAC, treated with two dose levels of the vaccine. According to the protocol, a complete surgical resection of the primary tumor was undertaken, if possible, if there was no evidence of metastatic disease. If HSP could be isolated after resection, patients were vaccinated within 8 weeks of surgery with 5 mg of Oncophage, every week, for 4 weeks. No adjuvant chemotherapy or radiation was administered. Subjects were followed clinically by CT scan and CA-19-9 assay, every 3 months for one year for evidence of recurrence, and approximately every 6 months thereafter. In this trial a vaccine could be successfully produced in only 5 of the first 15 patients. Subsequently, a change in the manufacturing process improved yield, and 5 additional patients with resected disease were treated with the vaccine. In total, between October 1997 and July 2001, 10 patients were administered a full vaccination course. No dose-limiting toxicity (DLT) was observed. At the time of

this report, 3 patients were alive without disease at 5.0, 1.7, and 1.6 years, and 1 patient was alive with disease after progressing on the first CT scan following the final vaccination. Median survival time (MST) is 2.0 years. Despite the possibility of selection bias, these results were deemed promising, and support the further evaluation of Oncophage in resected PDAC (Maki RG, etal, ECCO03, Abs. 48 and Eur J Cancer Supplements, Sep 2003;1(5):S19). According to more mature data, MST was 2.5 years, with one patient still alive disease free after more than 5 years, and two other patients alive disease-free 2.5 and 2.2 years after treatment.

Allogeneic Tumor-cell Vaccines

Allogeneic tumor cell vaccines use cells of a particular cancer type originally obtained from one or more patients, and then killed, grown in the lab, and preserved very much like an injectable cancer drug. These allogeneic cells are usually injected into patients in combination with one or more substances (adjuvants) known to stimulate the immune system. The fact that these vaccines are 'off-the-shelf' makes them far more practical to deliver than autologous cancer cell vaccines.

GVAX, under development by Cell Genesys (Foster City, CA), in collaboration with researchers at John Hopkins University (Baltimore, MD), is a vaccine based on GM-CSF-transfected allogeneic tumor cells. GM-CSF plays a central role in the recruitment and differentiation of APC, thereby generating an efficient immune response. Replication-defective viral vectors are used to insert the gene encoding for GM-CSF into tumor cells *ex vivo*.

In a dose-escalation, phase I clinical trial, conducted at Johns Hopkins University School of Medicine, 14 patients who had undergone pancreatic resection for Stage I, II, or III PDAC were vaccinated with allogeneic GVAX 6 to 8 weeks after surgery ($1x10^7$ vaccine cells=3, $5x10^7$ vaccine cells=3, $10x10^7$ vaccine cells=3, and $50x10^7$ vaccine cells=5); 12 patients were then treated with a 6-month course of adjuvant radiation and chemotherapy. Up to 1 month after completing adjuvant treatment, 6 patients still in remission were treated with 3 additional monthly vaccinations with the same original vaccine dose. All participants experienced Grade 1/3 local reactions at the vaccine site, although there was no DLT. DTH responses to autologous tumor cells post-vaccination were observed in 3 patients at the $10x10^7$ vaccine cell dose. DTH responses post-vaccination appeared to correlate with increased disease-free survival (DFS); 3 of 8 patients treated with the two highest vaccine doses were alive and free of disease more than 2 years following treatment, whereas the 6 patients at the two lowest doses relapsed. These findings are particularly significant given the fact that all 3 long term survivors were judged to be at high risk for recurrent disease because of microscopic evidence of pancreatic tumor following surgery or metastatic tumor in pancreatic lymph nodes. Eosinophil and macrophage infiltration was evident from immunohistochemical staining of skin biopsies from patients treated with GVAX vaccine at the vaccination sites, and eosinophil, lymphocyte, and macrophage infiltration at the DTH sites (Jaffee EM, etal, ASCO00, Abs. 1784, Jaffee EM, etal, J Clin Oncol, 1 Jan 2001;19(1):145-56, and Thomas AM, etal, Exp Med, 2 Aug 2004;200 (3):297-306).

As reported in August 2004, these 3 patients were still alive disease-free at 6 years after treatment. Of particular note is that in all these 3 long term survivors the specificity of the T-cell response to mesothelin was unique to each patient. In contrast, only 1 of the 11 patients who progressed and died from PDAC mounted a detectable immune response to mesothelin, and the level of response in this patient was minimal by comparison. These findings provide evidence that patient-specific immune responses can be generated following vaccination with an allogeneic GVAX cancer vaccine, and that such responses may correlate with clinical outcome.

An open label, phase II clinical trial (protocol IDs: CDR0000361806; JHOC-J9988) of GVAX in resectable Stage I, II, or III PDAC was initiated in October 2001, prompted by compelling results from an initial phase I clinical trial. This phase II trial is evaluating the safety and efficacy of GVAX, used in combination with surgical resection followed by standard adjuvant radiotherapy (RT) and chemotherapy. Primary trial objectives are to determine overall survival (OS) and DFS, while secondary objectives are to correlate specific *in vivo* parameters of immune response, i.e., post vaccination DTH reactions to autologous tumor and the degree of local eosinophil, macrophage, and T-cell infiltration at the vaccine site, with clinical responses in patients treated with this regimen, and determine the toxic effects associated with ID injections of this vaccine in these patients. The trial, being conducted at Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins under PI Daniel Laheru, MD, is to enroll about 60 patients to be administered the vaccine ID within 8-10 weeks after pancreaticoduodenectomy on day 0. Adjuvant chemoradiotherapy with IV fluorouracil (5-FU) is then initiated within 16 to 28 days after the first vaccination, to be administered continuously for 3 weeks. Approximately 1 to 2 weeks after completion of the 5-FU regimen, patients are treated with chemoradiotherapy comprising RT daily and IV 5-FU continuously for 26 to 28 weeks. Approximately 3 to 5 weeks after completion of chemoradiotherapy, patients are again treated with IV 5-FU continuously for 4 weeks. The 5-FU regimen repeats every 6 weeks for 2 courses. Within 4 to 8 weeks after the completion of chemoradiotherapy, patients are again treated with GVAX ID on days 0, 28, and 196. Treatment continues in the absence of unacceptable toxicity. Patients are followed every 3 months for 1 year, and then every 6 months thereafter. As of August 2004, this trial had enrolled approximately 50 out of the projected 60 patients.

In June 2004, Cell Genesys presented clinical data at the annual meeting of the Lustgarten Foundation for

Pancreatic Cancer Research regarding an exploratory phase II clinical trial of GVAX pancreatic cancer vaccine in patients with inoperable, metastatic PDAC. The trial enrolled 50 patients, the majority of whom had failed at least two prior chemotherapy regimens. Patients were divided into 2 cohorts, both administered up to 6 doses of GVAX pancreatic cancer vaccine at 21-day intervals. Cohort A (n=30) was treated with the vaccine alone, and cohort B (n=20) was administered the vaccine in combination with low dose cyclophosphamide. Disease stabilized in 40% of the patients in cohort B, compared with 16.7% in cohort A. Moreover, the median time-to-progression (TTP) was 57 days in cohort B and 42 days in cohort A, and MST in cohort B was 4.3 months compared with 2.3 months in cohort A. In addition, there was a correlation between survival and the patients' postvaccination blood level of GM-CSF produced by the vaccine. MST was 5.3 months for patients above the median peak level, and 1.8 months for patients below that level. Treatment with GVAX pancreatic cancer vaccine with or without cyclophosphamide was generally well tolerated.

In addition to an allogeneic vaccine expressing GM-CSF, GVAX is also developing a hybrid product that simplifies autologous cell transfection with GN-CSF. Although irradiated autologous tumor cells transduced with the gene encoding the cytokine GM-CSF have exhibited therapeutic promise in both animal models and early phase clinical trials, clinical development of autologous vaccines has been made difficult by the need to establish in culture the tumor of each patient, and to perform individualized gene transfer. To circumvent this problem, scientists at Johns Hopkins have generated an HLA-negative, genetically modified human cell line, producing large quantities of human GM-CSF, which can be easily propagated as a suspension culture in defined, serum-free medium, for use as a universal bystander cell to be mixed with unmodified autologous tumor cells in the formulation of a vaccine (Borrello I, etal, Hum Gene Ther, 10 Aug 1999;10(12):1983-91). This new GVAX construct is being currently evaluated in hematologic malignancies.

NemodDC is a standardized fully functional human DC line developed by Nemod Biotherapeutics (Berlin, Germany), based on a cell line from a patient with acute myeloid leukemia (AML). This cell line corresponds to precursor cells (prec-NMDC), which can be differentiated into immature DC (i-NMDC) and further to mature DC (m-NMDC) with all the phenotypic and functional characteristics typical of human DC. NemodDC has been developed for the immunotherapy of diseases such as cancer, and also as a tool for research and validation. Nemod's own development program focuses on NemodDC-based cancer vaccines.

NemodDC provides unlimited numbers of standardized fully functional DC. Based on preclinical data, NemodDC is being developed as an off-the-shelf semi-allogeneic DC vaccine inducing specific immune responses via syngeneic antigen presenting HLA (HLA A2, A3, and B44) and bystander nonspecific helper T-cell responses via allogeneic HLA. NemodDC is virus free, serum free, and safe. NemodDC does not grow *in vitro* or *in vivo* in the mature antigen presenting differentiation state (m-NMDC).

NemodDC is suitable for loading with antigens in the form of MHC class peptides, proteins, or cell lysates, or by transfection via electroporation with genetic material or via viral vectors, or by cell fusion with cancer cells. NemodDC is also attractive for a prime/boost vaccination setting in order to increase the effectiveness of other vaccines or break the tolerance of DNA, allogeneic whole cell, or peptide vaccines. NemodDC can be directly loaded with the vaccines as antigens, and can be used for *ex vivo* generation of specific T cells for adoptive T-cell therapy.

Nemod has a strong IP position on the NemodDC platform, and is looking for both codevelopment partners with proprietary antigens for cancer indications.

Peptide/Antigen Vaccines

Numerous vaccines have been created using various TAA associated with GI cancer, in general, and pancreatic cancer, in particular (Exhibits 1 and 2).

Avicine, under development by AVI BioPharma (Portland, OR), is a synthetic peptide formulation designed to elicit an anti-hCG immune response by hCG-producing cancer cells. This peptide is composed of carboxy terminal 37 amino acids (CTP-37) of the b-subunit of hCG that has been conjugated to diphtheria toxoid. It is effective in stimulating an immune response to hCG that does not crossreact with related glycoprotein hormones.

Avicine has been used to treat a variety of solid tumors including pancreatic cancer. In a multicenter, phase II clinical trial in patients with PDAC, initiated in June 1999, 1-year survival of patients treated with Avicine was similar to that reported for gemcitabine (Gemzar; Eli Lilly), but was associated with minimal side effects, compared to gemcitabine. However, the 1-year survival of patients treated with both Avicine and gemcitabine was significantly better than either treatment alone. In the combination arm, one has patient survived for 22 months.

In addition to survival, the trial's endpoints included response and influence of chemotherapy on antibody response. Virtually equivalent antibody responses to vaccination were observed in patients treated with Avicine and chemotherapy, indicating that gemcitabine had little or no impact on patients' ability to mount an immune response with the vaccine.

In 2005, AVI BioPharma de-emphasized its cancer-related efforts to concentrate on its Neugene technology against mostly viral infections.

GV1001, under development by GemVax (Oslo, Norway), is a vaccine based on several epitopes of the human telomerase catalytic subunit (hTERT). The vaccine is designed to activate both CD4+ and CD8+ cells.

Exhibit 2
Selected Antigens in PDAC Immunotherapeutics

Antigen Description	Role in Cancer Vaccines
Carcinoembryonic antigen (CEA)	
Carcinoembryonic antigen (CEA) belongs to a family of large and heterogeneous group of cross-reacting proteins, comprising 29 genes/pseudogenes. CEA is commonly expressed by a wide range of malignancies, including pancreatic cancer, and by several benign disorders including diverticulitis, pancreatitis, inflammatory bowel disease, cirrhosis, hepatitis, bronchitis, and renal failure; it is also overexpressed in individuals who smoke.	Several human-leukocyte-antigen-restricted epitopes (short peptides) within the CEA protein have been identified that are recognized by human T lymphocytes. However, CEA-expressing tumor cells are generally weakly recognized by the immune system, necessitating various approaches to enhance the immunogenicity of this antigen. Various strategies have been tried to endow CTL or NK cells with antibody activity against CEA.
Epithelial cell adhesion molecule (EpCam)	
EpCam, an antigen present on the surface of most normal epithelia, is a cell adhesion molecule (CAM) that mediates Ca(2+)-independent, homophilic adhesions. In intact epithelia, EpCam is shielded by a basement membrane, and is engaged in cell adhesion. Only malignant cells that have left their primary site and spread throughout the body expose free EpCam molecules on their surface. EpCam is abundantly expressed in many tumors and is present in all stages of tumor development, from primary tumor, to residual disease and metastasis.	When investigators analyzed 3,900 tissue samples of 134 different histologic tumor types and subtypes on a tissue microarray by immunohistochemistry, EpCam expression was detected in 98 of 131 tumor categories. A high fraction of strongly positive tumors was associated with adenocarcinoma of the colon (81%) and pancreas (78%), as well as hormone-refractory adenocarcinoma of the prostate (71%) (Went PT, etal, Hum Pathol, Jan 2004;35(1):122-8).
Gastrin	
Gastrin is a signaling hormone that stimulates the stomach mucosa to produce and secret hydrochloric acid, and the pancreas to secrete digestive enzymes. In normal adult tissue, gastrin is only produced in the antrum region of the stomach and its receptor is produced only on its target cells found in the normal stomach (parietal and ECL cells). Gastrin signals through its receptor, CCK-2/gastrin-R. In cancer cells, gastrin acts to signal growth and proliferation. Gastrin expression and the appearance of gastrin receptors have been associated with increasing malignant characteristics of GI tumors and poor prognosis. Inhibiting gastrin not only inhibits cell growth, proliferation, and metastasis directly, but also 'unblocks' a central pathway leading to apoptosis. This effect is amplified synergistically when the drug is administered in combination with a chemotherapeutic. Amidated and non-amidated gastrins (gastrin precursors) may play an important role in the proliferation and carcinogenesis of GI and pancreatic cancer, especially in the presence of DNA-damaging and/or infectious agents. Amidated gastrins appear to have a protective role, while progastrins exert growth-promoting effects in malignancy. Amidated gastrins additionally play an important role in the migration of GI epithelial cells, and in glandular morphogenesis, while progastrins may play an important role in invasion and metastasis. Several receptor subtypes and signal transduction pathways mediate the biological effects of the gastrin peptides. Progastrin and gastrins also exert antiapoptotic effects, which may additionally contribute to the growth and carcinogenic effects of these peptides on GI mucosal cells *in vivo* (Rengifo-Cam W and Singh P, Curr Pharm Des 2004; 10(19):2345-58).	Gastrin is involved in the progression of colorectal, stomach, liver and pancreatic cancer. It is established as a central hormonal growth factor that stimulates gastric cancer to proliferate and spread. PDAC expresses CCK-2 receptors and responds to amidated gastrins, resulting in transcriptional activation of EGFr ligands, matrix metalloproteinases (MMP), and antiapoptotic factors that collectively increase tumorigenic potential. The gastrin gene may also be activated resulting in gly-gastrin secretion (Gilliam AD, etal, ASCO04, Abs. 2511). Targeting CCK2-receptors has, so far, not provided optimal beneficial effects. It is expected that targeting precursor gastrins (progastrins and gly-gastrins), exclusively rather than amidated gastrins, may be more effective in treating GI cancer. Because advanced stage GI malignancies are largely responsive to autocrine and intracrine progastrins, downregulation of intracellular progastrins will likely be more effective (Rengifo-Cam W and Singh P, *ibid*).

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HEr2/neu	
HEr2/neu, also ErbB-2 (ErbB2), is a 185 kDa transmembrane receptor tyrosine kinase (RTK) belonging to the epidermal growth factor (EGF) superfamily. In human carcinoma, HEr2/neu may function solely as a shared coreceptor for multiple stroma-derived growth factors. HEr2/neu gene amplification and protein overexpression is seen in many different malignancies and may also contribute to chemotherapy resistance.	HEr2/neu is expressed in approximately 20% of PDAC. Recently, coamplification was reported of topoisomerase IIa and HEr2/neu in a subset of PDAC (Hansel DE, etal, Am J Clin Pathol, Jan 2005;123(1):28-35).
Human chorionic gonatoropin (hCG)	
The oncofetal antigen hCG is naturally produced during pregnancy, and is believed to stimulate growth and shield the developing embryo from immune attack (i.e., rejection). The role of hCG in cancer is believed to be analogous to that in pregnancy. In both cases, hCG serves as a growth factor, encouraging rapid cell division, making it a biochemical marker of malignancy associated with all the major types of cancer. It promotes implantation and tissue invasion, fosters angiogenesis, and facilitates immunosuppression, allowing the fetus or tumor to avoid rejection.	An immune response directed against hCG stimulates an attack against the tumor and neutralizes the hormonal benefits provided by hCG. In effect, an hCG vaccine that is effective in blocking fertility, should also be effective in treating cancer. Clinical trials using hCG vaccines in cancer indeed show that an immune response to hCG significantly improves patient survival.
K-ras	
K-ras is a proto-oncogene belonging to the ras family of genes. The mutant oncogene produces an abnormal ras protein that is distinct from the wild type.	K-ras mutations are the most common oncogene mutations in pancreatic cancer, occurring in 75% to more than 95% of pancreatic cancer tissues (Minamoto T, etal, Cancer Detect Prev 2000;24(1):1-12, Sakorafas GH, etal, Cancer Treat Rev, Feb 2000;26(1):29-52, and Lohr M, etal, Int J Pancreatol, Apr 2000;27(2):93-103). Therefore, K-ras is a potential target for vaccination.
Lewis y (Ley)	
Lewis y is a hexasaccharide selectively expressed on tumors of epithelial cell origin. LewisY (Ley equivalent to Leb) often occurs in sialylated form, but sulfated derivatives are also present.	Human pancreatic cancer is characterized by an alteration in fucose-containing surface blood group antigens such as Lewis b, Lewis y, and sialyl-Lewis.
Mesothelin	
Mesothelin is a differentiation antigen present on normal mesothelial cells and overexpressed in several human tumors, including mesothelioma, ovarian cancer, and PDAC. The mesothelin gene encodes a precursor protein that is processed to yield the 40-kDa protein, mesothelin, attached to the cell membrane by a glycosylphosphatidyl inositol linkage and a 31-kDa shed fragment named megakaryocyte-potentiating factor. The biologic function of mesothelin is not known.	Mesothelin is a promising candidate for tumor-specific therapy, given its limited expression in normal tissues and high expression in several malignancies, including pancreatic cancer. Also, see FO, page 1653
MUC1	
Polymorphic epithelial mucin (PEM), encoded by the MUC1 gene, is a membrane-bound (transmembrane) mucin (glycoprotein) with an extensively O-glycosylated core protein that is normally present at the apical surface of glandular epithelial cells of pancreas, breast, prostate, colon, and lung. MUC1 is upregulated and aberrantly glycosylated in most human epithelial cancers. MUC1 may play a role in adhesive functions and in cell-cell interactions, metastasis, and signaling. High levels of MUC1 expression and secretion are associated with a high risk for metastasis and, as a result, with a poor prognosis. The development of metastasis may be attributed to the disruption of the cell-cell adhesion system by MUC1, as some reports have indicated that MUC1 inhibits E-cadherin-mediated cell-cell adhesion or interferes with cellular adhesion by steric hindrance from the rigid ectodomain.	When produced by tumor cells, PEM is often cleaved into the circulation, where it is detectable as a tumor marker (CA 15.3) by various antibodies, permitting early detection of recurrence and assessment of treatment efficacy. Also, soluble MUC1 (sMUC-1) levels are elevated in many MUC1+ cancers. It is estimated that more than 700,000 of the 1.2 million tumors diagnosed in the USA every year overexpress MUC1 protein, making it one of the most common abnormalities associated with human cancer, including those of the lung, breast, prostate, pancreas, ovary, and bowel. Overexpression of MUC1 was found in approximately 90% of human primary pancreatic tumor tissues and three pancreatic cancer cell lines (CAPAN-1, CFPAC-1, and PANC-1) tested by immunohistochemistry and further confirmed by confocal microscope and flow cytometry analysis on the cell surface. Therefore, overexpression of MUC1 is a useful target for the treatment of micrometastases or minimal residual disease in pancreatic cancer patients with overexpression of MUC1 antigen (Qu CF, etal, Br J Cancer, 13 Dec 2004;91(12):2086-93).

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Telomerase	
Telomerase is ribonuclear protein that maintains the length of the telomere and induces cell immortality. Telomeres are involved in the cellular aging process, protecting chromosomes from degradation and fusion. Reactivation of telomerase is a very common feature in human malignancies, with high telomerase activity detectable in 85% to 90% of primary tumors, but not in most normal tissues. Abnormal telomerase upregulation has been associated with cell immortality, and although not sufficient in itself to induce neoplasia, is thought to be essential in maintaining the proliferative capacity of tumor cells. Human telomerase is composed of an RNA component (hTR) and a catalytic subunit encoded by the human telomerase reverse transcriptase (hTERT) gene.	Telomerase is present in 95% of PDAC and is associated with aggressive tumor behavior. Real-time quantitative PCR analysis revealed the presence of telomerase mRNA expression in 50% (10 of 20) of normal, 86% (31 of 36) of chronic pancreatitis, and 90% (26 of 29) of pancreatic cancer samples. Quantification of the expression data revealed that the relative increase above normal was 5.5 for chronic pancreatitis, and 23.9 for pancreatic cancer samples (Buchler P, etal, Pancreas, May 2001;22(4):331-340).

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), April 2005

GemVax is also developing a vaccine based on a combination of GV1001 and p21Ras peptides.

A single center, dose-escalation, phase I clinical trial was initiated at Ullevål Hospital (Oslo, Norway) under PI Trond Buanes, MD, in September 2000, to determine the safety and immunogenicity of GV1001 in 47 chemotherapy-naïve patients with newly diagnosed, histologically confirmed, inoperable PDAC, and to correlate immune responses with any clinical responses. Inclusion criteria were newly diagnosed histologically confirmed inoperable PDAC, Karnofsky performance status >70 and adequate bone marrow, liver, heart and renal functions. The peptide vaccine was injected ID 8 times over a period of 10 weeks, with 3 vaccinations the first week, followed by 5 more injections over the next 9 weeks. The vaccine was tested in two dose levels, using GM-CSF as an adjuvant. Selected patients were treated with monthly booster vaccinations thereafter. Review of preliminary biochemistry data did not indicate any treatment-related changes.

One of the aims of this trial was to see if there would be any toxic affects with this treatment, because low levels of telomerase activity are also found in stem cells of the bone marrow. Clinical development of a telomerase vaccine must, therefore, in addition to eliciting an immune response, must not interfere with the integrity of stem cells in the bone marrow. In this trial, there were no serious adverse events related to the treatment, indicating the possibility of testing the telomerase vaccine also for other cancer indications.

An antitelomerase immune response, measured as DTH, was induced in patients with the percentage of responders being higher in the group of patients treated with the highest dose of vaccine. Several patients in the high dose group developed 'flu-like' symptoms after vaccination. Telomerase specific helper T-cell clones restricted by HLA-DRB*1301 could be generated from a blood sample from one of the vaccinated patients (Buanes T, etal, ASCO02, Abs. 1891).

Based on the safety data from this trial, two parallel trials were performed in 10 patients with malignant melanoma, and in 20 with non-small cell lung cancer (nsclc). Based on a total of 505 vaccine injections (up to 18 injections in one patient) administered to 77 patients, no serious adverse events were noted related to treatment (Gaudernack G, etal, ASCO03, Abs. 666:166).

Two separate phase I clinical trials of GV1001 were initiated at Ullevål Hospital under PI Trond Buanes, MD, in PDAC. One was initiated in April 2003 in combination with imiquimod, an immune response modifier marketed as a topical antiviral (Aldara) by 3M Pharmaceuticals, in patients with inoperable PDAC. The other is a single agent trial, initiated in June 2003, in patients with resectable PDAC. In the latter trial, the vaccine is being administered pre and postresection and the resected tumor is being evaluated for tumor infiltrating lymphocytes.

In April 2005, Pharmexa (Porsgrunn, Norway) announced its intention to acquire GemVax.

Insegia (formerly Gastrimmune), under development by Aphton (Philadelphia, PA), is an oil-based vehicle incorporating a synthetic peptide fragment of hormone gastrin (G17), conjugated to a toxin (diphtheria toxoid), and administered in conjunction with an adjuvant. Insegia induces a directed antibody response against gastrin, and its precursor gly-gastrin, as well as other gastrin-related growth factors. Using human pancreatic cell lines, PANC-1, BxPC3, and an in-house cell line, PAN1, investigators demonstrated that anti-G17DT antibodies provide an additional antitumor effect over that which can be achieved by gemcitabine alone in the treatment of PDAC (Watson SA, etal, ASCO02, Abs. 37).

However, among the limitations of Insegia may be that only a subpopulation of individuals respond to the vaccine and produce antibodies; and even then development of antibodies is slow, a feature that is especially problematic in the case of this rapidly progressing disease. These shortcomings may be addressed by combination treatments, pairing Insegia with MAb-based approaches.

Researchers found that although gastrin and the gastrin receptor pathways are the predominant, central, proliferative pathways in GI adenocarcinoma, additional, but less 'central' receptors and pathways, also exist. These pathways include those stimulated by ligands to EGFr, and are thus capable of being disrupted by MAb, which target one of several of the members of the EGFr family. Other routes to proliferation, whether direct or indirect, are inhibited by a multitude of chemotherapies, albeit with varying degrees of toxicity. Therapies based on cytotoxics or MAb are noncompetitive with Insegia, and may be used in conjunction with this immunotherapy approach.

In February 2005, Aphton reported that the phase III clinical trial (protocol ID: PC4) of Insegia, in combination with chemotherapy, did not meet its primary efficacy endpoint of improving overall survival in patients with PDAC. This trial, initiated in April 2000, randomized 383 previously untreated patients with metastatic PDAC, to be treated with Insegia plus gemcitabine or gemcitabine alone. The toxicity profile in this trial was not significantly different between trial arms, and was similar to the observed events in previous clinical trials. While Insegia did not meet the primary endpoint, survival was prolonged in the approximately 70% of patients who responded compared to patients treated in the gemcitabine monotherapy arm, as well as to patients treated with the combination regimen but who did not mount an antibody response. Results from this trial will be important in designing future clinical trials of Insegia in combination with chemotherapy. In addition, these results further support the current monotherapy applications of Insegia, as a potential treatment for patients for whom chemotherapy is not an option.

In December 2003, Aphton began submissions of regulatory documentation first to the Australian Therapeutic Goods Administration (ATGA) for the registration of Insegia as monotherapy in patients with advanced PDAC who are either unable to tolerate or elect not to take chemotherapy. This submission was followed in June 2004, with one to the Swiss Agency for Therapeutic Products (Swissmedic), the regulatory authority in Switzerland, for the same indication. Submission was based on a European, randomized, multicenter, double blind, placebo-controlled, phase III clinical trial (protocol ID: PC6), completed in March of 2003, of Insegia administered to patients with advanced Stage II/IV PDAC, unsuitable or unwilling to take chemotherapy. Patients were administered the drug or vehicle at weeks 0, 1, 3, 24, and 52. Primary endpoint was survival; secondary endpoints included tolerability, weight change, quality of life (QoL) and tumor response. Among 154 patients (Insegia=79, and placebo=75), MST was 151 and 82 days, respectively. Insegia was well tolerated; 2 patients developed sterile abscess at the injection site (Gilliam AD, etal, ASCO04, Abs. 2511). In this trial, the overall MST was 111 days; it was 176 days for responders, 63 days for nonresponders, and 83 days in the placebo group (Broome P, etal, ASCO04, Abs. 4073). In August 2004, Aphton also submitted an application for regulatory approval of Insegia for the same indication to Health Canada, under the Notice of Compliance with conditions (NOC/c) policy.

In a phase II clinical trial, performed to evaluate the safety of Insegia in inoperable PDAC, 30 patients with histologically proven PDAC were treated with Insegia (100 µg or 250 µg) by IM injection into the thigh on weeks 0, 2, and 6. Patients were evaluated at 2 to 4 weekly intervals. Adverse events were limited to discomfort at the injection site; 11/30 had discomfort only, 4/30 discomfort with swelling, and 3/30 developed a sterile abscess and/or fever. Among 13 patients treated with a dose of 100 µg, 6 (46%) developed antigastrin antibodies, and among 17 treated at a dose of 250 µg, 14 (82.4%) developed such antibodies. Among 20 antibody-positive patients, weight increased in 5 (25%) at week 16 compared to baseline (excluding ascites and edema). Among 20 evaluable patients, disease stabilized in 5 (25%) at week 16 by CT scan. Among 18 antibody-positive patients with elevated CA19-9 at baseline, CA19-9 stabilized in 5/18 over 16 weeks. MST from first injection was 187 days for the whole group, 217 days in antibody producers, and 121 days in the antibody-negative group. Insegia was well tolerated, with 82% of patients producing antibody when treated with a dose of 250 µg (Smith S, etal, ASCO01, Abs. 1029:258a). In another phase II clinical trial, when patients were treated with only the higher dose of the vaccine, MST was 297 days. Over a third of these patients gained weight.

Insegia was granted orphan drug status for treatment of both pancreatic and gastric cancer, in July 2003, in the European Union (EU), by the TGA of Australia in December 2002 and, in July 2002, by the FDA. In September 2002, Aphton also received 'fast track' designation from the FDA for Insegia for the treatment of PDAC.

Mesothelin-specific vaccine was shown to elicit immune responses in patients with PDAC. In December 2003, rights to such a vaccine, originally designed at the Sidney Kimmel Cancer Center at Johns Hopkins University (Baltimore, MD), were licensed to Cerus (Concord, CA). Cerus is currently developing an active immunotherapy approach for treating pancreatic and ovarian cancer, combining this vaccine with the company's facultative intracellular bacterium Listeria monocytogenes vaccine platform that is capable of inducing potent innate and adaptive immunity. This vaccine is currently in development in PDAC, in collaboration with investigators at Johns Hopkins University. In December 2004, Cerus entered into an exclusive license with Chugai Pharmaceutical (Tokyo, Japan), relating to the DNA sequence of mesothelin for the development of cancer vaccines. Terms of this license agreement include an upfront payment, development milestone payments to Chugai, and royalties on product sales.

Cerus' Listeria-based cancer vaccines segregate immunogenicity from toxicity. Cerus has developed a recombinant live-attenuated vaccine platform strain that retains the potency of the fully virulent pathogen, combined with a >1,000-fold reduction in toxicity, as compared with wild-type Listeria. The *in vivo* toxicity of Listeria was diminished without any reduction in its immunopotency by selectively deleting two virulence factors, ActA (DeltaactA) and Internalin B (DeltainlB). Listeria DeltaactA/DeltainlB-based vaccines were rapidly cleared from mice after immunization, and induced potent and durable effector and memory T-cell responses with no measurable liver toxicity (Brockstedt DG, etal, PNAS USA, 21 Sep 2004;101(38):13832-7).

Immune responses to mesothelin have been documented in patients with PDAC treated with whole cell cancer vaccines. Mesothelin-specific CD8+ T-cell responses provide evidence of *in vivo* cross priming by APC in vaccinated patients with PDAC. Tumor-specific CD8+ T cells can potentially be activated by two distinct mechanisms of MHC class I-restricted antigen presentation, either direct presentation by tumor cells themselves, or indirect presentation by professional APC. However, controversy still exists as to whether indirect presentation, the cross-priming mechanism, can contribute to effective *in vivo* priming of tumor-specific CD8+ T cells that are capable of eradicating cancer in patients.

A clinical trial of vaccination with GM-CSF-transduced pancreatic cancer lines was used at the Sidney Kimmel Cancer Center to test whether cross-presentation by locally recruited APC can activate pancreatic tumor-specific CD8+ T cells. Previously, postvaccination DTH responses to autologous tumor cells were noted in 3 out of 14 treated patients. Consistent induction of CD8+ T cell responses to multiple HLA-A2, A3, and A24-restricted mesothelin epitopes were noted exclusively in the 3 patients with vaccine-induced DTH responses. Importantly, neither of the vaccinating pancreatic cancer cell lines expressed HLA-A2, A3, or A24. These results provide the first direct evidence that CD8+ T-cell responses can be generated via cross presentation by an immunotherapy approach designed to recruit APC to the vaccination site (Thomas AM, J Exp Med, 2 Aug 2004;200(3):297-306).

MUC1 100-mer peptide vaccine, composed of a 100-amino acid peptide corresponding to five 20-amino acid long repeats, was evaluated by investigators at the University of Pittsburgh Cancer Institute (Pittsburgh, PA), in a phase I clinical trial (protocol IDs: PCI-97-046, PCI-IRB-970871, NCI-G00-1888), in combination with adjuvant SB-AS2 (AS02A; GlaxoSmithKline Biologicals). The trial was initiated in January 2001, and completed in February 2003, in 16 chemotherapy and radiotherapy naïve patients with resected or locally advanced PDAC, to assess the safety, toxicity, and ability of this vaccine to elicit or boost MUC1-specific immune responses. According to the protocol, escalating doses of the peptide (100, 300, 1,000, and 3,000 mg), admixed with SB-AS2, were administered IM every 3 weeks, for a total of 3 doses, in cohorts of 4 patients. Common adverse reactions included Grade 1 flu-like symptoms, tenderness, and erythema at the injection site. DTH sites showed few or no T cells prevaccination, but increased T-cell infiltration postvaccination. There was an increase in the percentage of CD8+ T cells in the peripheral blood postvaccination. An increase in total MUC1-specific antibody was seen in some patients, and several patients developed IgG antibody. At follow-up, 2/15 patients with resected PDAC were alive and disease free for 32 and 61 months. The vaccine was safe and induced low but detectable mucin-specific humoral and T-cell responses in some patients. No difference was seen between different peptide doses (Ramanathan RK, etal, Cancer Immunol Immunother, Mar 2005;54(3):254-64).

MUC1/IL-18 DNA vaccine was investigated by scientists at Centocor (Malvern, PA) using a human MUC1 transgenic mouse (huMUC1 Tg) model. These mice express human MUC1 in the appropriate tissue-specific pattern, thereby making MUC1 a self antigen. Two MUC1+ tumor models were used to test MUC1 vaccine candidates, a SC tumor model and a lung metastasis model. Using the SC tumor model, ID vaccination with a DNA vaccine encoding MUC1 (pMUC1) alone was insufficient to break tolerance to MUC1, or to induce tumor protection in a subcutaneous tumor model. However, co-administration of pMUC1 with an adjuvant plasmid encoding interleukin-18 (pIL-18) resulted in significant protection from tumor development. This protection was durable in the absence of additional vaccination, as demonstrated by protection of vaccinated mice from a second challenge with MUC1+ tumors.

Evidence of epitope spreading was also observed when protected mice survived a third tumor challenge in which MUC1-expressing tumor cells were administered. Antibody-mediated depletion of specific lymphocyte subsets suggests that the mechanism of protection is attributable to the induction of a cellular immune response against MUC1. These results demonstrate that vaccination with pMUC1/pIL-18 can break tolerance to MUC1 to induce an immune response that mediates decreased tumor burden and increased survival. Furthermore, by mediating tumor regression of MUC1+ tumors, the cellular immune response induced by vaccination with pMUC1/pIL-18 'spread' to other tumor-associated determinants (epitope spreading) leading to the regression of MUC1- tumors. Therefore, it is likely that DNA vaccination could provide clinical benefit in patients with MUC1+ tumors (Snyder L, etal, AACR04, Abs. 4845).

Panvac-VF, under development by Therion Biologics, is a therapeutic vaccine comprising recombinant vaccinia and fowlpox viruses coexpressing CEA, MUC1, and the TRICOM genes for treatment of PDAC.

In June 2004, Panvac-VF entered a phase III clinical trial (protocol ID: TBC-PAN-003) for the treatment of

metastatic PDAC in patients with disease refractory to treatment with gemcitabine. This phase III trial will enroll 250 patients at 50 to 60 participating treatment centers across the USA. The trial's primary endpoint is overall survival compared with palliative chemotherapy, or best supportive care (BSC). The trial is being conducted under the guidance of a Special Protocol Assessment (SPA) provided by the FDA. Patients are being randomized 1:1 to either Panvac-VF or a control treatment. Patients in the Panvac-VF arm are treated with an initial 'priming' dose of Panvac-VF plus GM-CSF to initiate an anticancer immune response, followed by a series of 'booster' vaccinations to sustain the response. Control treatment consists of either BSC or palliative chemotherapy with capecitabine (Xeloda; Roche), irinotecan, or 5-FU. Secondary endpoints include safety, QoL, change in serum tumor antigen levels, response rate, and disease stabilization. John Marshall, MD, Associate Professor of Medicine at Georgetown University Medical Center (Washington, DC), is the trial's lead investigator. Commencement of this phase III trial comes less than one year after the company filed an SPA. The SPA was based on two promising phase I clinical trials in PDAC, as well as a number of clinical trials with the vaccine's components. These latter trials, sponsored by the NCI, involved over 200 patients with various tumor types.

In June 2004, two phase I clinical trials evaluated vaccines targeting MUC1 and CEA in patients with advanced Stage III or IV PDAC. The first trial tested an earlier generation of the vaccines while the second trial tested the current version of Panvac-VF. The trials accrued 22 patients who had prior therapy; 20 of these patients had metastatic disease. Safety was the primary objective of both trials. Patients were administered a 'prime' dose of vaccinia on day 0, followed by 'boost' doses of fowlpox on days 14, 28, and 42; all vaccinations were followed by administration of GM-CSF (100 µg) for 4 days. Patients who were clinically stable were able to continue these vaccinations on a monthly schedule. The most common adverse events were Grade 1 injection-site reaction, which included erythema, swelling, pruritus, blistering, induration, and pain. Other adverse reactions included fatigue, anorexia, nausea, vomiting, fever (Grade 3=1), headache, and myalgia. Of the 12 patients enrolled in the first trial, 6 have died; MST was 7.9 months and 4 patients remained alive at 13 months. For the second trial, MST has not yet been determined, but will be >5.3 months. Expected MST in this population with metastatic disease at baseline would have been 3 months, based on historical controls (Schuetz T, etal, ASCO04, Abs. 2564).

In addition to PDAC, Therion vaccines targeting CEA and MUC1 have been tested in patients with other tumors that are known to overexpress CEA and MUC1 antigens, including breast, lung, and colorectal cancer. Therion and the NCI are currently planning up to 18 additional studies with Panvac-VF in these indications.

SAI-TGF-α, under development by CancerVax (Carlsbad, CA), SAI-TGF-α is a specific active immunotherapy (SAI) approach targeting transforming growth factor-α (TGF-α) that binds to and activates EGFr. Increased stimulation, as a direct result of overexpression of EGFr, EGF or TGF-α, may contribute to dysregulation of the EGFr pathway. In addition, cancerous cells may secrete EGF and TGF-α, which in turn fuels their growth and proliferation by increased activation of the EGFr pathway.

SAI-TGF-α is one of three constructs targeting the EGF/EGFr pathway, licensed exclusively from the Centre of Molecular Immunology (CIMAB), in Cuba, in July 2004 by Tarcanta, a wholly owned subsidiary of CancerVax. This agent is currently in preclinical development.

MONOCLONAL ANTIBODIES (MAB)

Monoclonal antibodies (MAb) that stimulate humoral immunity represent a passive immunotherapy approach. Passive immunotherapy is used to reproduce the activity of the immune system by the introduction in the host of *ex vivo*-generated MAb targeting a specific malignancy. In this case, the host's immune system need not be activated because the immune system effectors, i.e. the MAb injected into the host, are expected to reproduce the action of the immune system. Antibody-dependent cellular cytotoxicity (ADCC) is considered to be one of the effector functions of exogenous MAb. Passive immunotherapy has the advantage of providing an instantaneous 'drug-like' treatment approach that may be beneficial in a rapidly evolving disease like cancer. In contrast, in specific immunotherapy, the host's immune system must be coaxed to produce immune effectors to destroy disease that may be rapidly disseminating, thus overwhelming the immune system.

MAb targeted to various molecular markers expressed in PDAC were covered in Part IV of this series. Of course, in addition to naked MAb acting as passive immunotherapy agents, MAb are being conjugated with various cytotoxic drugs, toxins, or radionuclides, to kill tumor cells expressing the MAb's target. Immunoconjugates being used for the treatment of PDAC were discussed in Part V of this series on pancreatic cancer.

Binding Bodies

Binding Bodies, invented by Pepscan Systems (Lelystad, Netherlands), are small molecules consisting of two or more peptides that represent complementary determining regions (CDR), which are the hypervariable and antigen-interacting parts of the binding site of an antibody, coupled covalently to a small chemical scaffold. These chemically synthesized Binding Bodies represent a novel approach for the production of protein-based molecules that are highly specific for their targets.

In February 2005, Pepscan Systems, Proteomika (Derio, Spain) and AlgoNomics (Ghent, Belgium) obtained research funding within the 6th framework scientific program of the EU, together with the University Medical Centre (UMC; Utrecht, Netherlands), the National Cancer

Research Center (CNIO; Madrid, Spain), and the University Louis Pasteur (Strasbourg, France). This project, which was initiated and is being coordinated by Pepscan Systems, is sponsored by the EU with a research grant of €1.2 million ($1.6 million).

The expertise of the six consortium partners will be combined in order to develop effective fully synthetic Binding Bodies against gastrin to treat pancreatic cancer. Pepscan Systems will develop and produce the Binding Bodies using its CLIPS (Chemically Linked Peptides on Scaffolds) technology. AlgoNomics will apply Tripole, its structural bioinformatics platform, to rationally design optimized Binding Bodies. Proteomika will apply the Binding Bodies to the development of new diagnostic tools. CNIO will apply its recombinant antibody technology. University Louis Pasteur will provide a detailed characterization of the Binding Bodies, and UMC Utrecht will assess their biological efficacy.

Other MAb-based Vaccines

PankoMab, under preclinical development by Nemod Biotherapeutics, recognizes a tumor-specific carbohydrate-induced MUC1 epitope. Nemod's GlykoMab technology is based on specific know-how in the generation of MAb and antibody fragments against highly complex carbohydrate, glycoprotein, and conformational structures, using in-house expertise in chimerization, humanization, and multimerization. Additionally, Nemod has developed a proprietary technology for the easy and fast generation of anti-idiotypic antibodies and surrogate molecules, as well as assay systems to test specificity and properties of these MAb and antibody fragments. Nemod employs its assays to test biochemical, physiochemical, and functional properties of MAb, including ADCC, complement-dependent cytotoxicity (CDC), endocytosis, apoptosis, shedded antigens, biodistribution, and tumor models.

PankoMab discriminates between MUC1 present on tumor cells and that on normal cells, particularly in colorectal carcinoma. PankoMab has many important advantages compared to other MUC1 antibodies in the clinic, including higher specificity, higher affinity, and lack of or largely reduced binding to shedded MUC1. PankoMab is internalized, and shows ADCC-mediated tumor cell killing. In tumor biodistribution models, PankoMab enriches in up to 80% in the tumor, with a more than 50-fold increase in specific tumor uptake.

HuABL-364 (IGN311), under development by igeneon (Vienna, Austria), is a humanized IgG1 MAb directed against Lewis y carbohydrate antigen. The company licensed this agent from Protein Design Labs (PDL; Freemont, CA) in July 2002, based on an option agreement signed by the companies in 2000. The company has exclusive worldwide rights to develop and market the antibody, while PDL will receive an upfront fee, milestone payments, and royalties on any product sales generated by the antibody. IGN311 destroys tumor cells by complement activation and by the activation of cytotoxic effector cells.

IGN311 blocked EGF- and heregulin-stimulated phosphorylation of mitogen-activated protein kinase (MAPK) in both human SKBR-3 breast and A431 vulval carcinoma cells. The effect was comparable in magnitude with that of trastuzumab (Herceptin; Genentech), and apparently noncompetitive with respect to EGF. Stimulation of MAPK by ErbB was dynamin-dependent and contingent on receptor internalization. ABL364 and IGN311 changed the intracellular localization of fluorescent EGF-containing endosomes, and accelerated recycling of intracellular EGF to the plasma membrane (Klinger M, etal, Cancer Res, 1 Feb 2004;64 (3):1087-93).

In an open label, single treatment arm, dose-escalating phase I clinical trial, initiated at the Augsburg Central Clinic, in Germany, in December 2002, IGN311 was administered to 10 evaluable patients (colorectal cancer=7, gastric cancer=1, pancreatic cancer=1, and breast cancer=1) with solid tumors expressing Lewis Y antigen. Patients were administered an IV infusion of IGN311 (50, 100 or 200 mg) on days 1 and 15 of a 43-day protocol. The primary objective was to deduce the safety and tolerability of the drug. A total of 5 adverse events were observed. In the 200 mg cohort, Grade 3 vomiting and skin reaction was observed in 1 patient. Furthermore, 4 serious adverse events occurred, and 2 patients died before protocol completion because of tumor progression. Further clinical trials are in preparation to substantiate the clinical profile of IGN311 (Oruzio DV, etal, ASCO04, Abs. 2624).

In March 2005, Aphton completed the acquisition of igeneon that became a wholly owned subsidiary of Aphton. Pursuant to the agreement, igeneon stockholders will receive 21.5 million shares of Aphton common stock in exchange for 100% of the equity of igeneon. Aphton plans to maintain its current operations as well as the new facilities in Vienna, Austria.

ING-1, under development by Triton BioSystems (Chelmsford, MA), is a high affinity human-engineered IgG1 MAb to the tumor-associated Ep-CAM antigen expressed on epithelial cell malignancies. Once bound to Ep-CAM, ING-1 recruits host immune cells to kill the cancer cell by ADCC. Originally developed by Xoma (Berkeley, CA), NG-1 antibody was produced as a recombinant protein in mammalian expression (CHO) cells using Xoma's Human Engineered (HE) technology. It was designed to reduce potential immunogenicity in patients, while maintaining potent ADCC.

In October 2004, Triton BioSystems in-licensed the exclusive worldwide rights to commercially use ING-1 with Triton's Targeted Nano-Therapeutics (TNT) System. The TNT System is an innovative product that ablates tumors by using tiny magnetic spheres delivered systemically with MAb. A localized externally applied magnetic field then causes these tiny spheres to become heated upon becoming internalized in the targeted tumors. The license to Triton includes USA and foreign patent rights related to

Xoma's ING-1 and Human Engineering technologies, along with several pending applications.

Xoma had completed two phase I clinical trials evaluating ING-1, administered IV, and one phase I clinical trial with ING-1, administrated SC, in patients with various adenocarcinomas.

In a multicenter, open label phase I clinical trial, conducted at the University of Alabama Comprehensive Cancer Center (Birmingham, AL), and the Cancer Therapy and Research Center (San Antonio, TX), ING-1 was investigated in the treatment of advanced adenocarcinoma, including breast, colorectal, pancreatic, gastric, esophageal, lung, ovarian, and prostate cancer. Trial objectives included evaluating safety, tolerability, immunogenicity, and pharmacokinetics. Among 25 patients enrolled in this trial, 22 were administered a single dose of IV ING-1 at 0.03, 0.1, 0.3, or 1 mg/kg over 1 hour. Treatment cycles repeated every 3 weeks. At 1 mg/kg, dose-limiting adverse events were amylase and lipase elevations with abdominal pain. At doses ≤0.3 mg/kg, adverse events were mild, including rigors, chills, or mild fever. There were no objective responses among 22 evaluable patients, but disease stabilized in 2. MTD was determined to be 0.3 mg/kg. Blood samples from the 22 participants in the phase I clinical trial were evaluated for antibody response to ING-1. Among 17 evaluable patients, only 2 had a detectable immune response to ING-1, 1 patient after 2 doses, and another after 3 doses at 0.3 mg/kg (de Bono JS, etal, ASCO02, Abs. 34:9a, and Better M, etal, ASCO02, Abs. 75:20a). This trial was initiated in November 2000, and was closed in August 2001.

IMMUNOMODULATION/IMMUNOSTIMULATION

Immunomodulation is a passive immunotherapy approach that attempts to stimulate the immune system in generalized rather than specific fashion. Immunomodulators include vaccine adjuvants and cytokines, including colony-stimulating factors that are used systemically to boost the immune system.

Cytokines

Various cytokines, including interferons and interleukin-2 (IL-2), delivered systemically, have been approved for the treatment of several malignancies, but they have not proven effective in PDAC. Also, in most cases, treatment with these cytokines causes severe side effects. A more targeted approach involves using various delivery methods to transfer cytokine-producing genes to exclusively to cancer cells, sparing the host from systemic toxicities.

Interleukin 12 (IL-12) is a 70 kD heterodimer cytokine consisting of p35 (IL-12A) and p40 (IL-12B) subunits. Coexpression of two distinct genes is required to generate secreted bioactive IL-12. IL-12 exhibits antiangiogenic effects and a broad spectrum of immunomodulatory activity including activation of CTL and NK cells and Th1 cells by induction of interferon γ (IFN-γ). However, its clinical applications as a systemically delivered immunomodulator have been hampered by severe side effects. Therefore, investigators are focusing on the development of locally delivered IL-12.

Intratumoral administration of the IL-12 gene, either directly using an adenoviral vector, or in cultured DC engineered to produce IL-12 by *in vitro* infection with recombinant adenovirus, eradicate established SC tumors derived from the CT26 murine colon carcinoma cell line. The elicited response is mainly mediated by CTL.

AD.IL-12 is an adenoviral vector encoding human interleukin-12 genes. In a phase I clinical trial, conducted at the University of Navarre (Pamplona, Spain) to evaluate the feasibility and safety of intratumoral injection of Ad.IL-12, the agent was administered in doses ranging from $2.5x10^{10}$ to $3x10^{12}$ viral particles, to 7 cohorts of patients with advanced pancreatic, colorectal, or primary liver malignancies. Patients were thoroughly assessed for toxicity. Antitumor response was evaluated by imaging techniques, tumor biopsy, and hypersensitivity skin tests. Patients with stable disease and no serious adverse reactions could be treated with up to 3 monthly doses of Ad.IL-12. A total of 21 patients (primary liver cancer=9, colorectal cancer=5, and pancreatic cancer=7) were treated with a total of 44 injections. Ad.IL-12 was well tolerated, and DLT was not reached. Frequent but transient adverse reactions, including fever, malaise, sweating, and lymphopenia, seemed to be related to vector injection rather than to transgene expression. No cumulative toxicity was observed. A significant increase in tumor infiltration by effector immune cells was apparent in 4/10 (40%) assessable patients. There was one objective partial response (PR) of the injected tumor mass in a patient with hepatocellular carcinoma (HCC), and disease stabilized in 29%, mainly those with primary liver cancer. Although intratumoral injection of up to $3x10^{12}$ viral particles of Ad.IL-12 to patients with advanced digestive malignancies is feasible and well tolerated, it exerts only a mild antitumor effects (Sangro B, etal, J Clin Oncol, 15 Apr 2004;22(8):1389-97).

Using a different approach, the same investigators evaluated the feasibility, safety, and biologic activity of intratumoral injection of autologous DC transfected with an adenovirus encoding interleukin-12 genes (AFIL-12) in 17 patients (pancreatic cancer= 3, colorectal cancer=5, or primary liver cancer=9). DC were generated from CD14+ monocytes obtained by leukapheresis that were cultured and transfected with AFIL-12 before administration. Doses from 10x106 to 50x106 cells were escalated in 3 patient cohorts. Patients were administered up to 3 doses at 21-day intervals. Intratumoral DC injections were mainly guided by ultrasound. Treatment was well tolerated. The most common side effects were lymphopenia, fever, and malaise. After each treatment IFN-γ and IL-6 serum concentrations increased in 15 patients, as well as

peripheral blood NK activity in 5 patients. DC transfected with AFIL-12 induced a significant increase of infiltrating CD8+ T lymphocytes in 3/11 analyzed tumor biopsies. Among the 17 patients enrolled, 15 (88%) were evaluable for toxicity, and 11 (65%) for response. There was 1 PR in 1 patient with PDAC, and disease stabilized in 2 and progressed in 8 patients, with 2 of the cases progressing fast during treatment. Intratumoral injection of DC transfected with an adenovirus encoding interleukin-12 to patients with metastatic GI malignancies is feasible and well tolerated, but further studies are necessary to define and increase clinical efficacy (Mazzolini G, etal, J Clin Oncol, 10 Feb 2005;23(5):999-1010).

Interferon α (IFN-α) may also play a roll in the immunotherapy of PDAC. Generally, it has been shown that daily administration of interferon α (IFN-α) inhibits angiogenesis in human PDAC growing orthotopically in nude mice. Therefore, the antitumor effect of PEG-interferon-α, a long lasting pegylated formulation of IFN-α, was examined in an orthotopic model of L3.6pl human PDAC. Mice were randomized to once per week SC injections of saline, PEG-IFN-α alone, gemcitabine alone, or combination of PEG-IFN-α and gemcitabine. Pancreatic tumors were analyzed for expression of βFGF, VEGF, and matrix metalloproteinase 9 (MMP-9). Exposure to PEG-IFN-α reduced the median tumor size by 53.5% compared to controls, and by 98% when combined with gemcitabine. Tumors treated with this combination were also significantly smaller (83.5% reduction) compared to those in mice treated with gemcitabine alone. Significantly lower levels of bFGF, VEGF, and MMP-9 were present in tumors from mice treated with PEG-IFN-α. Therefore, weekly administration of PEG-IFN-α inhibited growth of pancreatic cancer. Synergistic antitumor effect of PEG-IFN-α with gemcitabine was associated with decreased levels of the proangiogenic factors βFGF, VEGF, and MMP-9 (Hwang RF, etal, AACR02, Abs. 2603).

Other Immunomodulators

OncoVEX GM-CSF, under development by BioVex (London, UK), is a construct that combines the cell-killing ability of an oncolytic virus, with the generation of a host immune response. OncoVex is based on a novel modified herpes simplex virus (HSV) type 1 vector carrying the gene encoding human GM-CSF. The virus is based on a primary isolate of HSV, which replicates more effectively in human tumor cells than laboratory strains used elsewhere. Deletion of ICP34.5 provides tumor-selective replication. Additionally, the gene for human GM-CSF is inserted to boost the antitumor immune response following liberation of tumor antigens by virus replication. OncoVEXGM-CSF provides, therefore, an *in situ*, patient specific, GM-CSF enhanced, antitumor vaccine combined with oncolysis, and is intended to treat both injected tumors and disseminated disease (Hu J, etal, AACR04, Abs. 5360).

Versions of the OncoVEX virus expressing other active genes have also been constructed and tested in preclinical models. These include viruses expressing TNF-µ and TNF-µ combined with GM-CSF, intended to be synergistic with radiotherapy, and versions of the virus expressing prodrug activating genes combined with the delivery of a fusogenic glycoprotein, designed to maximize the properties of the virus for local tumor control. Promising results have been obtained with each of the newly constructed versions of OncoVex in preclinical tumor models, including in combination with chemotherapy where synergism has been demonstrated (Coffin RS, etal, AACR04, Abs. 1193, and Coffin RS, etal, ASCO04, Abs. 2601).

Reximmune, under preclinical development by Epeius Biotechnologies (Los Angeles, CA), comprises retroviral expression vectors that combine Rexin-G with a targeted vector bearing the GM-CSF (Reximmune-C) and/or IL-2 (Reximmune-L) genes. For information about Rexin G, see FO, p 1781.

In April 2005, Epeius Biotechnologies initiated a phase I clinical trial in New York to test the safety and efficacy of intra-arterial Rexin-G in the treatment of metastatic colon cancer. Epeius has also received FDA approved of its investigational new drug (IND) to initiate a phase I/II clinical trial in the USA with Rexin-G as an IV infusion for the treatment of metastatic PDAC.

Virulizin, a novel immunotherapeutic agent, under development by Lorus Therapeutics (Toronto, Canada) stimulates natural killer (NK) cell function. Virulizin was profiled in FO, p 1691. Lorus is also developing Neo Virulizin, a synthetic version of Virulizin.

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