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STATE-OF THE-ART IN THE MANAGEMENT OF CANCER

PANCREATIC CANCER — PART V

ANGIOGENESIS INHIBITORS/ANTIMETASTATIC AGENTS AND TARGETED DELIVERY/GENE TRANSFER APPROACHES

Next issue:
Immunotherapy and vaccine approaches in pancreatic cancer

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Previously, novel drugs and formulations used in the treatment of pancreatic ductal adenocarcinoma (PDAC) based primarily on cytotoxic principles, were described in Volume 7 #12 issue of FUTURE ONCOLOGY (pp 1702-1729) and those targeting various biomarkers were described in Volume 8 #1/2 (pp 1730-1560). In this issue new approaches in the treatment of PDAC are described that involve strategies in development to prevent metastatic spread in PDAC employing a variety of antivascular, antiangiogenic and antimetastatic agents, as well as agents based on targeted delivery/gene transfer approaches. Immunotherapy approaches are described in the upcoming Volume 8 #4 issue of FUTURE ONCOLOGY.

Agents discussed in this issue are listed in Exhibit 1. Targets for these agents are listed in Exhibit 2.

ANGIOGENESIS INHIBITORS/ANTIMETASTATIC AGENTS

Although some localized tumors can be devastating to the host by damaging vital organs, tissue invasion and the ability to metastasize is what confers cancer its lethality. Prevention of invasion and metastasis represents the most rational target of anticancer treatment. Most interventions, including drugs on the market and in development, attempt to stave off malignant spread by destroying the primary tumor, but in most cases micrometastases may have occurred even before the diagnosis is confirmed.

The process of metastasis needs to be further elucidated in order to be adequately address by pharmacologic intervention. Currently, two putative mechanisms associated with metastasis, i.e. cell adhesion and angiogenesis, are the primary targets of antimetastatic agents.

Disruption of cellular junctions and interactions between cells and the extracellular matrix (ECM) are considered essential prerequisites for cell detachment from the primary tumor, invasion of the bloodstream, and growth at distant sites. Several families of proteases are implicated in these processes, with the largest and best characterized being the family of matrix metalloproteinases (MMP).

Growth of new vessels, i.e. angiogenesis, is necessary to provide nourishment for these new tumor-cell colonies so they can continue to grow. Therefore, both tumor growth and metastasis are angiogenesis dependent, making them an attractive target in the attempt to arrest tumor progression and spread. Angiogenesis also has prognostic importance in PDAC.

Numerous antiangiogenesis/antivascular agents are in development to prevent or reverse angiogenesis, with several having been evaluated in PDAC (Exhibit 1). Although the information presented here is confined to such approaches in the treatment of PDAC, naturally, strategies discussed in this article are also applicable to most solid tumors.

Endothelial Monocyte-activating Protein II (EMAP-II)

Investigators at the Cancer Institute of New Jersey (New Brunswick, NJ) tested a novel antiangiogenic treatment approach in a murine model of xenotransplanted human pancreatic cancer cells, using the antiendothelial cytokine EMAP-II as a therapeutic agent. EMAP-II is a novel cytokine that is antiangiogenic in tumor vascular development, strongly inhibiting tumor growth. However, although its mechanism pertains to angiogenesis inhibition, its precise mode of action is uncertain.

In an attempt to evaluate the effect of this factor in PDAC, human Panc-1 PDAC cells were injected subcutaneously into nude mice, and human recombinant EMAP-II was administered through daily intraperitoneal (IP) injections. Comparisons were made between untreated xenotransplanted mice with those treated with IP gemcitabine alone or with EMAP-II alone. While subcutaneous Panc-1 tumors grew in control animals by a factor of 3.4, EMAP-II, which did not affect Panc-1 proliferation *in vitro*, induced a significant antitumor effect *in vivo*. Mean net tumor growth by day 14 in the control and monotherapy groups was 87 ± 35 (control), 50 ± 21 (gemcitabine), and 9 ± 38 (EMAP-II). There was no noticeable toxicity associated with EMAP-II administration. EMAP-II therapy significantly reduced both intratumoral microvessel counts and tumor cell proliferation, and increased the frequency of vascular thrombosis. In combination therapy, high dose EMAP enhanced the gemcitabine-induced antitumor effect, with an average net tumor change of -8 ± 15. Relative tumor reduction was 38% with gemcitabine versus 81% with the combination of gemcitabine and EMAP-II. Therefore, EMAP-II exerts potent antitumor activity in this xenograft model of PDAC, and may be beneficial in combination with cytotoxic therapy (Konduri S, etal, AACR04, Abs. 60).

Matrix Metalloproteinase Inhibitors (MMPI)

Early in the attempt to limit cancer metastasis, one of the most aggressively pursued class of drugs were members of the large matrix metalloproteinase (MMP) family that has been implicated in solid tumor growth, atherosclerosis, rheumatoid arthritis, and Alzheimer's disease, as well as in corneal, periodontal, and dermatologic disorders.

MMP are zinc-dependent proteolytic enzymes with different substrate specificities for such ECM molecules as collagen, fibronectin, laminin, and elastin. Among the various family members, MMP-2 and MMP-9 are most commonly implicated in tumor angiogenesis. MMP activity may be inhibited by members of the family of proteins known as tissue inhibitors of metalloproteinases (TIMP). Another protein, membrane Type 1 matrix metalloproteinase

(MT1-MMP), is an enzyme that plays a major role in remodeling of the ECM, by cleaving ECM components such as fibronectin and Type 1 collagen, and activating other ECM remodeling enzymes such as progelatinase A and procollagenase 3. MT1-MMP is overexpressed both in stromal cells surrounding malignant tumors and in invasive tumor cells.

Overexpression of several MMP has been demonstrated in PDAC, suggesting that an imbalance between MMP and TIMP plays a role in pancreatic tumor progression. Also, MMP-2 activation correlates with more advanced pathologic stages of PDAC, and early recurrence after resection.

Synthetic MMP inhibitors (MMPI) have been developed and tested against a variety of tumors. However, phase III trials with MMPI, alone or in combination with gemcitabine, produced disappointing results in PDAC. Therefore, at this point, the consensus is that MMPI have no role in the treatment of advanced PDAC. However, because of their cytostatic effect, it is conceivable that use of MMPI in early stage disease may improve existing therapies. Also, a new generation of MMP inhibitors is in development, and MMP are being used as delivery approaches to target other anticancer agents to tumor vasculature.

In March 2004, Aventis (now sanofi-aventis) entered into an agreement with Protein Mechanics (Mountain View, CA) to advance identification, discovery, and validation of highly selective, orally available MMPI for treatment of a broad array of pathologies involving the ECM. Protein Mechanics will use its Imagiro predictive simulation technology to provide sanofi-aventis with insights into important aspects of specific target-ligand interaction, including novel binding sites, binding modes, mechanisms of action, and induced-fit conformational changes, in order to drive design and optimization of highly selective drug leads.

BAY 12-9566, a biphenyl MMP-2 and MMP-9 inhibitor, proved inferior to gemcitabine in a randomized trial of 277 patients with advanced PDAC. This phase III clinical trial, initiated in May 1998 was discontinued in September 1999. It was expected to enroll 900 patients to be treated with BAY 12-9566 (800 mg) PO, twice daily, in comparison to gemcitabine.

Marimastat, an orally bioavailable synthetic MMPI, was shown to be active against several MMP. In preclinical models, marimastat and other MMPI delayed tumor growth and prolonged animal survival. In phase I clinical trials, inflammatory polyarthritis was the dose-limiting toxicity (DLT).

Unfortunately, although initial results from phase I/II clinical trials in PDAC suggested a clinical effect, marimastat did not show efficacy in a multicenter phase IIb/III trial as a monotherapy for PDAC. In February 1999, results were reported from a randomized, multicenter phase III clinical trial (protocol ID: BB-C03/IVB/128), conducted by the Marimastat Pancreatic Cancer Study Group, which compared marimastat to gemcitabine as first line therapy in 414 patients with pancreatic cancer. The primary end point was overall survival; secondary endpoints included progression-free survival (PFS), patient benefit, and safety. The trial was designed to detect a ≥16% reduction in mortality in patients treated with marimastat dose of 10 mg or 25 mg, twice daily, as compared to treatment with gemcitabine. The trial did not meet its primary endpoint. There was no significant difference in survival between 5, 10, or 25 mg of marimastat or gemcitabine. Median survival time (MST) was 111, 105, 125, and 167 days, respectively, and 1-year survival rate was 14%, 14%, 20%, and 19%, respectively. Both agents were well tolerated, although Grade 3/4 toxicities were reported in 22% of those treated with gemcitabine, compared to 12% in marimastat-treated patients. The major toxicity of marimastat was musculoskeletal, seen in 44% of patients treated with marimastat, compared with 12% of those treated with gemcitabine; musculoskeletal toxicity was severe in only 8% of patients treated with marimastat. In a secondary analysis, which adjusted for baseline variables, survival did not differ significantly between the two arms. The 1-year survival rate for patients treated with a marimastat dose of 25 mg was similar to that of patients treated with gemcitabine (Bramhall SR, etal, JCO, Aug 2001;19(15): 3447-3455).

Although results from this trial provided evidence of a dose response for marimastat in patients with advanced PDAC, they came up short compared to gemcitabine. Median PFS among patients treated with marimastat was approximately half that of patients treated with gemcitabine. Likewise, reported response rates were lower in the marimastat arms of the trial (3% versus 26% for gemcitabine). However, in the group treated with marimastat, according to a subgroup analysis of patients with metastatic disease (approximately 65% of the cases), MST of patients with nonmetastatic disease was longer than that of patients with metastatic disease. No such differences were seen in the gemcitabine group.

A large, placebo-controlled phase III multicenter trial that investigated efficacy of marimastat in combination with gemcitabine also failed to show clinical benefit in terms of response, or a significant increase in survival. In this trial, 239 patients were treated with gemcitabine (in the same schedule as in the preceding trial) and were concurrently randomized to marimastat (10 mg), twice daily, or placebo. Median and 1-year survival was nearly identical in the two groups, and no differences were observed in overall survival or PFS, with hazard ratios of 0.99 and 0.95, respectively. In this trial, no interaction was observed between marimastat and disease stage.

MMI-166, an N-sulfonyl amino acid derivative, is a third-generation compound under development by Shionogi (Osaka, Japan), designed to selectively inhibit MMP-2, MMP-9, and MMP-14. *In vitro*, MMI-166 inhibits gelatinase activity of MMP-2 and MMP-9 derived from the PDAC cell line PGHAM-1, and dose-dependently inhibits invasion of PGHAM-1 through a basement membrane-like

barrier. In hamsters orthotopically implanted with PGHAM-1, MMI-166 significantly reduced incidence of liver surface metastasis from 66.7% to 20.0%, and also reduced the number of liver surface metastases per animal from 6.17 to 2.00, although this result was not statistically significant. MMI-166 significantly reduced the volume of pancreatic tumors from 718.3 mm^3 to 222.8 mm^3, reduced microvessel density from 37.90 m^2 to 16.16 m^2, and increased the apoptotic index from 1.75% to 3.96%. However, even with these results, there was no significant difference between tumor-cell proliferation in the MMI-166 group and the control group (Matsushita A, etal, Int J Cancer, 1 May 2001;92 (3):434-40).

Ro28-2653, a pyrimidine-2,4,6-trione under development by Roche, is a novel synthetic inhibitor of MMP with high selectivity for MMP2, MMP9, and MT1-MMP.

In a preclinical study, treatment with either Ro28-2653 orally, once-a-day for 21 days, or with gemcitabine IP, every 48 hours for 21 days, alone or in combination, was initiated one week after tumor cell implantation of the human PDAC cell line PancTu1 into the pancreas of SCID mice. Treatment with Ro28-2653 monotherapy reduced tumor volume significantly by 64%, treatment of gemcitabine alone by 84%, and combination therapy by 94%, compared to controls. Induction of necrosis in pancreatic tumors was significant in the group treated with combination therapy. In controls treated with vehicle alone, the poorly differentiated PDAC invaded into adjacent organs and metastasized to different sites in the abdomen and lungs. Invasion into duodenum, stomach, liver, and spleen was not observed in mice treated with gemcitabine alone, or in combination with the MMP-inhibitor. Metastases were not detected in the group treated with combination therapy. Treatment with Ro28-2653 or gemcitabine alone, as well as in combination, prolonged survival of the tumor-bearing mice to 13 more days (Ro28-2653 alone), 26 more days (gemcitabine alone), and 33 more days (combination therapy). Therefore, treatment with Ro28-2653 during early neoplastic progression was highly effective in inhibiting PDAC growth and progression. Also, this MMPI prolonged survival of tumor-bearing mice, and enhanced the antitumor efficacy of gemcitabine (Alves F Sr, etal, AACR03, Abs. 4626).

Vascular Endothelial Growth Factor (VEGF) Inhibitors

Members of the vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) family of proteins are important angiogenic cytokines, playing critical roles in tumor angiogenesis. VEGF variants are among the most potent angiogenic molecules described thus far. These proteins include growth factors VEGF (VEGF-A), VEGF-C (VEGF-2, or Flt4 ligand), and VEGF-D, and their receptors such as kinase-insert domain-containing receptor (KDr), also referred to as VEGFr2, or Flk-1, VEGFr1 (Flt-1), and VEGFr-3 (Flt-4).

VEGF and its receptors appear to be co-expressed in pancreatic tumors; VEGF expression correlates positively with local recurrence, metastatic potential, and overall survival in PDAC. Although the VEGF pathway has emerged as a therapeutic target in pancreatic cancer, laboratory results are often contradictory as to the validity of VEGF as a target of treatment for PDAC. Immunohistochemical measures of VEGF expression in pancreatic cancer have typically been qualitative or semi-quantitative and, furthermore, VEGF levels in primary versus metastatic sites have not been well characterized.

Investigators at the Yale School of Medicine (New Haven, CT) developed a set of algorithms referred to as automated quantitative analysis (AQUA), to assess tissue microarrays (TMA) that use fluorescent tags to define tumors, localize subcellular compartments, and grade intensity of specific markers on a continuous scale. AQUA was used to compare VEGF levels of a pancreatic cancer TMA involving tumors removed from the pancreas and metastatic sites. TMA were constructed by arraying 1.5-mm cores from 80 cases of PDAC, deposited in the Yale archives between 1996 and 2002, with 14 normal pancreatic cores serving as controls. Of the 80 cases examined, 58 were evaluable, of which 35, 9, and 14 were from pancreas, liver, and other intra-abdominal contiguous or metastatic sites, respectively. VEGF expression was similar in normal pancreatic and malignant tissue as measured by quantitative immunohistochemistry. Stromal VEGF expression however, appeared to be lower in malignant tissue than in normal pancreas, and tumor expression of VEGF was lower in nonhepatic contiguous or metastatic sites compared with primary pancreatic cancer (Yoon HH, etal, AACR04, Abs. 424).

Investigators at Merck KgaA (Darmstadt, Germany) and the University of Regensburg, in Germany, demonstrated that blocking murine KDR/flk-1 activation with an antimouse KDR/flk-1 MAb led to significant reduction of primary pancreatic tumor growth and metastasis, as compared to untreated control animals following orthotopic injection of human pancreatic cancer cells in nude mice. Based on these findings, antitumor and antiangiogenic efficacy of an antihuman VEGF MAb (R&D MAP 293) was evaluated on human pancreatic cancer cells growing orthotopically in nude mice. Groups of these mice were treated IP with escalating doses of antihuman VEGF MAb by 7 injections over 19 days, and then sacrificed on day 22. In this mouse model, *in vivo* therapy with a low dose antihuman VEGF MAb lead to a significant regression of primary pancreatic tumor growth and a 50% reduction of lymph node metastases. This effect was attributed to inhibition of tumor-induced angiogenesis (Bruns CJ, etal, AACR02, Abs. 1277).

Numerous agents that inhibit the VEGF family of proteins are in development, with several being specifically evaluated in PDAC (Exhibit 1). Monoclonal antibody (MAb) inhibitors that bind to VEGF are in more advanced phases of clinical development than many other potential

strategies to block signal transduction associated with VEGF receptors.

2C3, under development by Peregrine Pharmaceuticals (Tustin, CA) is an antibody that blocks the action of VEGFr, and may be used both as a potential targeting agent for the Vascular Targeting Agents (VTA) platform and also as a stand-alone antiangiogenesis therapeutic. MAb 2C3 blocks binding of VEGF to VEGFr2 (KDR/Flk-1) without blocking VEGFr1 (FLT-1/flt-1).

In a preclinical study the effect of 2C3 in PDAC was evaluated in cell lines Mia PaCa-2, Capan-1, and Panc-1, both *in vitro* and when xenografted in nude mice. Although 2C3 demonstrated specific binding to VEGF, it did not inhibit growth of these tumor cells *in vitro*. However, after 7 weeks of systemic therapy, tumor burden of mice bearing Mia or Panc-1 xenografts treated with 2C3 was reduced >50% compared to controls. Complete tumor regression occurred in 3/5 mice bearing Panc-1 tumors after 7 weeks of therapy with 2C3. In contrast, mice bearing Capan-1 tumors did not respond to therapy with 2C3 after 6 weeks of treatment. Therefore, systemic therapy with 2C3 significantly inhibited establishment of tumors using Panc-1 and Mia PDAC cell lines. However, tumors established from the Capan-1 cell line appeared to be resistant to therapy with 2C3. Systemic administration of 2C3 to tumor-bearing mice did not induce any apparent toxicity such as weight loss (Holloway S, etal, AACR03, Abs. 3037).

Bevacizumab (Avastin; Genentech) is a humanized IgG1 MAb that blocks binding of VEGFA to its receptors, VEGFr1 (FLT-1) and VEGFr2 (KDr), resulting in inhibition of angiogenesis. In February 2004, Avastin was approved in the USA for first line treatment of metastatic colorectal cancer in combination with 5-fluorouracil (5-FU)-based chemotherapy. Subsequently, the drug was also approved in Israel and Switzerland in 2004, and in the European Union (EU) in January 2005. The drug can be used safely, either alone or in combination with a number of standard cytotoxic regimens. Bevacizumab has a favorable toxicity profile, with asthenia, fever, and headaches being the most common side effects. However, hypertension and bleeding have also been reported.

Several combination trials are ongoing with bevacizumab in the treatment of PDAC (Exhibit 3). Also, two combination trials, one with gemcitabine and one with capecitabine and radiation therapy, have been completed for this indication. In January 2005, in a webcast to investors, it was reported that the phase III clinical trial (Exhibit 3) of Avastin, in combination with gemcitabine, in PDAC is continuing to accrue patients. Depending on the results of this trial, Genentech may file for approval for Avastin in advanced PDAC. If Avastin is approved in PDAC, it would be the first targeted therapeutic to be commercialized for a pancreatic cancer indication.

In a phase II trial (protocol IDs: UCCRC-NCI-2675; NCI-2675), initiated in November 2001, in patients with metastatic (Stage IV) PDAC, IV bevacizumab (10 mg/kg) was administered over 30-90 minutes on days 1, and 15 in combination with IV gemcitabine (1000 mg/m^2) administered over 30 minutes on days 1, 8, and 15, every 28 days for up to 6 courses. Patients with a partial response (PR), complete response (CR), or stable disease (SD) after completion of 6 courses of therapy may continue on bevacizumab alone until disease progression. Hedy L. Kindler of the University of Chicago Cancer Research Center is Study Chair. CT scans were obtained every 8 weeks. This trial was closed in May 2004. Among 45 patients enrolled in the trial, 42 were evaluable for response. There were 9 (21%) PR lasting a median of 9.4 months, and disease stabilized in 19 (45%) lasting a median of 5.4 months. Median survival time (MST) was 9.0 month, time-to-progression (TTP) was 5.8 months, and overall survival (OS) was 74% at 6 months. Observed Grade 3/4 toxicities included neutropenia (33%), leukopenia (30%), thrombocytopenia (7%), thrombosis (12%), bowel perforation (5%), hypertension (2%), proteinuria (2%), and headache (2%). Observed Grade 5 toxicities included one case of gastrointestinal bleed and one of bowel perforation (Kindler HL, etal, ASCO03, Abs. 1037:259 and Kindler H, etal, ASCO04, Abs. 4009). According to interim analysis involving the first 26 patients entered in the trial, the estimated one-year survival rate was 53%, which compares favorably with the historical control of approximately 18%.

An open label phase I clinical trial (protocol ID: ID02-146) to determine the safe dose of bevacizumab or capecitabine, in combination with chemoradiation for the treatment of locally advanced, inoperable PDAC, was initiated in September 2002 at the University of Texas M. D. Anderson Cancer Center under PI Christopher Crane, MD. Bevacizumab (5 mg/kg IV) was administered to all patients 2 weeks prior to the start of radiotherapy (50.4 Gy), treating the primary and gross adenopathy, then every 2 weeks thereafter, at a dose of 2.5 mg/kg in cohorts 1-2, then 5 mg/kg in cohort 3. Capecitabine was administered orally continuously with radiotherapy on days 14-52 (650 mg/m^2) twice daily in cohort 1 and 900 mg/m^2 in cohorts 2-3. Patients with stable or responding disease were offered maintenance bevacizumab (5 mg/kg IV q 2 wks) until progression. Functional CT was performed on days 0, 14, and at the time of restaging, 5 weeks after radiation therapy. According to a preliminary report, no significant hematologic or gastrointestinal toxicity, thrombosis, proteinuria, or hypertension were observed in 9 treated patients. In one patient, a tumor-related duodenal ulcer that bled two weeks after discontinuing therapy, subsequently healed after the tumor responded to therapy. Among 3 evaluable patients who completed treatment, there was 1 PR and disease stabilized in 2 patients. In 1 patient with stable disease, a baseline Ca 19-9 level of 1000 dropped to 177 (nadir not reached). Results from perfusion imaging indicated increased blood flow after the initial dose of bevacizumab. Treatment with this novel combination of bevacizumab and chemoradiation is well tolerated

and has activity in patients with PDAC (Crane C, etal, ECCO03, Abs. 980).

According to another interim report, among 18 patients treated, no significant hematologic toxicity, thrombosis, proteinuria, or hypertension was observed. Among 9/18 patients evaluable at a median follow-up of 6 months, there was 1 radiographic CR, and disease stabilized locally in the remaining 8 patients. There was no objective local tumor progression. No patients have died, 4 have developed distant disease and 2 of the first 3 patients treated were distant progression free at 9 months. Results from perfusion imaging indicated increased blood flow after the initial dose of bevacizumab. Treatment continued with escalating doses of bevacizumab at 2.5 mg/kg increments to 10 mg/kg (Crane CH, etal, ASCO04, Abs. 85).

Subsequently, a total of 24 patients were treated with this regimen; 8/24 had been previously treated with chemotherapy. There were 5 PR among 6 patients treated with bevacizumab (5 mg/kg) and capecitabine (825 mg/m^2). None of the patients experienced local disease progression. Gastrointestinal side effects included an 8% incidence of Grade 3 toxicity. Grade 3 side effects relating to bevacizumab consisted of hypertension (n=1), thrombosis (n=1), and bleeding (n=1). A multicenter trial is planned by the Radiation Therapy Oncology Group (RTOG) to test the role of bevacizumab (5 mg/kg) combined with chemoradiation in the treatment of locally advanced PDAC.

CEP-7055, under development by Cephalon (West Chester, PA), is the lead compound of a new class of synthetic orally active molecules that block growth of solid tumors by preventing angiogenesis via the VEGF cascade. CEP-7055 is a dimethylglycine ester of CEP-5214, a water-soluble compound that is converted rapidly to CEP-5214 *in vivo*. CEP-7055 is a highly specific angiogenesis inhibitor. In preclinical studies CEP-7055 inhibited specific kinases in the signaling cascade in prostate and pancreatic cancer.

In December 2001, Sanofi-Synthelabo (now sanofi-aventis) and Cephalon signed a collaborative agreement to develop and market angiogenesis inhibitors, including a number of orally active molecules based on Cephalon's proprietary kinase inhibitor technology. Under the terms of this agreement, Sanofi-Synthelabo acquired co-promotion rights in the USA, Canada, and Mexico for the marketing of the drugs and exclusive marketing rights in Europe and the rest of the world, excluding Japan. In return, Sanofi-Synthelabo made an upfront payment to Cephalon, will make milestone payments, and pay royalties on sales. Sanofi-Synthelabo and Cephalon will share development expenditures.

Chronic PO administration of CEP-7055 resulted in significant growth inhibition, 50% to 90% maximum inhibition relative to controls, of a variety of established murine and human tumor xenografts in nude mice, including ASPC-1 pancreatic carcinoma. The antitumor efficacy of chronic CEP-7055 administration was independent of initial tumor volume in the ASPC-1 model, and was reversible on withdrawal of treatment. Chronic PO administration of CEP-7055 in preclinical efficacy studies for periods of up to 65 days was well tolerated with no apparent toxicity or significant morbidity (Ruggeri B, etal, Cancer Res, 15 Sept 2003; 63, 5978-91).

In July 2001, Cephalon initiated an open label, dose-escalation, phase I clinical trial (protocol IDs: 01108, J0146) with CEP-7055 in patients with locally advanced solid tumors not amenable to known therapies. Objective of this trial, being conducted at Duke University Medical Center (Durham, NC) and Johns Hopkins University (Baltimore, MD) under PI R. Pili, MD, is to determine safety, tolerability, and MTD. A single cycle of treatment consists of CEP-7055, administered continuously for 28 days, followed by a 14-day washout period. Patients are eligible for further cycles subject to tolerability and tumor status. Among the first 19 patients treated at doses of 10, 20, 40, 80 and 120 mg, administered twice daily, adverse events have been generally mild, and DLT has not been observed to date. Hypertension observed in one patient at 120 mg, occurred towards the end of the washout period (Pili R, etal, ASCO03, Abs. 831).

CX-3543, a cationic porphyrin under development by Cylene Pharmaceuticals (San Diego, CA), is a small molecule compound designed to interact with a structurally defined cluster targeting the c-Myc/VEGF quadrome. CX-3543 targets a specific subset of oncogenes, the c-Myc/VEGF quadruplex cluster, downregulating both c-Myc and VEGF.

CX-3543 binds to the c-Myc quadruplex motif with significant molecular selectivity relative to the human telomeric quadruplex motif, or other forms of DNA such as single stranded or duplex plasmid DNA. Small molecule compounds such as CX-3543 are designed to interact with structurally defined clusters within the quadrome, which represents a quadruplex motif grouped into clusters related by sequence, structure, function, and other features that regulate oncogene expression. These G-quadruplex structures regulate transcription of certain oncogenes, and can be targeted with small molecules, leading to the selective suppression of oncogene expression (Siddiqui-Jain etal, PNAS 2002;99:11593–11598). CX-3543, designated an 'oncogene inhibitor', belongs to a class of small molecule compounds that targets G-quadruplex regulatory elements in genes involved in growth and proliferation. This new approach to cancer treatment allows the inhibition of targets such as c-Myc that are not druggable (Jin C, etal, AACR04, Abs. LB-243).

In vitro, CX-3543 displayed antitumor activity over a broad range of tumor types. *In vivo*, CX-3543 induced significant tumor growth inhibition in refractory prostate cancer (PC-3), colorectal cancer (HCT-116), and pancreatic cancer (MiaPaCa) xenograft models, which express high levels of c-Myc.

Exhibit 1
Novel Targeted Therapeutics Evaluated Either Clinically or Preclinically for the Treatment of Pancreatic Cancer

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Development Status ❑ Indication(s)
Æterna Zentaris ❑ Tulane U	AN-238 and AN-162	Synthesized targeted cytotoxics consisting of AN-201 or doxorubicin, respectively, linked to octapeptide RC-121 ❑ IV	Preclin (ongoing 7/04)➢Europe (Germany) ❑ solid tumors
Antisoma ❑ Imperial Cancer Research Fund (ICRF), Cancer Therapeutics, National Cancer Institute (NCI)	AS1406 ❑ Theranase	Humanized monoclonal antibody (MAb) huHMFG1 recognizing polymorphic epithelial mucin (PEM), conjugated with recombinant cytotoxic RNase ❑ injection	Preclin (ongoing 10/04)➢Europe (UK) ❑ solid tumors, lymphoma
AstraZeneca	ZD6474, AZD6474	Potent vascular endothelial growth factor receptor 2 (VEGFr2) tyrosine kinase inhibitor ❑ PO	Phase II (ongoing 1/05)➢USA ❑ various solid tumors
Bayer ❑ Onyx Pharmaceuticals, Chiron	Sorafenib ❑ BAY 43-9006	Small molecule drug that inhibits tumor-cell proliferation by targeting the RAF/MEK/ERK signaling pathway at the level of RAF kinase and exerts an antiangiogenic effect by targeting the receptor tyrosine kinases VEGFr 2 and PDGFr and their associated signaling cascades ❑ PO	Phase II (begin 10/04)➢USA (combination) ❑ advanced or metastatic 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 GM-CSF ❑ intratumoral, intralesional	Phase I/II (begin 6/02, ongoing 5/04) ➢Europe (UK) ❑ solid tumors
Cell Genesys ❑ Geron, Genetic Therapy, Novartis	CG5757	Oncolytic adenovirus for the treatment of retinoblstoma (Rb) pathway-defective and telomerase-positive malignancies ❑ injection	Preclin (ongoing 1/05)➢USA ❑ solid tumors
Centocor	CNTO 859	Humanized MAb derived from TF8-5G9, a previously described mouse antihuman tissue factor (TF) MAb ❑ injection	Preclin (ongoing 1/05)➢USA ❑ solid tumors
Cephalon ❑ Ricerca Biosciences, Sanofi-Aventis	CEP-7055	CEP-7055 is the lead compound of a new class of orally active molecules that block growth of solid tumors by preventing angiogenesis via the VEGF cascade ❑ PO	Phase I (ongoing 8/04)➢USA ❑ solid tumors
Cylene Pharmaceuticals ❑ U Arizona	CX-3543	Cationic porphyrin, a small molecule compound designed to interact with a structurally defined cluster targeting the c-Myc/VEGF quadrome ❑ PO	Preclin (ongoing 10/04)➢USA ❑ advanced solid tumors
Eisai	E7080 (ER-203492-00)	Synthetic highly potent novel orally active KDR tyrosine kinase inhibitor ❑ PO	Preclin (ongoing 10/04)➢Japan ❑ solid tumors
Epeius Biotechnologies ❑ BioFocus	Retroviral Expression Vectors Bearing Inhibitory Genes (Rexin-G)	Combination of a proprietary targeted vector system and a mutant cell-cycle control gene; accumulates in tumors and metastatic sites, and induces acute arrest of tumor growth and/or tumor regression ❑ IV, intra-arterial, intrahepatic	Phase I/II (begin 6/03)➢Philippines ❑ metastatic (Stage IV) pancreatic cancer

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EntreMed	p520, 547m	Peptide inhibitors of proteinase activated receptor-2 (PAr2) ❑ IV	Preclin (ongoing 5/04)➢USA ❑ solid tumors
Enzon Pharmaceuticals	SS1P, SS1(dsFv)-PE38, SS1-PE38	Immunotoxin composed of a single chain MAb targeting mesothelin, genetically engineered and linked to *Pseudomonas* exotoxin PE-38 ❑ IV	Phase II (planned 05)➢USA ❑ pancreatic cancer
GenVec ❑ Varian Medical Systems, Asahi Chemical Industry, BioReliance, Targeted Genetics	TNFerade	Proprietary replication-deficient adenoviral vector carrying the transgene encoding tumor necrosis factor (TNF) α, regulated by the radiation-inducible promoter Egr-1, a molecular switch that allows maximum gene expression and therapeutic protein secretion only when the target tissue is exposed to standard radiation therapy ❑ intratumoral, endoscopic ultrasound (EUS) or percutaneous injection	Phase II (begin 7/02, ongoing 1/04) ➢USA (combination) ❑ locally advanced pancreatic cancer, first line
GlycoGenesys ❑ Wayne State U, Barbara Ann Karmanos Cancer Institute	GCS-100 (formerly GBC-590)	A carbohydrate lectin inhibitor, which recognizes the galectin 3 receptor; disrupts cellular recognition ❑ IV	Phase IIa (begin 6/99, completed 7/01)➢USA ❑ refractory or relapsing pancreatic carcinoma
GlycoGenesys ❑ Wayne State U, Barbara Ann Karmanos Cancer Institute	GCS-100LE	Substantially ethanol-free formulation of GCS-100 ❑ IV	Phase I (begin 5/04)➢USA ❑ solid tumors
ImClone Systems	IMC-1121b (previously IMC-1CIIb, IMC-2C6, c-P1C11, IMC-1C11)	Fully human MAb directed against kinase insert domain-containing receptor (KDr) ❑ IV	Phase I (begin 1/05)➢USA ❑ advanced solid tumors
Immunomedics ❑ Center for Molecular Medicine and Immunology (CMMI)	Labetuzumab ❑ hMN-14, hCEA-Y-90, hCEA-I-131, 90Y-MN-14 ❑ CEA-Cide	Humanized MAb hMN-14 directed against carcinoembryonic antigen (CEA) being evaluated as either naked or labeled with yttrium-90- or idodine-131 ❑ IV	Phase I/II (begin 1/00, ongoing 1/04)➢Europe (Germany, Hungary, the Netherlands), USA (yttrium-90 label) ❑ advanced, refractory or metastatic pancreatic cancer
Immunomedics	IMMU-107	Humanized anti-MUC1 IgG (PAM4) MAb linked to yttrium 90 ❑ IV	IND (approved 04)➢USA (combination) ❑ pancreatic cancer
MedImmune ❑ Purdue Research Foundation	Anti-ephA2 MAb	Antibodies designed to interact with ephA2 that may both regulate tumor cell growth and prevent metastasis, while sparing normal cells ❑ injection	Preclin (ongoing 1/05)➢USA ❑ solid tumors
MedImmune	Anti-ephA4 MAb	Antibodies designed to interact with ephA4 that may both regulate tumor cell growth and prevent metastasis, while sparing normal cells ❑ injection	Preclin (ongoing 1/05)➢USA ❑ solid tumors
Oncolytics Biotech ❑ BioReliance, U Calgary, Institut Catala d'Oncologia, Alberta Cancer Board	Reolysin (formerly Reosyn)	Human reovirus that replicates specifically in tumor cells bearing an activated Ras pathway ❑ intralesional, intratumoral, SC, intracranial, intracerebral, intravesical, IV	Phase I (begin 5/04)➢Europe (UK) ❑ refractory, advanced or metastatic solid tumors
Panacea Pharmaceuticals ❑ Massachusetts Institute of Technology (MIT), Rhode Island Hospital, M. D. Anderson Cancer Center		MAb inhibitors of human aspartyl (asparaginyl) beta-hydroxylase (HAAH), a membrane-associated enzyme overexpressed by many malignant cells, associated with cancer-cell proliferation, motility, and invasiveness ❑ injection	Preclin (ongoing 10/04)➢USA ❑ solid tumors

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Peregrine Pharmaceuticals ❑ U Texas Southwestern Medical Center	2C3	MAb that blocks the action VEGFr2; may be used both as a potential targeting agent for the Vascular Targeting Agents (VTA) platform and also as a stand-alone antiangiogenesis therapeutic ❑ IV	Preclin (ongoing 10/04)➢USA ❑ solid tumors
Protein Design Labs ❑ Icos, U California San Diego	Volociximab ❑ Eos 200-4, M200	MAb against α5β1 integrin (AAB1), a member of the integrin family of proteins known to be involved in angiogenesis ❑ IV, PO	Phase I (begin 5/03, ongoing 9/04)➢USA ❑ advanced, refractory solid tumors
Protein Design Labs	F200	Derivative Fab fragment of MAb M200 ❑ IV, PO	Preclin (ongoing 1/05)➢USA ❑ solid tumors
Roche	Ro 28-2653, Ro28-2653	Pyrimidine-2,4,6-trione, is a novel synthetic inhibitor of matrix metalloproteinases (MMP) with high selectivity for MMP2, MMP9 and membrane type 1-MMP ❑ PO	Preclin (ongoing 10/03)➢USA ❑ solid tumors
Shionogi	MMI-166	N-sulfonyl amino acid derivative, third-generation MMP inhibitor, designed to selectively inhibit MMP-2, MMP-9, and MMP-14 ❑ PO	Preclin (ongoing 3/04)➢Japan ❑ solid tumors
Sugen ❑ Esteve SA, Taiho Pharmaceutical	SU6668, SU-6668, SU006668, TSU-68 (Japan)	Synthetic inhibitor of receptor tyrosine kinases (RTK) associated with various growth factors involved in tumor angiogenesis, including VEGF, fibroblast growth factor (FGF) and platelet derived growth factor (PDGF) ❑ IV, PO	Phase I (completed 11/04)➢USA ❑ advanced solid tumors
Threshold Pharmaceuticals ❑ German Cancer Research Centre, Baxter Oncology	Glufosfamide ❑ D-19575	Novel alkylating agent in which the active metabolite of isophosphoramide mustard is covalently linked to β-D-glucose to target the glucose transporter system and increase intracellular uptake in tumor cells ❑ IV	Phase II (begin 12/99, closed 5/01) ➢Europe; phase III (begin 9/04) ➢USA, Europe ❑ metastatic pancreatic cancer, first line
Twinstrand Therapeutics	TST10088	Cytotoxic prodrug, consisting of a catalytic toxin, targeting MMP2 in tumor cells ❑ IV	IND (filed 12/04)➢USA ❑ solid tumors
Wilex	WX-UK (WX-UK1)	Small molecule inhibitor of the urokinase-type plasminogen activator (uPA) system, which plays a key role in the progression and metastasis of various solid tumors ❑ PO	Phase Ib/IIa (begin 10/02, ongoing 10/04)➢Europe (Germany) ❑ advanced solid tumors
Wilex	WX-671	Orally available small molecule serine protease inhibitor, targeting the uPA system ❑ PO	Phase I (ongoing 10/04)➢Europe (Germany) ❑ metastatic solid tumors
Wilex ❑ VU U Medical Center	MAb K931	MAb targeting the Ep-CAM antigen which is widely expressed on solid tumors ❑ IV	Preclin (ongoing 10/04)➢Europe (Germany, The Netherlands) ❑ solid tumors
Xenova	XR303 (formerly KSB303)	Fully human MAb radiolabeled with iodine 131 for localized radioimmunotherapy of gastrointestinal malignancies, enabling higher deposited radiation dose to the solid tumor with minimized toxicity to normal tissues ❑ IV, locoregional	Phase I/II (begin 10/02, ongoing 9/04)➢Europe ❑ inoperable pancreatic cancer
YM BioSciences ❑ Centre of Molecular Immunology, Oncoscience	YM-1001 ❑ TheraCIM HR3, TheraCIM h-R3, TheraCIM hR3; OSAG101, h-R3, Theraloc	Radiolabeled humanized MAb directed at epidermal growth factor receptor (EGFr) ❑ IV	Phase II (begin 11/04)➢Europe ❑ metastatic pancreatic cancer

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), December 2004

E7080, under development by Eisai (Tokyo, Japan), is a synthetic, highly potent, novel, orally active tyrosine kinase inhibitor, targeting VEGFr2 (KDr). E7080 also prevents human umbilical vein endothelial cells (HUVEC) from responding to VEGF stimulation, such as phosphorylation of KDr, tube formation in three dimensional type I collagen gel, and proliferation, with cells being arrested in G1 phase, resulting in apoptosis in a dose-dependent manner. With respect to selectivity to other tyrosine kinases, E7080 inhibited fibroblast growth factor receptor 1 (FGFr1), but did not affect epidermal growth factor receptor (EGFr). E7080 also inhibited FGF2-induced proliferation and FGF2-induced tube formation of HUVEC. *In vivo*, E7080 was evaluated in a mouse dorsal air sac model using human pancreatic cancer cells KP-1 stably transfected with VEGFA. Oral administration of E7080 clearly reduced KP-1/VEGF-induced angiogenesis. It also reduced FGF2-stimulated angiogenesis. Growth of KP-1/VEGF was almost completely inhibited by the treatment with E7080. Retreatment with E7080 suppressed regrowth of KP-1/VEGF after cessation of treatment with E7080. These *in vitro* and *in vivo* antiangiogenic activities of E7080 were comparable or superior to those of other KDr tyrosine kinase inhibitors (Matsui J, etal, AACR03, Abs. 51).

IMC-1121b, under development by ImClone Systems (New York, NY), is a fully human MAb directed against KDr. By targeting and binding to KDr, IMC-1121b blocks VEGFA/KDr interaction, efficiently neutralizing VEGF-induced receptor activation and mitogenesis of human endothelial cells. IMC-1121b replaced IMC-1C11, which was chimerized, and IMC-1CIIb, IMC-2C6, and c-P1C11, which were discontinued.

In January 2005, ImClone Systems initiated a phase I clinical trial of IMC-1121b in patients with solid tumors. This 2-site trial, to be performed at Fox Chase Cancer Center (Philadelphia, PA) and the University of Colorado Health Sciences Center (Aurora, CO), was designed to examine the safety and pharmacology of IMC-1121b administered weekly by IV infusion. Expected enrollment is 33 patients.

Sorafenib, a novel bi-aryl urea, is a small molecule drug that inhibits tumor-cell proliferation by targeting the RAF/MEK/ERK signaling pathway at the level of RAF kinase and exerts an antiangiogenic effect by targeting receptor tyrosine kinases (RTK) VEGFr2 and PDGFr and their associated signaling cascades. The drug is being developed by Bayer and Onyx Pharmaceuticals (Richmond, CA).

Raf kinase represents the first step in the ras oncogene signaling pathway and is a significant contributor to the malignant phenotype driven by activated ras signaling. The Ras/Raf signaling pathway is an important mediator of tumor cell proliferation and angiogenesis. This pathway is often aberrantly activated in human tumors because of the presence of activated ras, mutant B-RAF, or elevation of growth factor receptors. Raf kinase inhibitors that eliminate Raf kinase function, reverse the Ras-transformed phenotype.

BAY 43-9006, is a potent inhibitor of both c-RAF and B-RAF (wildtype and V599E mutant) *in vitro*, and is active against several RTK involved in neovascularization and tumor progression such as VEGFr2, VEGFr3, PDGFrβ, Flt3, and c-KIT, along with p38α, a member of the MAPK family. In cellular mechanistic assays, BAY 43-9006 demonstrated inhibition of the MAPK pathway in colon, pancreatic, and breast lines expressing mutant k-ras, or wildtype, and/or mutant B-RAF. Potent inhibition of VEGFr2, VEGFr3, and PDGFrβ cellular receptor autophosphorylation was also observed. Broad-spectrum antitumor activity was observed in colon, pancreatic, breast, and lung xenograft models with once daily oral dosing of BAY 43-9006. BAY 43-9006 demonstrated dose-dependent inhibition of neovascularization (Wilhelm S, etal, AACR-NCI-EORTC03, Abs. A78; Clin Cancer Res, 1 Dec 2003;9(16).

In clinical trials, toxicities included anorexia, fatigue, alopecia, diarrhea, and mainly skin toxicity such as rash, hand and foot syndrome, folliculitis, and dryness of skin. Skin toxicity limited dose escalation and reduced dose intensity at the highest dose levels (600 and 800 mg). All toxicities were rapidly reversible. There was no myelosuppression.

A multicenter phase II clinical trial (protocol ID: UCCRC-13169B, NCI-6567) of sorafenib and gemcitabine in patients with locally advanced or metastatic PDAC was initiated in October 2004 in the USA to determine response rate, toxicity, 6-month survival, and overall survival for this regimen. Patients are administered oral sorafenib twice daily on days 1-28 and IV gemcitabine, over 30 minutes, on days 1, 8, and 15. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. A total of 12-35 patients will be accrued for this trial. Hedy Kindler, MD, of the University of Chicago Cancer Research Center is the PI.

SU6668, under development by Sugen (Pfizer), is a small molecule inhibitor of Flk-1/KDr, FGFr, and platelet-derived growth factor (PDGFr) RTK. Several phase I clinical trials have been completed in the USA and Japan, evaluating SU6668 in advanced solid tumors, mostly as an orally administered agent. Responses in these trials were exclusively represented by stable disease of about 6 months duration. SU6668 may prove more effective when used in combination with other anticancer agents, or as a radiosensitizer.

According to investigators at M. D. Anderson Cancer Center (Houston, TX), SU6668 inhibits tumor growth and induces endothelial cell apoptosis in an orthotopic model in which L3.6pl human pancreatic cancer cells are injected into the pancreas of nude mice. A decrease in tumor weight and volume was noted with increasing daily doses of IP SU6668. Endothelial cell apoptosis increased from an average of 0% in controls to 9% in the high dose group, corresponding to a 26% decrease in microvessel density. A

dose-dependent increase in tumor cell apoptosis ranging from 6.9% to 11.7% was also observed. Targeting KDr, FGFr, PDGFr with SU6668 increases endothelial cell apoptosis, and suggesting that VEGF, FGF, and PDGF, alone or in combination, serve as survival factor(s) for endothelial cells. The observed increase in tumor-cell apoptosis and decrease in tumor burden with SU6668 treatment is likely secondary to blockade of the above receptors and resultant angiogenesis inhibition (Takamori RK, etal, AACR02, Abs. 2611).

ZD6474, under development by AstraZeneca, is a novel, orally available inhibitor of VEGFr2 with additional activity against EGFr-1 RTK. Consistent, highly significant inhibition of subcutaneous tumor growth in a histologically diverse panel of human tumor models has been achieved with chronic once-daily administration of ZD6474.

ZD6474 was evaluated in a mouse model of metastatic PDAC. Nude mice were injected with L3.6pl human pancreatic cancer cells into the pancreas. Eight days after tumor cell injection, treatment was initiated with vehicle alone, IV gemcitabine, or oral ZD6474. Animals were culled 24 days after starting treatment. Compared with tumor size in controls (1231 mg), tumors reached a mean weight of 836 mg with gemcitabine and 541 mg with ZD6474. Lymph-node metastases occurred in 10/10 controls and 9/9 in gemcitabine-treated animals, but only 3/10 animals treated with ZD6474, while liver metastases occurred in 6/10 controls, in 4/9 gemcitabine-treated mice and 1/10 ZD6474-treated mice. In the proliferating areas at the periphery of the tumor, microvessel density and proliferation were significantly reduced by ZD6474. ZD6474 decreased primary pancreatic tumor growth and reduced both lymph-node and liver metastases compared with control or gemcitabine treatments in this model. Therefore, ZD6474 was more effective than gemcitabine in this model of human PDAC (Bruns C, etal, AACR03, Abs. 3041).

ZD6474 completed phase I clinical trials as monotherapy in advanced solid tumors and is in phase II clinical trials in various types of malignancies.

Urokinase Plasminogen Activator (uPA) System Inhibitors

Urokinase-type plasminogen activator (u-PA), the receptor u-PAr (CD87), and plasminogen activator inhibitor-1 (PAI-1) and PAI-2 comprise the urokinase plasminogen activator system, an extracellular enzyme system overexpressed on certain aggressive metastasizing solid tumors. The uPA system plays a key role in tumor metastasis of various solid tumors including breast, ovarian, gastric, colon, and pancreatic cancer. The uPA system enables tumor cells to degrade their surrounding tissue, i.e. the ECM, and invade into healthy tissue and blood vessels and, thus, migrate and form new tumors at distant sites. In addition, the uPA system plays an important role in promoting growth of primary tumors.

Urokinase plasminogen activator (uPA) is a serine protease similar to those in the blood coagulation cascade, shown to play an important role in the metastasis of certain solid tumors. It is a key initiator of ECM degradation, which precedes cell migration involved in tumor metastasis, angiogenesis, and restenosis.

Several approaches have been evaluated to target uPA to treat cancer, including inhibition of uPA synthesis, blocking the interaction of uPA with its receptor, uPAr, and interfering with its activity using small molecule inhibitors. Other approaches include transfer of genes into tumor cells to switch off uPA or uPAr gene expression, thus reducing invasive properties of these tumor cells, or using MAb to block interaction of uPA and uPAr to prevent tumor cell invasion. Also, a soluble form of uPAr, by scavenging uPA, limits proteolysis at the cancer cell surface, thereby inhibiting invasion and growth of cancer cells.

Because of the role of the uPA system in cancer progression, the European Organization for Research and Treatment of Cancer (EORTC) classified uPA and PAI-1 as prognostic markers for the highest level of evidence (LOE-1). Also, expression levels of uPA and PAI-1 are being used in diagnosis and disease monitoring.

WX-UK1, under development by Wilex (Munich, Germany), is a small molecule inhibitor of the uPA system.

A multicenter phase I clinical trial (protocol IDs: WX/50-003) of WX-UK1 in combination with capecitabine in advanced malignancies was initiated in late 2003 at Fox Chase Cancer Center under PI Lori Goldstein, MD. The trial's primary objectives are to determine MTD of four weekly infusions of WX-UK1 in combination with one 14-day cycle of capecitabine, and define the DLT of this regimen. Secondary objectives are to determine safety, tolerability, and pharmacokinetics of WX-UK1, capecitabine, and their principal metabolites in plasma and in urine; determine potential effects of WX-UK1 on biomarkers, including levels of PAP, D-dimer, uPA/PAI-1-complexes and soluble uPAr (suPAr) in plasma and suPAr and suPAr-D2D3 in urine; determine potential effects of WX-UK1 on tumor markers in blood; and describe any preliminary evidence of antitumor activity of this combination.

In October 2002, Wilex initiated a multicenter, dose-escalation, phase Ib/IIa clinical trial with WX-UK1, to evaluate tolerability and biological activity of WX-UK1 in patients with advanced gastric, pancreatic and ovarian cancer. The trial is being conducted in Germany under PI Christian Peschel, MD, at the Technical University of Munich. In phase Ib, patients with late stage malignancies for which currently no standard therapy exists, were treated for 4 weeks with WX-UK1 to determine MTD and pharmacokinetics. In the phase IIa part, 30 patients will be treated up to 12 weeks at MTD to evaluate and confirm the safety and tolerability of this dosing schedule. In addition, biologic activity of the substance will be analyzed in order to assess biologic markers in blood and urine, which might indicate effects of WK-UK1 on the tumor. Finally, a tumor

assessment will be carried out to evaluate changes in tumor growth.

Wilex is also developing a series of novel orally active serine protease inhibitors pharmacologically similar to WX-UK1, representing a distinct new class of chemicals. WX-682 is the lead compound in this series.

WX-671, under development by Wilex, is another orally available small molecule modulator of the urokinase plasminogen activator (uPA) system and other serine proteases. WX-671 is a second generation orally available serine protease inhibitor targeting the uPA system. In September 2004, Wilex started a 'first-in-man' open label, dose-escalation, phase I clinical trial with WX-671, to investigate the oral bioavailability, pharmacokinetics, and safety of this agent in 12 healthy male volunteers.

Other Antiangiogenesis Agents

CNTO 859, under development by Centocor (Malvern, PA), is a humanized MAb derived from TF8-5G9, a previously described mouse antihuman tissue factor (TF) MAb. TF is a cell-surface receptor that plays a critical role in hemostasis by acting as the primary initiator of the extrinsic clotting cascade.

Tissue factor is produced by certain tumors and is increased in both tumor-associated macrophages and blood monocytes (mTF) and in urine (uTF). TF is overexpressed on tumor cells and intratumoral endothelial cells in most major tumor types. Overexpression of TF has been observed to induce proangiogenic factors such as VEGF, and promote angiogenesis in experimental models. TF activity is thought to be a major contributor to thrombotic complications of cancer, and plays an important role in tumor growth, metastasis, and angiogenesis. Recently, the intracellular function of TF has been revealed to be involved in cancer invasion, independent of the blood coagulation pathway.

CNTO 859 binds specifically to TF on the surface of cells in the presence or absence of Factor VIIa. This prevents the binding and catalytic conversion of Factor X to Factor Xa and, therefore, downstream production of thrombin and fibrin. Inhibition of TF has been shown to reduce experimental metastasis.

CNTO 859 was shown to significantly inhibit tumor growth in two xenograft tumor models using MDA-MB-231 human breast carcinoma cells and BxPC3 PDAC cells. CNTO 859 significantly inhibited tumor growth of BxPC3 PDAC cells; treatment starting on day 1 following tumor cell implantation resulted in 47% inhibition of tumor growth relative to control animals. Results reflected MAb binding to human TF on the implanted tumor cells and not to host vasculature (Tawadros R, etal, AACR-NCI-EORTC03, Abs. B230).

Proteinase-activated receptor-2 (PAr2) inhibitor 547m, under development by EntreMed (Rockville, MD), is a peptidomimetics that blocks PAr2 signaling. Peptidomimetic antagonists, including 547m, were designed using structure of p520, a peptide antagonist of PAr2, as a template. These peptomimetics exhibited a ten-fold increase in antitumor activity when compared to the parental p520 peptide. *In vivo* studies demonstrate that this small molecule mimetic is significantly more potent than the peptide. These antagonists of PAr2 signaling are specific for the PAr2, with no inhibitory activity against other G-protein coupled receptors (GPCr), including PAr1 and ATP receptors.

These novel PAr2 antagonists were potent inhibitors of angiogenesis in the mouse Matrigel angiogenesis model, demonstrating dose-dependent inhibition of greater than 75% at the highest doses tested (Hembrough TA, etal, ASH03, Abs. 1946).

Thalidomide analogs, under development by Celgene (Warren, NJ), are represented by two distinct groups of compounds, SelCID, which are phosphodiesterase (PDE) type IV inhibitors, and IMiD, with unknown mechanism(s) of action. These thalidomide analogs exhibit enhanced activity compared to the parent compound against solid tumors, in which caspase-dependent apoptosis is associated with altered expression of bcl-2 family proteins.

Investigators at St. George's Hospital Medical School, Tooting (London UK) found that SelCID-3, one of the SelCID analogs, was consistently effective at reducing tumor cell viability in a variety of solid tumor cell lines, but had no effect on normal cells. Antitumor activity was independent of known PDE4 inhibitory activity and did not involve cAMP elevation. Growth arrest was preceded by early induction of G2/M cell cycle arrest, which led to caspase 3-mediated apoptosis. This was associated with increased expression of proapoptotic proteins and decreased expression of antiapoptotic bcl-2. Furthermore, extensive apoptosis *in vivo* was detected during SelCID-3-mediated inhibition of tumor growth in mice. These results suggest that SelCID-3 represents a novel antitumor agent distinct from thalidomide, and from previously characterized analogs with therapeutic potential against a range of solid tumors. This effect appears to be mediated via alterations in the expression of bcl-2 family proteins (Marriott JB, etal, Cancer Res, 1 Feb 2003;63(3):593-9).

Volociximab (M200), under development by Protein Design Labs (PDL; Freemont, CA), is a MAb against a5b1 integrin, a member of the integrin family of proteins involved in angiogenesis. This integrin is functionally downstream to the activities of highly redundant angiogenic growth factors secreted by tumors, and is likely a final common pathway necessary for angiogenesis. A derivative of M200, F200, a MAb fragment is also under development by PDL.

The mechanism of action of M200 is distinct from other antiangiogenic candidates in clinical trials. M200 targets a $\alpha 5\beta 1$ integrin expressed on the surface of activated endothelial cells, and plays a critical role in angiogenesis.

By functionally blocking the interaction between the integrin and the ECM, M200 prevents two key steps in the angiogenic process and, subsequently, induces apoptosis in these same activated cells.

M200, and its derivative Fab F200, inhibit binding in an angiogenesis model, of fibronectin to α5β1 with similar potencies. *In vitro*, both M200 and F200 significantly inhibited VEGF- and bFGF-dependent tube formation. M200 inhibited human umbilical vein endothelial cell (HUVEC) proliferation more effectively than an anti-VEGF MAb. M200 exerted novel cytotoxic effects in addition to recapitulating the antiproliferative properties of anti-VEGF. M200 selectively induced apoptosis in proliferating, but not senescent, endothelial cells (Ramakrishnan V, etal ASCO04, Abs. 3187).

A phase I clinical trial, initiated in May 2003 at the University of Wisconsin Comprehensive Cancer Center (Madison, WI) and Brooks Army Medical Center (San Antonio, TX), is administering escalating doses of M200 to patients with refractory solid tumors. M200 is infused over 1 hour on days 1, 15, 22, 29, and 36, at a dose of 0.5, 1, 2.5, 5, 10, or 15 mg/kg. Trial endpoints include determination of MTD, DLT, safety profile, immunogenicity, pharmacokinetics, and monocyte saturation. As of September 2004, 16 patients had been enrolled and 15 treated with M200. No DLT was observed with doses of 0.5 mg/kg administered to 1 patient, 1 mg/kg to 2, 2.5 mg/kg to 3, 5 mg/kg to 3, and 10 mg/kg to 6. Adverse events possibly related to M200 were mild-to-moderate nausea (n=5), fever (n=2), vomiting (n=2), headache (n=2), anorexia (n=2), and asthenia (n=2). There were no infusion reactions; 2/16 patients developed human antichimeric antibodies (HACA), which was not associated with any apparent adverse effects. Disease stabilized in 9 patients and progressed in 6. Disease stabilized in 5/6 patients treated at a dose of 10 mg/kg. The 10 mg/kg dose, administered every 2 weeks, was well tolerated achieving monocyte saturation and, therefore, is the recommended dose regimen for subsequent clinical trials. However, because DLT was not observed, dose escalation is continuing with additional patients being treated at a dose of 15 mg/kg (Ricart A, etal, EORTC-NCI-AACR04, Abs. 166).

Other Antimetastatic Agents

Anti-ephA2 and anti-ephA4 MAb, under development by MedImmune (Gaithersburg, MD), are agonists that inhibit malignant behavior by interacting with EphA2 (Carles-Kinch K, etal, Cancer Res May 2002;62(10):2840-7), or EphA4 RTK.

Eph receptors comprise the largest family of tyrosine kinases. Together with their membrane bound ligands, ephrins, they are involved in normal embryonic development and blood vessel morphogenesis. Eph RTK and their ligands are critical determinants of embryonic patterning, neuronal targeting, and vascular assembly. They are involved in vascular assembly, angiogenesis, tumorigenesis, and metastasis. Elevated expression of Eph receptors and ephrin ligands is associated with tumors and tumor vasculature, suggesting that they play critical roles in tumor growth and angiogenesis (Cheng N, etal, Cytokine Growth Factor Rev, Feb 2002;13(1):75-85). Based on the identity of their ligands, Eph receptors have been divided into two distinct subfamilies, EphA and EphB.

EphA2 is a transmembrane RTK that is upregulated on many aggressive carcinoma cells. Significant changes in subcellular localization and function of EphA2 are linked with cancer. Despite being overexpressed in cancer cells, unstable cancer cell-cell contacts prevent EphA2 on malignant cells from binding its ligand, ephrinA1, anchored to the membrane of adjacent cells. Although, unlike other receptor kinases, EphA2 demonstrates kinase activity that is independent of ligand binding, ligand binding causes EphA2 to negatively regulate tumor cell growth and migration, and also induce EphA2 degradation. On the basis of these findings, EphA2 on tumor cells is being targeted with agonist MAb, which mimic the consequences of ligand binding. MAb targeting of EphA2 decreases tumor-cell growth, as measured using xenograft tumor models.

Investigators at Purdue University Cancer Center (West Lafayette, IN), using a novel approach to preserve extracellular epitopes and optimize antibody diversity, generated agonist MAb that identify epitopes on the extracellular domain of EphA2. These EphA2 MAb selectively bind epitopes on malignant cells, which are not available on normal epithelial cells, inhibiting activities unique to metastatic cells while minimizing damage to nontransformed cells. These epitopes arise from differential cell-cell adhesions. Stable intercellular junctions of normal epithelial cells occlude the binding site for the ligand, as well as this subset of EphA2 MAb. These MAb-binding sites on EphA2 are selectively available on cancer cells, but are not accessible on normal cells (Coffman KT, etal, Cancer Res, 15 Nov 2003;63(22):7907-12).

In October 2001, MedImmune licensed worldwide rights to ephA2 technology from Purdue Research Foundation at Purdue University. MedImmune is responsible for developing, manufacturing and commercializing therapeutics that target ephA2. As a part of the agreement, Purdue will receive certain upfront payments and future milestones and royalties.

AZM475271, under development by AstraZeneca, is a novel, orally available Src kinase inhibitor. Src inhibition by AZM475271, either alone or in combination with gemcitabine, demonstrated significant antitumor and antimetastatic activity in an orthotopic nude mouse model of human pancreatic cancer. The combination of AZM475271 with gemcitabine also sensitized tumor cells to the cytotoxic effect of gemcitabine.

AZM475271 is a potent inhibitor of L3.6pl tumor cell migration at submicromolar dose levels *in vitro*. In L3.6pl human pancreatic cancer cells, AZM475271 was shown to significantly inhibit migration of L3.6pl tumor cells; however, significantly higher doses were needed to inhibit tumor

Exhibit 2
Selected Targets of Antiangiogenesis Inhibitors/Antimetastasis Agents and Drug Delivery/Gene Transfer Approaches for Treatment of PDAC

Primary Developer	Generic Name or Number or Brand Name	Target
Immunomedics	Labetuzumab	Carcinoembryonic antigen (CEA)
Xenova	XR303	CEA
Wilex	MAb K931	Ep-CAM
MedImmune	Agonistic MAb	EphA2 or ephA4 RTK
YM BioSciences	TheraCIM/Theraloc	Epidermal growth factor (EGF) receptor (EGFr)
GlycoGenesys	GCS-100 (formerly GBC-590) and GCS-100LE	Galectin 3 receptor
Panacea Pharmaceuticals	MAb inhibitors	Human aspartyl (asparaginyl) beta-hydroxylase (HAAH)
Protein Design Labs (PDL)	Volociximab and F200	Integrin $\alpha5\beta1$
Roche	Ro 28-2653	Matrix metalloproteinases (MMP)
Shionogi	MMI-166	MMP-2, MMP-9, and MMP-14
Twinstrand Therapeutics	TST10088	MMP-2
Enzon Pharmaceuticals	SS1P	Mesothelin
Immunomedics	IMMU-107	MUC1
Antisoma	AS1406	MUC1
EntreMed	547m	Proteinase activated receptor-2 (PAr2)
Oncolytics Biotech	Reolysin	Ras
AEterna Zentaris	AN-238 and AN-162	Somatostatin
Centocor	CNTO 859	Tissue factor (TF)
Wilex	WX-671	Urokinase plasminogen activator (uPA) system
Wilex	WX-UK (WX-UK1)	uPA system
AstraZeneca	ZD6474, AZD6474	Vascular endothelial growth factor receptor 2 (VEGFr2)
Sugen	SU6668	VEGFr2; fibroblast growth factor receptor (FGFr) 1; platelet-derived growth factor receptor-β (PDGFrβ)
Eisai	E7080	VEGFr2
Peregrine Pharmaceuticals	2C3	VEGFr2
ImClone Systems	IMC-1121b	VEGFr2
AstraZeneca	ZD6474, AZD6474	VEGFr2; EGFr
Cylene Pharmaceuticals	CX-3543	VEGF; c-myc
Bayer	Sorafenib (BAY 43-9006)	VEGFr; raf-1 kinase; RAF/MEK/ERK signaling pathway
Cephalon	CEP-7055	VEGFr1;VEGFr2;VEGFr3

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), December 2004

cell proliferation. In contrast to observations *in vitro*, there was an antiproliferative component of Src kinase inhibition *in vivo*. In an orthotopic setting, administration of oral AZM475271 in nude mice injected with L3.6pl tumor cells into the pancreas resulted in a small, but significant effect represented by a ~40% inhibition of primary tumor growth, compared to controls. AZM475271 inhibited the growth of the primary tumor and, in addition, there were no liver metastases apparent in AZM475271-treated animals, although such metastases were seen at sacrifice in 60% of mice treated with vehicle alone. AZM475271 did not have a significant effect on tumor vasculature density, indicating that antitumor effects were unlikely to be attributable to inhibition of tumor angiogenesis. These results are consistent with an involvement of Src kinase signaling pathways in tumor-cell adhesion and migration phenotypic changes. They also support the concept that Src kinase inhibitors may have potential as anti-

invasive agents for clinical development (Yezhelyev M, etal, AACR03, Abs. R1718).

Investigators at AstraZeneca and the University of Munich-Großhadern LMU (Munich, Germany) observed a synergistic effect of AZM475271 and gemcitabine in human pancreatic cancer cells *in vitro* and *in vivo,* growing orthotopically in nude mice. AZM475271, alone or in combination with gemcitabine, also completely inhibited development of liver metastases *in vivo*, suggesting that Src kinase inhibitors may have potential as anti-invasive agents in the clinical setting. In this experiment, mice were treated with AZM475271 by oral feeding alone or in combination with IP injection of gemcitabine. Although in sacrificed animals pancreatic tumor weight was reduced by monotherapy with AZM475271 or gemcitabine, compared with untreated controls, the largest reduction was observed in combination groups indicating synergism between the two agents. Also, in contrast to untreated controls, no liver metastases were detected following therapy with AZM475271 alone or in combination with gemcitabine. *In vitro* studies also showed that AZM475271 and gemcitabine had synergistic activity in L3.6pl cells. Although the percentage of apoptotic tumor cells was increased after treatment with AZM475271 or gemcitabine compared with untreated controls, it was considerably greater after treatment with the combination compared with either treatment alone. The number of proliferating cells was reduced in tumors treated with the combination compared with untreated controls (Bruns CJ, etal, AACR-NCI-EORTC03, Abs. B198).

GCS100, and GCS100LE, under development by GlycoGenesys (Boston, MA), are complex carbohydrates that modulate cell processes such as cell-cell adhesion, cell migration, and proliferation, through interference with carbohydrate-binding receptors such as galectin-3. GCS-100 is an antiadhesive that disrupts cellular recognition processes. It binds lectins on cancer cells, thus blocking the binding site and preventing individual cancer cells from metastasizing. Based on bioassays and animal models, GCS-100 acts by multiple modes of action, affecting apoptosis via mitochondrial depolarization, antiangiogenesis by interfering with VEGF from binding to endothelial cells, and metastasis via interaction with galectin-3.

GCS-100, when administered as a single agent, produced a significant response in the PANC-1 human pancreatic tumor cell model, with 44% of the mice in the GCS-100 group surviving the 90-day experiment, and 33% demonstrating a complete eradication of the tumor. No mice in the control groups that were administered saline solution or gemcitabine survived past 43 days, with an MST of <30 days.

The safety and efficacy of GCS-100 was investigated in 13 patients (locally advanced tumors=4, metastatic=9) with advanced PDAC (one prior treatment=12, untreated =1) in a multicenter phase IIa clinical trial, initiated in April 2000 at Beth Israel Deaconess Medical Center (Boston, MA), under PI Keith E. Stuart, MD. Other participating centers included the University of Chicago Medical Center, and the University of Rochester Cancer Center in New York under PI Corliss Newman, MD. Treatment consisted of IV GCS-100 (20 mg/m^2) over 3 hours twice weekly. CT scans were performed every 4 weeks. Patients were treated with a median of 7 doses (range=2 to 10). Toxicity was minimal, with the most common adverse event being progressive fatigue. There was no gastrointestinal toxicity and no alopecia; 3 patients experienced asymptomatic drops in diffusing lung capacity for carbon monoxide (DLCO), requiring dose adjustments; 2 discontinued treatment, 1 because of hyperbilirubinemia. Disease stabilized in 1 patient after the first month of treatment, and progressed in 9 patients. Although GCS-100 was well tolerated in this setting, it did not demonstrate antitumor efficacy at the dose tested (Stuart KE, etal, ASCO01, abs. 2312:140b). Enrollment in this phase IIa clinical trial was completed in July 2001, after 20 patients entered the trial.

GCS-100LE is a substantially ethanol-free formulation of GCS-100. Preclinical and toxicology data support dose escalations above the 80 mg/m^2 reached with GCS-100 in previous clinical trials, as well as the use of a daily dosing regimen to potentially achieve the maximal biological effect in humans. Therefore, GlycoGenesys developed the GVS-100LE formulation of GCS-100 to avoid potential ethanol-related adverse events at higher dose levels in future trials. Furthermore, this ethanol removal may have increased GCS-100's potential for development as an anticancer therapeutic administered alone or in combination with a variety of other anticancer agents.

In May 2004, GlycoGenesys initiated a dose-escalation phase I clinical trial of GCS-100LE in the treatment of various solid tumors, at Sharp Clinical Oncology Research (San Diego, CA), under PI Charles Redfern, MD. This phase I trial has been designed to evaluate the safety, pharmacokinetics, and MTD of GCS-100LE in patients with advanced disease. The trial's primary objective is to establish MTD of GCS-100LE, or the recommended dose for phase II trials, in this setting. Secondary objectives are to observe patients for any evidence of antitumor activity, and investigate the pharmacokinetics of GCS- 100LE. Up to 30 patients are expected to enroll in the trial, 3 to 6 patients per dose level, until MTD is reached. Each treatment cycle consists of 5 daily infusions of GCS- 100LE followed by two weeks rest. Cycles are repeated until disease progression or unacceptable toxicity.

Human aspartyl (asparaginyl) β-hydroxylase (HAAH) inhibitors, under development by Panacea Pharmaceuticals (Gaithersburg, MD), are recombinant human antibody fragments targeting the catalytic domain of HAAH (Yeung A, etal, AACR-NCI-EORTC03, Abs. B244). These constructs were engineered by Panacea, in collaboration with scientists at the Massachusetts Institute of Technology (MIT; Cambridge, MA).

HAAH, encoded by the ASPH gene, is a highly conserved enzyme that hydroxylates EGF-like domains in transformation-associated proteins involved in either cellular signaling such as notch, or cell/ECM interactions such as tenascin. HAAH overexpression has been detected in primary tumor tissue of pancreatic, breast, ovarian, liver, colon, prostate, lung, brain, and bile duct cancer. Most normal tissues, including brain, exhibit low or undetectable levels of this protein. According to recent findings overexpression of HAAH is sufficient to induce cellular transformation, increase cell motility and invasiveness, and establish tumor formation *in vivo*. When certain neuronal cells are transfected with the gene for HAAH, and the enzyme is overexpressed, transfected cells go through malignant transformation and act like cancer cells. When these transfected cells are injected into mice, tumors form at the injection site.

HAAH is normally localized to the endoplasmic reticulum. However, upon cellular transformation, it is translocated to the cell surfaces so HAAH in tumor cells is localized to the cell surface. Even a partial inhibition of HAAH expression, or its activity, restores normal cellular phenotype in relation to cellular proliferation and morphology. HAAH overexpression tumor cells and its functional relevance for tumorigenesis, growth, and metastasis represent an important and novel target for cancer therapy and diagnosis.

MAb K931, under development by Wilex, is a chimeric MAb targeting the epithelial cell adhesion molecule (EpCam) widely expressed on solid tumors. In preclinical studies, MAb K931 was shown to be a very promising drug candidate for the treatment of various malignancies.

EpCam is an antigen present on the surface of most normal epithelia. However, in intact epithelia, EpCam is shielded by a basement membrane, and is engaged in cell adhesion (Mol Cell Biol 2001;21:2570-80). 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 types of human adenocarcinoma, and is present in all stages of tumor development, from primary tumor, residual disease to metastasis.

In May 2003, Wilex acquired an exclusive license from VU University Medical Center (VUmc; Amsterdam, The Netherlands) for MAb K931 for the treatment of various solid tumors. Under the agreement, Wilex received full exclusive licenses to both the chimeric and the murine K931 antibody developed by VUmc, and to the cell lines producing the antibody in return for undisclosed upfront, milestone and royalty payments.

Targeted Drug Delivery

One important application of oncology-related biomarkers is as delivery systems to target various toxic substances to cancer cells. Among agents targeted to cancer cells are toxins, cytotoxic drugs, radioisotopes, oncolytic viruses, and other approaches that may confer certain desirable characteristics to cancer cells to sensitize them to certain treatments, make them more vulnerable to the host immune system, etc. In addition, pretargeting approaches further enhance delivery of toxic substances to cancer cells, sparing healthy tissues.

Targeted Toxins

Targeted toxins use a delivery approach combining a targeting molecule with a toxin. Toxins being evaluated in the treatment of cancer include bacterial toxins such as diphtherial toxin (DT) or *Pseudomonas* exotoxin (PE), or plant toxins such as ricin A or saporin. A very effective approach is the development of immunotoxins, using MAb to target toxins to cancer cells. Such an immunotoxin is the commercialized antileukemic drug gemtuzumab ozogamicin (Mylotarg; Wyeth), a recombinant humanized anti-CD33 MAb hP67.6, conjugated to the toxic antibiotic calicheamicin.

AS1406 (formerly Theranase), under development by Antisoma (London, UK), is humanized HMFG1 (huHMFG1) MAb fragment recognizing polymorphic epithelial mucin (PEM), also known as MUC 1, combined in a single fusion molecule with the enzyme RNase. HMFG1 is a murine MAb isolated in response to human milk fat globule, a component of human milk. RNase is a cytotoxic endoribonuclease with specificity for transfer RNA (tRNA).

SS1P [SS1(dsFv)-PE38, SS1-PE38], under development by Enzon Pharmaceuticals (Bridgewater, NJ), is an immunotoxin composed of a single chain MAb targeting mesothelin, genetically engineered, and linked to *Pseudomonas* exotoxin (PE)-38.

The native PE exotoxin protein consists of three domains, domain I targets cell surface receptors, domain II translocates the molecule across the cell membrane and into the cell interior, and domain III catalyzes irreversible ADP ribosylation and inactivation of elongation factor 2 (EF2), shutting down protein synthesis and leading to cell death. A single PE molecule can kill a cell. In the SS1P molecule, domain I is replaced by the disulfide-linked antibody fragment targeting mesothelin. Thus, SS1P targets the highly lethal toxin only to cells expressing mesothelin. Mesothelin is highly expressed in the majority of PDAC, and not in benign pancreatic diseases (see FO pp 1653).

In November 2003, Enzon entered into a collaborative development program with the National Institutes of Health (NIH), under a Collaborative Research and Development Agreement (CRADA), to develop SS1P in pancreatic and ovarian cancer. The CRADA initial period is for 3 years with an option to extend. In addition to the clinical development of SS1P the CRADA also includes a research project to further optimize SS1P using Enzon's proprietary macromolecular engineering technologies. A phase II clinical trial of SS1(dsFv)-PE38 is being planned in patients with pancreatic cancer, in collaboration with the NCI.

TST10088 and TST1000, in development by Twinstrand Therapeutics (Burnaby, BC, Canada), are prodrugs consisting of a catalytic toxin, targeting matrix metalloproteinase 2 (MMP2) in tumor cells. Twinstrand's therapeutic technology is based on derivatives of the plant toxins known as class II ribosome inhibiting proteins. Twinstrand targets these toxins to diseased cells and tissues by engineering short peptide 'switches' into the molecules to transform them into latent or inactive precursors or prodrugs. These switches are triggered by proteases that are specifically associated with a diseased cell. Therefore, the prodrug is activated by the appropriate protease resulting, in turn, in the death of the diseased cell. In essence, the diseased cell triggers its own destruction. In the treatment of cancer, Twinstrand prodrugs can be tailored or reprogrammed through simple changes to the peptide switch, to be activated by different proteases associated with different diseases.

Twinstrand submitted an IND to the FDA in December 2004 with the objective of initiating a phase I safety trial of TST10088 in solid tumors in early 2005.

Radioimunoconjugates

Various targeting molecules may also be conjugated to radioactive sources to either image tumors *in vivo* or selectively kill cancer cells. Radioimmunotherapy (RIT) refers to anticancer treatment with agents linking a MAb to a radioisotope.

IMMU-107, under development by Immunomedics (Morris Plains, NJ) consists of humanized yttrium-90 (^{90}Y)-labeled MAb PAM4 that is reactive with MUC1 expressed by >85% of human PDAC. Significant antitumor effects have been demonstrated using radiolabeled PAM4 as RIT in experimental pancreatic cancer.

Combined chemoimmunotherapy and RIT using gemcitabine and low dose ^{90}Y-PAM4 provided significantly increased antitumor efficacy than was observed for either treatment alone. Importantly, the enhanced antitumor efficacy was achieved with minimal toxicity to normal tissues. Athymic nude mice bearing CaPan1 human pancreatic cancer xenografts were administered gemcitabine with concurrent ^{90}Y-DOTA-cPAM4 on day 0. A second group of mice were treated with a second cycle of treatment 5 weeks after the start of the first cycle. Control groups of mice included those treated with either treatment arm alone, a combined modality treatment using a nontargeting control antibody, and an untreated group. Gemcitabine administered as a single agent did not elicit an antitumor effect. A single cycle of the combined ^{90}Y-DOTA-cPAM4 and gemcitabine resulted in a greater inhibition of tumor growth than was observed for any other treatment approach, with tumor growth delayed for 7 weeks. Two cycles of gemcitabine with concomitant 90Y-DOTA-cPAM4 yielded significant tumor regression and increased MST to 21 weeks versus 12 weeks for mice treated with a single cycle of therapy. Median tumor volume doubling-times were 18 weeks in mice treated with 2 cycles of therapy, compared to 7 weeks in mice treated with only 1 cycle, and 3.5 weeks for the group treated with 2 cycles of gemcitabine concomitant with an equitoxic nontargeting ^{90}Y–labeled agent. Therefore, addition of ^{90}Y-DOTA-cPAM4 to a gemcitabine treatment regimen may provide enhanced antitumor efficacy for the treatment of pancreatic cancer (Gold DV, etal, Clin Cancer Res, 1 Sep 2003;9(10 Pt 2):3929S-37S, and Int J Cancer, 20 Apr 2004;109(4):618-26).

In January 2004, Inmmunomedics was granted an IND by the FDA to initiate a multicenter phase I/II clinical trial of ^{90}Y-hPAM4 in pancreatic cancer. Subsequently, a dose-escalation phase I clinical trial with IMMU-107 was initiated at one cancer center, to be expanded to other institutions. This trial will evaluate IMMU-107 as monotherapy before an evaluation in combination with gemcitabine is undertaken.

Labetuzumab, under development by Immunomedics, in collaboration with the Center for Molecular Medicine and Immunology (CMMI; Belleville, NJ) is humanized MAb hMN-14 directed against carcinoembryonic antigen (CEA), being evaluated as either naked or labeled with ^{90}Y or iodine-131 (131I). Immunomedics obtained orphan drug status for labetuzumab for the treatment of PDAC in December 1998. It is estimated that more than 80% of PDAC express CEA.

The phase I portion of a multicenter (n=8), multinational, open label, dose-escalation, phase I/II clinical trial (protocol ID: IM-T-hMN14-03) of ^{90}Y-hMN14, administered as a single dose, initiated in January 2000 to treat patients with refractory advanced or metastatic pancreatic cancer, was completed and results were reported in June 2004 at the Society of Nuclear Medicine (SNM) meeting, in Seattle, WA. Treatment with labetuzumab achieved tumor targeting in the 15 to 18 patients enrolled in the trial, delivering acceptable normal organ radiation doses. MTD for a single administration was 25 mCi/m^2. Bone marrow suppression was the expected DLT.

A multicenter (n=8), multinational, open label, phase I/II clinical trial (protocol ID: IM-T-hMN14-03) of ^{90}Y-hMN14, administered as a single dose, was initiated in January 2000, to treat patients with refractory advanced or metastatic pancreatic cancer. Expected total enrollment is 75 patients. William Wegener, MD, of Immunomedics is Study Chair. Institutions participating in this trial include Hoag Cancer Center (Newport Beach, CA), Washington Hospital Center, (Washington, DC), Bay Pines VA Medical Center (St. Petersburg, FL), University of Pittsburgh Cancer Institute (Pittsburgh, PA), Virginia Mason Medical Center (Seattle, WA), Universitaetsklinikum Leipzig and Medizinische Fakultaet der Charité Berlin, in Germany, Semmelweis University (Budapest, Hungary), Medical University of Szeged, in Hungary and Academic Medical Center (Amsterdam, Netherlands).

A phase I/II trial (protocol IDs: CMMI-C-033-98, NCI-H99-0042, NCI-V99-1571) was initiated in March 1997 to assess the effectiveness of ^{90}Y-hMN14 (CEA-Cide) plus autologous peripheral blood stem cell transplantation (PBSCT) in treating patients with metastatic or recurrent pancreatic cancer that have not responded to previous treatment; a total of 24-30 patients were to be accrued for this trial. This trial was completed in October 2004. Participating institutions included Garden State Cancer Center (Belleville, NJ), St. Joseph's Hospital and Medical Center (Paterson, NJ), University of Pennsylvania Cancer Center (Philadelphia, PA) under Study Chair Jack D. Burton, MD, of the Garden State Cancer Center.

XR303 (formerly KSB303), under development by Xenova (Slough, Berkshire, UK), is a fully human MAb radiolabeled with ^{131}I for localized RIT of gastrointestinal malignancies. In May 2003, XR303 was awarded orphan drug status by the FDA and the European Commission for the treatment of pancreatic cancer.

A dose-escalation phase I/II clinical trial of XR303 was initiated in October 2002 in patients with inoperable pancreatic cancer. The trial has been designed to assess whether locoregional administration of XR303 results in improved efficacy and reduced systemic effects. This trial is expected to be completed in 2005.

YM-1001, trademarked TheraCIM hR3 in the USA and Theraloc in Europe, is a humanized MAb that targets EGFr, under development by YM Biosciences (Mississauga, Ontario, Canada). YM BioSciences obtained the license for TheraCIM from the Centre of Molecular Immunology (CIM; Havana, Cuba). TheraCIM blocks EGFr, thereby preventing EGF and TGFα from binding to their receptors, achieving either direct inhibition of cell growth or, possibly, cell destruction by the immune system. The drug may also be a radiosensitizer. According to results from phase II clinical trials in head and neck cancer, the drug doubles response to radiation treatment. It has also demonstrated a superior side effect profile to functionally equivalent already approved EGFr-targeted MAb-based agents.

In November, 2004, Oncoscience (Wedel, Germany), the European licensee of Theraloc, initiated a multicenter (n=6) rolling phase I/II trial in Germany with the drug in patients with metastatic pancreatic cancer. The trial is to enroll 60 patients with metastatic PDAC refractory to first line treatment with chemotherapy. Initially, 30 patients will be enrolled after which, subject to the achievement of a predetermined response rate, an additional 30 patients would be enrolled if needed. The results of the first portion of the trial are anticipated by the third quarter of 2005.

Targeted Cytotoxics

AN-238 and AN-162, under development by AEterna Zentaris (Quebec City, Canada), are synthesized targeted cytotoxics consisting of 2-pyrrolinodoxorubicin (AN-201), or doxorubicin, respectively, linked to octapeptide analog RC-121. Octapeptide analogs bind with high affinity to certain somatostatin receptors (SSTr) expressed in various human neoplasms. AN-238 and AN-162 are in development for treatment of endocrine pancreatic tumors (EPT), not PDAC. Somatostatin analogs are well established in the treatment of EPT.

Cytotoxic somatostatin analog AN-238 efficaciously inhibits growth of human breast or prostate cancer expressing SSTr2 and SSTr5 and can be used for receptor-targeted chemotherapy in the treatment of pancreatic, colorectal, and gastric cancer, as well as brain tumors and nsclc.

When expression of SSTr was examined on tumor cells and in intratumoral vessels in 28 tumor tissues from malignant EPT, SSTr2 and SSTr4 stained positive in 90% and SSTr1 in 70% of the tumor tissues, while SSTr3 and SSTr5 stained positive in only 50% of tumor tissues. However, tumors belonging to the same subgroup of EPT showed a variable expression of receptor subtypes. No differences in receptor-subtype expression could be seen between poorly and well differentiated tumors, or between primary tumors and metastases. These differences indicate the importance of determining each tumor's subset of receptors before treatment with receptor-subtype-specific analogs is initiated (Fjallskog LM, etal, Med Oncol, Feb 2003;20(1):59-68).

In vitro tests on human gastric cancer cell line MKN-45, breast cancer cell line MDA-MB-231, prostate cancer cell line PC-3, and pancreatic cancer cell line MIA PaCa, demonstrated that the cytotoxic radicals in the AN-238 and AN-162 conjugates retained their antiproliferative activity. When SST-like activities of AN-238 and its carrier, RC-121, were compared in the rat pituitary superfusion system, both compounds were found to suppress a stimulated growth hormone release at nanomolar concentrations. Preliminary studies in animal models of breast and prostate cancers showed that AN-238 is less toxic than AN-201 and more potent in inhibiting tumor growth (Nagy A, etal, PNAS USA, 17 Feb 1998, 95(4):1794-9).

Glufosfamide, under development by Threshold Pharmaceuticals (Redwood City, CA), is a novel alkylating agent in which the active metabolite of isophosphoramide mustard is covalently linked to β-D-glucose to target the glucose transporter system, thus increasing intracellular uptake in tumor cells. Cellular uptake of glufosfamide is mediated by this sodium-dependent transmembrane transporter protein of glucose and, possibly, also by other transporter proteins. Together with the elevated glucose use by tumor cells, this targeting mechanism probably contributes to the selectivity of glufosfamide.

In a phase I clinical trial with glufosfamide, there was clear evidence of antitumor activity, with a long-lasting CR in a patient with advanced PDAC. The principal toxicity of a 6-hour infusion of glufosfamide at MTD (6 g/m^2) was reversible renal tubular acidosis; the recommended phase II dose is 4.5 g/m^2. Close monitoring of serum potassium and creatinine levels is suggested for early

detection of possible renal toxicity (Briasoulis E, etal, J Clin Oncol 2000 Oct 15;18(20):3535-44).

In September 2004, Threshold received a Special Protocol Assessment (SPA) from the FDA for a pivotal phase III clinical trial to evaluate glufosfamide in patients with metastatic PDAC refractory to first line treatment. The primary endpoint will compare MST of patients treated with glufosfamide and 'best supportive care' (BSC) to those managed by BSC alone. Expected survival of these patients managed by BSC is approximately 3 months. At the same time, Threshold initiated a randomized, multicenter, multinational, phase III clinical trial to enroll approximately 306 patients with metastatic PDAC to evaluate the safety and of glufosfamide as first line therapy. Eligible patients will be randomized to either glufosfamide every three weeks in addition to BSC or BSC alone.

The effectiveness of glufosfamide with or without hydration in treating patients with metastatic or inoperable locally advanced PDAC was evaluated in a multicenter (n=13), phase II clinical trial (protocol IDs: EORTC-16994P, ASTA-D-19575-3166), initiated in Europe, in February 2000. Trial objectives were to determine response rates, and duration of response, assess the drug's toxic effects, and evaluate the nephroprotective effect of forced diuresis via hydration on the toxicity profile of this treatment in this setting. Patients were randomized to one of two treatment arms. In arm I, IV glufosfamide (5 g/m^2) was administered over 1 hour on day 1. In arm II patients were treated with glufosfamide (5 g/m^2) as in arm I and hydrated with excess physiological saline solution 4 hours before and for 3 hours after treatment. The regimen was repeated every 3 weeks in the absence of disease progression or unacceptable toxicity. Patients with an objective CR continued treatment for a maximum of 2 courses beyond confirmation of response. Patients were followed every 6 weeks until disease progression. This trial was reported closed to patient recruitment in May 2001. Axel R. Hanauske from the EORTC Early Clinical Studies Group was the PI, and Nicholas Pavlidis, MD, of EORTC New Drug Development Group was Protocol Chair.

A total of 35 patients were enrolled in this trial over a 13-month period between February 2000 and March 2001, and 114 treatment cycles (median=3, range=1-8) were administered to 34 patients with 18 patients allocated to the hydration arm. Treatment was discontinued for safety reasons in patients with a serum creatinine increase of >0.4 mg/dl (>35 mmol/l) compared with baseline value. Overall hematologic toxicity was mild. Metabolic acidosis occurred in 2 patients treated in the active-hydration arm, Grade 3 hypokalemia was recorded in 5 patients and Grade 3 hypophosphatemia in 4 patients; a Grade 4 serum creatinine levels increase was noted in 1 patient, concomitantly to disease progression. Active hydration did not show a nephroprotective effect and the plasma pharmacokinetics of glufosfamide was not significantly influenced by hydration. According to RECIST criteria, there were 2 (5.9%) confirmed PR, and disease stabilized in 11 (32.4%) patients. An extramural review panel confirmed all of the responses. MST was 5.3 months, and time-to-progression (TTP) was 1.4 months. Glufosfamide's activity was modest in this setting. Although hematologic toxicity is particularly mild, regular monitoring of renal function is recommended (Briasoulis E, etal, Eur J Cancer, Nov 2003;39(16):2334-40). An updated analysis of the survival shows an estimated 9% 2-year survival, which compares to ≤1% or reported with other first line therapies.

Gene Transfer

Gene transfer approaches confer certain desirable attributes to cancer cells. In some cases, cancer cells are made more vulnerable to certain treatments, such as a particular cytotoxic agent or irradiation, or to the host's immune system. In addition, various antiangiogenic/antimetastatic factors or tumor suppressor proteins delivered by gene transfer may be produced *in situ* to prevent tumor formation and metastasis.

In vivo gene transfer of regulatory genes has been demonstrated in animals with considerable success. As an example, investigators at Harvard Medical School and Children's Hospital (Boston, MA) demonstrated that *in vivo* adenovirus-mediated delivery of a soluble form of the VEGF receptor Flk1 (Flk1-Fc) delayed growth of murine and human PDAC in mice (Tseng JF, etal, Surgery, Nov 2002;132(5):857-65). Three animal models were used to test this approach, immunocompetent C57Bl/6 mice injected subcutaneously (SC) with Panc02 murine PDAC cells, immunodeficient severe combined immunodeficiency (SCID) mice injected SC with BxPc-3 human PDAC cells, and C57Bl/6 mice injected with Panc02 cells through an intrasplenic route in an effort to mimic metastatic disease. In each model, half the tumor-bearing mice were injected IV with 109 Flk1-Fc adenovirus particles, and half with a control adenovirus. In the SC tumor models, 6 weeks after vector administration, animals treated with Ad Flk1-Fc had 75% smaller murine and 78% smaller human pancreatic tumor volumes, relative to tumor volumes of controls (Ad Fc-treated animals). In the animals injected with tumor through the intrasplenic route, pathologic and histologic analyses of hepatic, pancreatic, and splenic tumors, together with a desmoplastic response, were consistent with pathologic findings of human PDAC. Cohorts of these tumor-bearing mice treated with Ad Flk1-Fc lived significantly longer, and there was less liver replacement with tumor at the time of death, relative to animals treated with Ad Fc.

Recombinant adenovirus NK4 (Ad-NK4), which encodes a secreted form of human NK4, suppresses growth of orthotopically implanted pancreatic cancer cells in nude mice. NK4, a four-kringle fragment of hepatocyte growth factor (HGF), acts as both an HGF antagonist and an angiogenesis inhibitor. Investigators at Kyushu University (Fukuoka, Japan), Osaka University Graduate School in

Exhibit 3
Ongoing Combination Clinical Trials with Bevacizumab in PDAC

Regimen	Clinical Status ❑ Clinical Indication Enrollment	Protocol Description and ID	Institution
Regimen 1 Bevacizumab + docetaxel **Regimen 2** Bevacizumab	Phase II (begin 7/03, ongoing 12/04)➢USA ❑ metastatic pancreatic cancer Enrollment: 46 patients (23 per treatment arm)	Patients are randomized to 1 of 2 treatment arms. In arm I, patients are treated with IV bevacizumab over 30-90 minutes on days 1 and 15, and IV docetaxel over 1 hour on days 1, 8, and 15. Patients in arm II are administered bevacizumab as in arm I. In both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Protocol ID: FCCC-03003	Fox Chase Cancer Center (Philadelphia, PA)
Regimen 1 Gemcitabine + bevacizumab **Regimen 2** Gemcitabine + placebo	Phase III (begin 7/04, ongoing 12/04)➢USA ❑ locally advanced or metastatic pancreatic adenocarcinoma Enrollment: 590 patients (295 per treatment arm)	Patients are stratified according to ECOG performance status (0-1 or 2), disease extent (metastatic or locally advanced), and prior radiotherapy (yes or no), and randomized to 1 of 2 treatment arms. In arm I, patients are administered IV gemcitabine over 30 minutes on days 1, 8, and 15, and IV bevacizumab, over 30-90 minutes, on days 1 and 15. In arm II, patients are administered IV gemcitabine as in arm I and IV placebo. In both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months for 1 year and then every 6 months for 3 years. Protocol ID: CALGB-80303 (CTSU)	Cancer and Leukemia Group B (Durham, NC)
Regimen 1 Bevacizumab + gemcitabine + cetuximab **Regimen 2** Bevacizumab + gemcitabine + erlotinib	Phase II (begin 8/04, ongoing 12/04)➢USA ❑ advanced pancreatic cancer Enrollment: 54-126 patients (27-63 per treatment arm)	Patients are stratified according to participating center (University of Chicago versus other) and ECOG performance, and randomized to 1 of 2 treatment arms. In arm I, patients are administered IV cetuximab IV over 1-2 hours on days 1, 8, 15, and 22; IV gemcitabine over 30 minutes on days 1, 8, and 15; and IV bevacizumab IV over 30-90 minutes on days 1 and 15. In arm II, patients are administered gemcitabine and bevacizumab as in arm I, and oral erlotinib once daily on days 1-5, 8-12, and 15-26. In both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months. Protocol IDs: UCCRC-NCI-6580, NCI-6580, UCCRC-13200A	University of Chicago Cancer Research Center (Chicago, IL)

Japan, and Tohoku University (Miyagi, Japan), evaluated the antitumor effect of Ad-NK4 gene delivered IP, peritumorally, or intrasplenically. Weekly IP injections of Ad-NK4 suppressed development of tumor nodules in a nude mouse model of peritoneal dissemination. NK4 expression was seen in the liver, pancreas, spleen, mesenterium, and disseminated nodules. There was a remarkable decrease in microvessel density and an increase of apoptotic tumor cells in the Ad-NK4-treated mice, and survival of these mice was significantly improved (Saimura M, etal, AACR02, Abs. 2961).

Similar results were attained with Ad-NK4 gene transfer to orthotopically implanted SUIT2 pancreatic cancer cells in nude mice. Treatment with Ad-NK4 induced con-

stitutive high expression of NK4 in pancreas, significantly suppressing pancreatic tumor growth and disease extension. No toxicity was observed in the Ad-NK4-treated mice (Saimura M, etal, AACR03, Abs. 759).

Another study evaluated *in vivo* the antitumor effect of Ad-NK4 gene transfer to liver metastasis in nude mice bearing SUIT2 human pancreatic cancer cells transplanted into their spleen. Mice were treated by intrasplenic injection of Ad-NK4 on day 3 after implantation. On day 28 after implantation, a few metastatic nodules were noted in the liver of Ad-NK4-treated mice, whereas metastases were seen in almost all parts of the liver in controls (Murakami M, etal, AACR03, Abs. 6460).

Rexin-G, under development by Epeius Biotechnologies (Los Angeles, CA), combines a proprietary targeted vector system with a proprietary mutant cell-cycle control gene. Rexin-G accumulates in tumors and metastatic sites, inducing acute arrest of tumor growth and/or tumor regression without appreciable toxicity.

Rexin-G was developed using Epeius Targeted Delivery System (TDS), which is based on established principles of hemostasis and wound healing. Dr. Frederick L. Hall, Epeius founder, engineered a series of targeted injectable retroviral vectors incorporating a physiologic surveillance function inherent in von Willebrand factor, which facilitates vector accumulation in tumors with exposed and/or newly deposited collagenous matrix proteins as a result of tumor invasion and tumor-associated angiogenesis. When injected IV, these tumor-targeted 'smart' nanoparticles, engineered to transport genetic medicine, are selectively and efficiently delivered to metastatic sites. In preclinical trials, the genetically expressed drug induced acute arrest of tumor growth and/or tumor regression without appreciable toxicity in both experimental animal models and human studies.

Uniquely suited by design to function within the human circulatory system, Epeius' pathotropic TDS technology is an enabling platform technology for the targeted delivery of many molecular and genetic medicines. It can be readily adapted for the strategic delivery of therapeutic genes, recombinant proteins, and pharmaceutical agents. In July 2004, Epeius licensed a retroviral display technology from BioFocus (Saffron Walden, Essex, UK) to be used to target Rexin-G to sites of tumor growth and metastasis.

In June 2003, a dose-escalation phase I/II clinical trial of Rexin-G was initiated at Makati Medical Center (Manila, the Philippines), under PI Gerardo H. Cornelio, MD, and Conrado Lorenzo III, MD, to evaluate the safety/toxicity and potential antitumor response/efficacy of IV Rexin-G in Stage IV pancreatic cancer. According to an intrapatient dose escalation regimen, increasing doses of Rexin-G were administered IV, daily, for 8 to 10 days. Completion of this regimen was followed by a once weekly evaluation period for toxicity, after which Rexin-G was administered at MTD for another 8 to 10 days. In a second protocol, IV Rexin-G was administered frontline for 6 days followed by 8 doses of weekly gemcitabine. Rexin-G arrested tumor growth in all 3 patients treated thus far without emergence of DLT. No bone marrow suppression, significant alterations in liver and kidney function, nausea or vomiting, mucositis or hair loss were observed; 2 patients were alive with stable disease approximately 5 and 14 months from diagnosis, and 1 was alive with progressive disease 20 months from diagnosis (Gordon EM, etal, Int J Oncol, Jan 2004;24(1):177-85).

In September 2003, the FDA designated Rexin-G as an orphan drug for pancreatic cancer. In the USA, REXIN-G was approved by the FDA for use in a phase I clinical trial for metastatic colorectal cancer.

TNFerade, under development by GenVec (Gaithersburg, MD), consists of a proprietary replication-deficient adenoviral vector carrying a transgene encoding tumor necrosis factor α (TNFα), regulated by the radiation-inducible promoter Egr-1, a molecular switch that allows maximum gene expression and therapeutic protein secretion only when the target tissue is exposed to standard radiation therapy. Under a CRADA signed with the National Cancer Institute (NCI) in October 2003, the two organizations will work together to test mutually agreed upon second-generation TNF-related product candidates that target tumors when administered systemically or regionally. These second-generation product candidates are based on GenVec's proprietary targeted gene delivery technology.

Although TNFα has been shown to possess antitumor activity, its use is limited by the toxicity of systemic administration. However, gene transfer to the tumor may be used to generate high concentrations of TNFα within the tumor environment.

A multicenter, randomized, controlled, phase IIb clinical trial (protocol IDs: UCLA-0207023, NCI-G02-2131, GENVEC-GV-001.004) of TNFerade as first line treatment of inoperable, locally advanced pancreatic cancer was initiated in July 2002. Key endpoints of the trial, to enroll approximately 140 patients, are tumor shrinkage, TTP, survival, and QoL. The trial will also keep track of the number of patients who may become eligible after TNFerade therapy for surgical removal of tumors previously classified as inoperable. This trial is divided into two parts. Part 1 is an open label, dose-escalation trial that adds TNFerade, administered by injection once weekly for 5 weeks, to 5-FU and radiation therapy as first line treatment in patients with locally advanced pancreatic cancer. In part 2, patients are being randomly assigned to standard care (5-FU + radiation therapy) with or without TNFerade injections.

The open label, dose-escalation portion (run-in) of this trial was conducted at the University of Kentucky Medical Center (Lexington, KY), Virginia Commonwealth University (Richmond, VA), UCLA School of Medicine, University of Florida (Gainesville, FL), University of South Florida (Tampa, FL), US Oncology (Dallas, TX), University

of Chicago Medical Center, University of California (Irvine, CA), among others, to select appropriate doses of TNFerade in combination with chemoradiation consisting of 5-FU (200 mg/m^2/day) and radiation (50.4Gy), to use in the randomized phase of the trial. The run-in phase explored up to 3 dose levels ($4x10^9$ pu, $4x10^{10}$ pu, and $4x10^{11}$ pu) of TNFerade. Weekly intratumoral injections of TNFerade were administered using either percutaneous transabdominal injections guided either by CT scan or percutaneous endoscopic ultrasound (EUS) techniques, for 5 weeks concomitant with 5-FU/radiation (Hanna N, ASCO03, Abs. 1086:271).

Among 37 patients, treated in three cohorts with doses of $4x10^9$ pu (n=10), $4x10^{10}$ pu (n=20), and $4x10^{11}$ pu (n=7), there was 1 DLT manifested as Grade 3 hypotension, that occurred in a patient at the $4x10^{10}$ pu dose, who was subsequently able to resume treatment. All other adverse events potentially related to TNFerade were Grade 1/2 fever (27%), fatigue (19%), and rigors (19%). At 3 months post treatment, the $4x10^{11}$ pu dose was associated with 100% locoregional control of treated tumors, and a greater patient survival without progression. Also, disease stabilized or CA19-9 levels decreased in a greater proportion of patients as compared to the other doses at 3 months post treatment. Survival without progression at 3 months was 25% at the $4x10^9$ pu dose, 50% at the $4x10^{10}$ pu dose and 67% at the $4x10^{11}$ pu dose; local tumor shrinkage or stabilization was seen in 67%, 70%, and 100% patients, respectively, while objective responses (> 25% reduction in tumor area) were 33%, 20%, and 67%, respectively, and CA19-9 levels decreased or stabilized in 57%, 53%, and 83%, respectively. Among 6 patients who were surgically explored, 4 underwent resection, with 1 achieving a complete pathologic response. TNFerade combined with chemotherapy shows promise in treatment of locally advanced pancreatic cancer. Also, increased doses beyond $4x10^{11}$ may be feasible (Senzer N, etal, ASCO04, Abs. 3038).

According to additional data, MST was 200 days (6.6 months) at $4x10^9$ pu and 235 days (7.8 months) at $4x10^{10}$ pu. In the $4x10^{11}$ pu dose level, MST had not been reached at 6 months and follow-up continues. As of May 28, 2004, more than half of the patients treated at the highest dose level were alive at 340 days (11.2 months). At 6 months post treatment, 80% of treated tumors stabilized in the $4x10^{11}$ cohort compared to approximately 40% at the two lower doses.

Oncolytic Viruses

Oncolytic viruses that are engineered to preferentially replicate in and destroy cancer cells represent a new approach to cancer therapy. These engineered viruses can be delivered either by intratumoral or IV injection, and are designed to be thousands of times more specific for killing cancer cells than standard chemotherapeutic drugs. In these constructs, the virus is designed to replicate within the target cancer cell until the cell bursts, thereby destroying the cell and spreading newly created viruses throughout the tumor, so that the cycle is repeated in neighboring cancer cells. After destroying cancer cells, the virus is cleared by the body's immune system.

In order for this approach to work in humans, highly specific cell- or tissue-specific targeting methods are necessary. A novel approach is use of conditional replication-competent virus vectors such as the herpes simplex virus 1 (HSV-1). This vector replicates in and is cytotoxic only to a specific cell type attributed to the regulated expression of an essential immediate early viral gene product. Investigators at Kyoto University and Osaka Medical College, in Japan, constructed a HSV-1 vector (d120.surv) in which the survivin promoter drives expression of ICP4, a major trans-activating factor for viral genes, so that replication of the vector is restricted to surviving-expressing cells. Survivin, a member of the inhibitor of apoptosis (IAP) family widely expressed during fetal development, is not expressed in normal adult tissues, but becomes highly expressed in transformed cell lines, and in most of the common human cancers including pancreatic cancer *in vivo*. Survivin mRNA expression level correlated with survivin promoter activity in PDAC cell lines. ICP4 protein was expressed in PDAC cells infected by d120.surv. In three PDAC cell lines, the ability to replicate and the cytotoxic activity of d120.surv correlated with survivin mRNA expression levels of the host cell lines. Among cells infected with d120.surv, 90% of Panc-1(low expression of survivin) and 15% AsPC-1(high expression of survivin) cells survived. Therefore, the conditional replication-competent HSV-1 vectors regulated by the survivin promoter may be a new therapeutic strategy for treatment of pancreatic cancer (Kami K, etal, AACR04, Abs. 4603).

A combination of oncolysis and immunotherapy is represented by OncoVEX GM-CSF, under development by BioVex (London, UK). OncoVEX GM-CSF is a construct combining the cell-killing ability of an oncolytic virus with the generation of a host immune response. It is based on a novel modified herpes simplex virus (HSV) type 1 vector, carrying the gene encoding human GM-CSF. OncoVEX GM-CSF provides an *in situ*, patient specific, granulocyte macrophage colony stimulating factor (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).

CG5757, under development by Cell Genesys (South San Francisco, CA) is an oncolytic virus derived from adenovirus that grows selectively in and destroys cancer cells. CG5757 has been genetically modified with two tumor-specific gene switches referred to as 'promoters' that make it highly selective for killing multiple types of cancer cells. Approximately 85% of cancer cells have a defective retinoblastoma (Rb) pathway, and also overexpress the enzyme telomerase, which aids in replication of cancer cells. CG5757 preferentially replicates in cells with both of these defects. CG5757 was effective in laboratory studies in killing multiple types of cancer cells, including lung,

bladder, liver, colon, prostate, and pancreatic cancer. The studies also showed that CG5757 replicated extensively in tumor cells, causing cell death. No DLT was observed in tumor models following treatment with CG5757. Approximately 1,000 to 100,000 cancer cells are killed for every normal cell. In contrast, standard chemotherapeutic drugs destroy approximately six cancer cells for every normal cell killed and, as a result, cause more treatment-related toxicity.

In July 2003, Cell Genesys announced a global alliance with Novartis for development and commercialization of oncolytic virus therapies. In addition, Cell Genesys acquired exclusive worldwide rights to the oncolytic virus therapy products and certain related intellectual property of Genetic Therapy (GTI), an affiliate of Novartis, as well as related intellectual property of Novartis, including an agreement with Geron (Menlo Park, CA) for telomerase-related promoters.

Reolysin, under development by Oncolytics Biotech (Calgary, Alberta, Canada), is a human reovirus that replicates specifically in tumor cells bearing an activated Ras pathway. Ras is an important component of a pathway controlling normal growth and differentiation of a cell, and when mutated, may account for 30% to 40% of all human malignancies. Activating mutations in Ras are found in 90% of cases of pancreatic cancer.

Tumors bearing an activated Ras pathway are unable to activate the antiviral response mediated by the host cellular protein, double stranded RNA (dsRNA)-dependent protein kinase (PKR). Because PKR is responsible for preventing reovirus replication, tumor cells lacking PKR activity are susceptible to reovirus infections. Because Ras is not activated in normal cells, these cells stop reovirus infection through normal PKR activity. Reovirus replicates freely, and eventually kills the host tumor cells with an activated Ras pathway. As cell death occurs, progeny viruses are then free to infect surrounding cancer cells. This cycle of infection, replication and cell death is believed to be repeated until there are no more tumor cells remaining carrying an activated Ras pathway.

When reovirus (serotype 3) was used as an antihuman pancreatic cancer agent) *in vitro*, it infected human pancreatic cancer cell lines Panc1, MIApaca-2, PK1, PK9, and BxPC3. Ras activity in these cancer cell lines was elevated compared with that in the normal cell line and susceptibility to reovirus was associated with Ras activity of these cells. Tumor growth was also suppressed by intratumoral injection of reovirus, in a unilateral murine xenograft model using Panc1 and BxPC3 cell lines. Furthermore, local injection of reovirus also had systemic antitumor effects in a bilateral xenograft model using the Panc1 cell line. Reovirus replication was observed within the tumor but not in surrounding normal tissue (Etoh T, etal, Clin Cancer Res, Mar 2003;9(3):1218-23).

In May 2004, Oncolytics Biotech initiated an open label, dose-escalation phase I clinical trial in the UK to investigate the IV delivery of Reolysin as a treatment for patients with advanced or metastatic solid tumors refractory to standard therapy, or for which no curative standard therapy exists. The primary objective of the trial is to determine the MTD, DLT and safety profile of Reolysin. Secondary objectives include evaluation of viral replication, immune response to the virus, and any evidence of antitumor activity. Up to 40 evaluable patients are expected to be enrolled in this trial, depending upon the number of dose levels tested. The principal investigator for the trial is Dr. J. de Bono of the Royal Marsden Hospital (London, UK).

In March 2002, the company reported results from the phase I clinical trial of Reolysin that was initiated in June 2000, and completed in October 2001. Regarding safety, the trial's primary endpoint, none of the patients treated with Reolysin experienced any serious adverse events related to the virus, and there were no DLT detected in any patient. In terms of secondary endpoints, tumor responses were measured at both the immediately treated lesion, as well as remote tumor sites. Activity was defined as a transitory, or lasting tumor regression of at least 30% measured in two dimensions and compared to tumor size prior to the injection on the first day of treatment. Evidence of viral activity was detected in 11/18 patients (61%), with tumor regression ranging from 32% to 100%. Disease stabilized at the injected tumors in 11/17 evaluable patients, on day 28 after the first, and in some cases, the only injection of Reolysin. By day 98, 1/10 evaluable patients experienced a PR, and disease stabilized in 4/10. In addition, there was evidence of remote tumor responses in several patients.

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