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STATE-OF-THE-ART IN THE MANAGEMENT OF CANCER

PANCREATIC CANCER — PART III

NOVEL CYTOTOXICS, HORMONE MODULATORS, FORMULATIONS/PRODRUGS AND RELATED AGENTS IN DEVELOPMENT

Next issue: *Part IV, the last part of this series on pancreatic cancer, will cover novel targeted therapeutics, other regulatory agents, radioimmunoconjugates, and immunotherapy approaches, encompassing over 151 agents with 95 having entered clinical trials for various indications.*

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Currently, commercialized cytotoxic agents used either as monotherapies, or in various combination or multimodality approaches in inoperable, advanced or metastatic pancreatic ductal adenocarcinoma (PDAC), result in only a few extra months of median survival time (MST), 6 months with monotherapies, and 12 months for chemoradiotherapy (CRT), compared to about 3 1/2 months for best supportive care (BSC). Therefore, although cytotoxic chemotherapy has demonstrated some activity in PDAC, results have been marginal at best. Hope lies in the discovery and development of novel agents that may lead to a more favorable outcome for this devastating disease.

This part III of the series on pancreatic cancer describes agents acting on broad mechanisms such as cytotoxicity, cell-cycle modulation, and apoptosis. Description of the agents' development status is specifically addressing their application in the treatment of pancreatic cancer (Exhibit 1). However, most of these agents are broadly applicable across numerous malignancies. Comprehensive profiles of these drugs are found on NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), a subscription-based resource in oncology residing at www.nmok.com. Agents in development against PDAC with novel mechanisms of action including those targeting specific markers, and immunotherapy approaches, are discussed in Part IV of this series on pancreatic cancer.

NOVEL CYTOTOXICS

Numerous traditional cytotoxics have been tested against all stages of pancreatic cancer, mostly in combination (see Part II of this series). Several novel cytotoxics are in development for this indication, being evaluated either as monotherapies or combination therapies. Recent developments in the field of traditional cytotoxic drugs also involve creation of novel formulations that improve the pharmacokinetics of these agents, reduce their toxicity, and enhance their overall effectiveness.

Topoisomerase I Inhibitors

Topoisomerase I (topo I) is a nuclear enzyme that acts to relax supercoils generated during transcription and DNA replication. Topo I inhibitors that interfere with this enzyme disrupt DNA synthesis and ultimately cell division. Use of commercially available topo I inhibitors in PDAC was described in Part II of this series.

Various formulations of camptothecin (CPT)-based cytotoxics are also in development. Evaluation of one such agent, Prothecan, a pegylated version of a camptothecin analog under development by Enzon (Piscataway, NJ), was discontinued in PDAC in July 2003.

DX-8951f, a topoisomerase I inhibitor under development by Daiichi Pharmaceutical (Tokyo, Japan), is a synthetic water-soluble CPT analog with a potent, broad spectrum antitumor activity. According to completed phase I evaluation, DX-8951f shows activity against a wide range of tumor types. DX-8951f may be of particular interest because it may overcome the usual mechanism by which cells acquire resistance to CPT analogs.

Results from two clinical trials indicate significant activity of DX-8951f in advanced PDAC. Between April 1999 and April 2000, 39 patients [20 (51%) had prior chemotherapy, 8 (21%) prior radiation therapy (RT), 2 (5%) prior biologic therapy, and 18 (46%) no prior treatment] with advanced or metastatic PDAC, were enrolled in a phase II clinical trial (protocol IDs: DAIICHI-8951A-PRT009, JHOC-99062405, MSKCC-99014) of single agent DX-8951f, at Memorial Sloan-Kettering Cancer Center (New York, NY), Cancer Treatment and Research Center (CTRC; San Antonio, TX), and Johns Hopkins Oncology Center (Baltimore, MD). DX-8951f (0.5 mg/m^2) was administered IV over 30 minutes, daily, for 5 days, with courses repeating every 21 days. Patients were restaged after every 2 cycles. Treatment continued in the absence of unacceptable toxicity or disease progression. Patients were followed every 3 months until death.

Final results of this trial were based on a total of 164 cycles of DX-8951f. Among 34 (87%) patients treated with ≥2 cycles of therapy, there were 2 (5%) PR, lasting for 10+ and 20 cycles of therapy, respectively. The first occurred in a gemcitabine-refractory patient and, in another patient who had no prior therapy, there was a complete disappearance of bulky liver metastases. Also, there was 1 (3%) minor response (MR), and disease stabilized in 15 (39%) patients for ≥4 cycles. Grade 3/4 toxicities included neutropenia (56% of patients), thrombocytopenia (11%), fatigue (8%), and nausea/vomiting (8%); 9 (23%) patients were hospitalized for cholangitis, neutropenic fever, hemoptysis, DVT/PE, and pain. For all 39 patients, MST was 5.5 months and 1-year survival was 27%. For the 19 patients who had not been previously treated with chemotherapy, MST was 10.6 months and 1-year survival was 35%. These results compare favorably with those of gemcitabine monotherapy with an MST of untreated patients with PDAC of 5.6 months and a 1-year survival of 18%, and show that DX-8951f has significant activity in advanced untreated pancreatic cancer (D'Adamo D, etal, ASCO01, Abs. 532:134a).

Subsequently, when a subset of 23 patients who had not been previously exposed to chemotherapy was analyzed further, there were 3 (13%) confirmed PR lasting 2.8, 4.3 and 10.1 months. MST for these 23 patients was 9.3 months. The 6-, 12-, and 24-month survival was 70%, 39% and 5%, respectively. Toxicity was primarily myelosup-

pression and fatigue [O'Reilly EM, etal, ASCO 2004 Gastrointestinal Cancers Symposium (ASCOGI04), Abs. 138].

Two large scale, randomized trials with DX-8951f in PDAC, undertaken in North America and Europe, have completed accrual targets. In the North American trial, a combination of DX-8951f and gemcitabine was compared to gemcitabine alone, while in the European trial DX-8951f as a single agent was compared to gemcitabine monotherapy. The European randomized, open label, multicenter, phase III clinical trial was initiated in August 2001 at Airedale General Hospital (Steeton, Keighley, UK), under PI Michael Crawford, MD, to compare the efficacy and safety of IV DX-8951f monotherapy against gemcitabine monotherapy in chemotherapy-naive patients with locally advanced or metastatic PDAC. This trial was completed in November 2002. A total of 339 patients were recruited to provide 306 evaluable patients. Primary endpoint for this trial was overall survival rate.

The North American multicenter (n=75), randomized, open label, phase III clinical trial (protocol ID: DAIICHI-8951A-PRT031, MSKCC-02011) of DX-8951f in combination with gemcitabine versus single agent gemcitabine, was initiated in September 2001, as first line therapy of locally advanced or metastatic cancer of the exocrine pancreas. Trial objectives are to compare overall survival, clinical benefit, antitumor efficacy, and safety profile of this combination regimen in this setting. Approximately 349 patients were accrued for this trial, stratified according to performance status (60% versus 70-80% versus 90-100%), extent of disease (locally advanced versus metastatic), and prior RT for pancreatic cancer (yes or no), and randomized to one of two treatment arms. In arm I, patients were treated with DX-8951f IV over 30 minutes immediately followed by gemcitabine IV over 30 minutes on days 1 and 8. Treatment repeated every 3 weeks in the absence of disease progression or unacceptable toxicity. In arm II, patients were treated with gemcitabine IV over 30 minutes, once weekly, for up to 7 weeks, followed by one week of rest (course 1). For all subsequent courses, patients were treated with gemcitabine, once weekly, for 3 weeks, followed by one week of rest. Treatment repeated every 4 weeks in the absence of disease progression or unacceptable toxicity. Patients are followed monthly. Accrual in this trial was completed in January 2003.

Dosing in this trial is based on results from a phase I clinical trial of DX-8951f, in combination with gemcitabine, conducted in advanced solid tumors, that yielded phase II/III clinical trial doses of 2.0 mg/m^2 for DX-8951f and 1,000 mg/m^2 for gemcitabine when both drugs were administered on a days 1, and 8, every 3 weeks (O'Reilly EM, etal, ASCO02, Abs. 394:99a). In this phase I clinical trial, among 31 chemotherapy-naïve patients with PDAC, there were 7 (23%) major tumor responses, 1 CR and 6 PR, with a median duration of response of 9.3 months. MST for these 31 patients was 8 months. The 6-, 12- and 24-month survival was 55%, 39% and 19%, respectively.

DE-310 is a water-soluble conjugate of DX-8951, linked by a peptidyl spacer to a carboxymethyldextran polyalcohol polymer; about 1/15 of DE-310 by weight is DX-8951, which is slowly released in cells by cathepsins. DE-310 is internalized in tumor cells and macrophages by endocytosis. Subsequently, endosomes containing DE-310 transfer it to lysosomes, whereupon DX-8951 and G-DX-8951 are released from DE-310 by lysosomal enzymes (Ochi Y, etal, AACR03, Abs. 1740). Pharmacokinetic analysis demonstrated that the DX-8951 moiety is eliminated faster than the carrier macromolecule. Therefore, DE-310 undergoes biodegradation to smaller carbohydrate fragments releasing the free drugs from conjugated DX-8951. Disposition of conjugated DX-8951 and free DX-8951 is determined by the tissue uptake rate of conjugated DX-8951 from blood (Masubuchi N, etal, AACR03, Abs. 1727).

While treatment with DX-8951f, administered once daily for 5 days at MTD was required to shrink tumors in a murine Meth A (fibrosarcoma) model, and DX-8951f at a similar schedule at 1/4 MTD was required to inhibit tumor growth, a single treatment with DE-310 at MTD or 1/4 MTD shrank the tumor, with no body weight loss occurring at 1/4 MTD. DE-310 inhibited tumor growth even at 1/16 MTD. In a long term assay, Meth A solid tumors disappeared in mice treated with DE-310 once daily at MTD and 1/2 MTD, and all 6 mice remained tumor free on the 60th day after administration. Repeated injection (4 times) every 3 days, for 7 days or 14 days, demonstrated that multiple treatments with DE-310 produced greater tumor growth delay than a single treatment (Kumazawa E, and Ochi Y, Cancer Sci, Feb 2004;95(2):168-75).

The University of Texas Health Science Center (San Antonio, TX) and the CTRC conducted a dose-escalation phase I clinical trial of DE-310 (1.0 mg/m^2 to 7.5 mg/m^2), administered as a 3-hour infusion, every 4 weeks, to 23 patients with advanced solid tumors, including colorectal cancer (n=6), non-small cell lung cancer (nsclc; n=4), gastric cancer (n=4), liver cancer (n=3), thymoma (n=2), and pancreatic, breast, cholangial, and unknown primary cancer or lymphoma. DLT was thrombocytopenia, observed at 7.5 mg/m^2. Some Grade 3/4 toxicities in the 6 mg/m^2 cohort included neutropenia (n=1), anemia (n=3), and thrombocytopenia (n=2). Other common toxicities observed across all dose cohorts included reversible liver transaminitis, mild nausea, vomiting, anorexia, and fatigue. Among 15 evaluable patients, disease stabilized in 5, and progressed in 10 (Takimoto CHM, etal, ASCO03, Abs. 522).

LE-SN-38, under development by NeoPharm (Lake Forest, IL), consists of SN-38 (7-ethyl-10-hydroxy-camptothecin), an active metabolite of camptothecin CPT-11, encapsulated in cardiolipin liposomes. This approach bypasses the need to convert CPT-11 to SN-38 in the body. CPT-11 has limited activity on its own but acts as a prodrug that is converted into its active metabolite SN-38 in the body; however, in colorectal cancer cells, only 6-12% of

CPT-11 is converted into SN-38. Also, because of variability in conversion rates among patients, the amount of SN-38 actually available to kill tumor cells can be unpredictable.

Despite its more attractive profile than CPT-11, clinical use of SN-38 has not been feasible because it is not soluble in pharmaceutically acceptable solvents. To overcome this problem, LE-SN-38, a novel lyophilized, liposome-based formulation with greater than 95% drug entrapment, was developed by employing NeoPharm's proprietary NeoLipid technology. NeoLipid technology combines drugs or compounds with proprietary lipids, such as cardiolipin. In its natural form, cardiolipin occurs as a negatively charged lipid found in cardiac tissue. Tumor cells recognize the NeoLipid liposomal drug as a potential source of nutrition, allowing the active drug to be delivered directly to tumor cells. In addition, NeoLipid technology allows for the creation of stable liposomes, an especially important physical property for drug storage and reconstitution for administration to patients. NeoLipid formulations reduce toxicity of potent chemotherapeutic drugs, and may represent a more comfortable to administer, safer, and well tolerated treatment for cancer patients.

In vivo, in comparative single and multiple dose therapeutic efficacy studies of LE-SN38 and CPT-11, conducted in a SCID mouse xenograft model of human pancreatic cancer (Capan-1), single dose IV treatment with LE-SN38 at 15, 30 and 45 mg/kg resulted in 53%, 80% and 95% growth inhibition, respectively. In contrast, CPT-11 did not produce any tumor inhibition at these dose levels. Multiple dose treatment of mice for 5 consecutive days with LE-SN38 at 4, 8 and 12 mg/kg resulted in 65%, 98% and 99% tumor growth inhibition, respectively, in comparison to growth inhibition in animals treated with 8 and 12 mg/kg of CPT-11 of 48% and 64%, respectively. This enhanced antitumor efficacy of LE-SN38 as compared to CPT-11 may be attributable to the sustained release of SN-38 in the circulation or at the target site (Pal A, etal, AACR03, Abs. 1785).

An open-label phase I clinical trial (protocol ID: LE-SN38-101) of IV LE-SN38, administered over 90 minutes in patients with advanced solid tumors refractory to conventional therapy, was initiated in October 2002 at the University of South Florida H. Lee Moffitt Cancer Center (Tampa, FL), Wayne State University Karmanos Cancer Institute (Detroit, MI), and Ohio State University Arthur G. James Cancer Hospital and Research Institute (Colombus, OH), under PI Fishman Mayer, MD, Patricia LoRusso, MD, and Eric Kraut, MD, respectively. This trial was designed to also deal with one of the serious side effects of treatment with CPT-11, i.e., unpredictable severe diarrhea and neutropenia. Metabolism of SN-38 is thought to be a significant factor in this toxicity. The enzyme UDP-glucuronosyltransferase (UGT) converts SN-38 to its inactive metabolite, SN-38 glucuronide (SN-38G). However, presence of a polymorphism in the promoter region of the UGT1A1 gene (UGT1A1 28) decreases SN-38 glucuronidation and is associated with increased risk of severe diarrhea and neutropenia following CPT-11 administration. Patients entering this trial are stratified prospectively according to their UGT1A1 genotype to determine the maximum tolerated dose (MDT) and safety profile for homozygous wild-type (WT/WT), heterozygous (WT/28), and homozygous UGT1A1 28 (28/28) genotypes.

In this dose-escalation phase I clinical trial, designed to assess the pharmacogenomics, pharmacokinetics, and safety of LE-SN38, separate patient cohorts are treated with LE-SN38 doses ranging from 2.5 to 90 mg/m^2. Tumor progression is monitored radiographically after every second cycle. As of August 2003, dose escalation had reached 20 mg/m^2 for the WT/WT stratum. Patients with WT/28 and 28/28 genotypes were administered doses up to 10.0 mg/m^2 and 5.0 mg/m^2, respectively. Among 55 patients screened, the genotype frequencies were 45% WT/WT, 38% WT/28, and 16% 28/28, consistent with reported values. The pharmacokinetic profile of total SN-38 (both free and liposome encapsulated) was evaluated for up to 96 hours after first administration of the drug. Values for Cmax and AUC were linear for WT/WT patients across doses from 2.5 to 10 mg/m^2. SN-38 clearance ranged from 304 to 1385 ml/min, and half-life ranged from 5.06 to 35.8 hours.

To date, based on analysis of results from patients treated with 2.5 mg/m^2 LE-SN38, the pharmacokinetic profile of SN-38 in WT/28 patients is similar to that in WT/WT patients. Importantly, diarrhea and neutropenia have not been dose limiting. LE-SN38 appears to be safe and well tolerated in all patients. Patient accrual in all three strata continues. Up to 40 patients are expected to enroll. Treatment continues on a 21-day schedule in the absence of progressive disease or unacceptable toxicity (Fishman MN, etal, AACR-NCI-EORTC03, Abs. B250, and Fishman MN, etal, ASCO03, Abs. 600:150). Subsequent preliminary results indicate that disease stabilized in 9 of 27 (33%) patients. LE-SN38 appears to be safe and well tolerated at doses up to 20 mg/m^2 and patient accrual is continuing at doses up to 30 mg/m^2; further dose escalation is also planned.

MBT-0312, under development by Munich Biotech (Neuried, Germany), is lyophilized, cationic lipid-complexed CPT targeted to tumor blood vessels. MBT-0312 has an overall positive charge and contains a high amount of CPT. MBT-0312 was created using Munich Biotech's neovascular targeting properties of the EndoTag technology platform, that encapsulates CPT in cationic lipid complexes and targets it to tumor blood vessels. Cationic lipid complexes preferentially accumulate in angiogenic endothelial cells of pancreatic tumors in transgenic RIP-Tag2 mice (Thurston G, etal, J Clin Invest, 1 Apr 1998;101(7):1401-13) and in tumor blood vessels of the amelanotic hamster melanoma A-Mel3 cell line (Krasnici S, etal, Int J Cancer, 1 Jul 2003;105(4):561-7). Rhodamine-labeled MBT-0312 strongly accumulated in tumor blood

vessels in the A-375 melanoma model, and strongly inhibited tumor growth in the subcutaneous mouse tumor models RenCa and B-16 at well tolerated doses (Brill B, etal, AACR04, Abs. 4107).

Rubitecan (Orathecin, RFS2000), a 9-nitrocamptothecin (9NC) under development by SuperGen (Dublin, CA), is an oral cytotoxic investigated as a treatment of patients with PDAC who have failed at least one prior chemotherapy regimen. Although rubitecan is not a cure, it can be of benefit to some patients with PDAC. Rubitecan completed phase III clinical trial development in PDAC and, in March 2004, the FDA officially accepted SuperGen's NDA for filing for rubitecan in the form of oral capsules. The FDA indicated that the user fee goal date for this NDA is November 26, 2004, which is the target date for the completion of FDA's review and resulting action letter for the filed NDA.

SuperGen's NDA filing contains data on more than 1,000 patients with refractory PDAC. Among these patients, more than 600 were treated with rubitecan with the remainder administered control therapies. In January 2004, SuperGen submitted the clinical module to complete submission to the FDA of a rolling NDA for rubitecan. The two other portions of the NDA, covering preclinical and the chemistry/manufacturing/controls data, were submitted in February 2003, and December 2002, respectively. In November 2002, the FDA designated rubitecan as a 'fast track' product for the treatment of patients with locally advanced or metastatic PDAC that is resistant or refractory to standard chemotherapy.

SuperGen's phase III clinical program in PDAC is believed to be the largest clinical development program for this indication ever conducted worldwide. The submission is also supported by data from a phase II clinical trial of rubitecan in refractory PDAC.

Rubitecan has demonstrated activity *in vitro* against human cancer cells grown in culture and *in vivo* in xenografts in nude mice. Apoptosis of cancer cells, induced by 9NC, is mediated by topo I, and executed by pathways that involve cytochrome c release from mitochondria and/or activation of death receptors, depending on the cell type. Alternatively, 9NC induces differentiation or senescence of certain cell types *in vitro*.

According to investigators at the University of Miami (Coral Gables, FL), in several instances, activities of 9NC are regulated by the Bcl-2 family of proteins and cell cycle-associated proteins, p53, p21 and Cdk. Development of resistance to 9NC, associated with mutations in the topo I gene, can be overcome by regulating specific proteins other than topo I, such as raf kinase inhibitor protein (RKIP). Finally, derivatives of 9NC, such as alkyl esters, liposome-encapsulated 9NC, and combination treatment of 9NC with ionizing radiation or hyperthermia, represent other approaches to enhance the apoptotic activity of 9NC against human cancer cells (Pantazis P, etal, Anticancer Res, Sep-Oct 2003;23(5A):3623-38).

Scientists at the Cancer Institute of New Jersey, Robert Wood Johnson University of Medicine and Dentistry of New Jersey (New Brunswick, NJ), investigated if overexpression of wild type as well as certain mutant forms of breast cancer resistance protein (BCRP)/MXR/ABCG2, that confers resistance to camptothecin analogs used clinically, including topotecan and irinotecan, is also involved in multiple drug resistance associated with the camptothecins 9-aminocamptothecin (9AC) and 9NC. BCRP is a new member of the family of ATP-dependent drug efflux proteins. In studies with 9AC and 9NC using mammalian cells stably transfected with constructs expressing a variety of efflux proteins, including wild type BCRP and a mutant BCRP that contains a threonine rather than an arginine at position 482 (R482T), overexpression of either P-gp multidrug resistance protein type 1 (MDR1), or MDR2 had little effect on the cytotoxicity of 9NC or 9AC. In contrast, overexpression of either wild type or R482T BCRP conferred resistance to 9AC, but not to 9NC. In addition, overexpression of wild type or mutant BCRP was associated with reduced intracellular accumulation of 9AC, but not 9NC and, whereas increased BCRP expression is evident in cells selected for resistance to irinotecan, BCRP expression is not detectable in two different cell lines selected for resistance to 9NC. These findings suggest that wild type and R482T BCRP mediate cellular efflux of 9AC but not 9NC (Rajendra R, etal, Cancer Res, 15 Jun 2003;63(12):3228-33).

In April 2002, results of a multicenter phase II clinical trial of rubitecan in 58 patients with refractory PDAC, were followed-up and independently reviewed by a third party. Among 45 evaluable patients, there were 10 independently verified objective PR, 3 cases of >50% reduction in tumor size, and disease stabilized in 7 patients, for an overall clinical benefit rate of 44.5%. MST for the 10 patients who responded was more than 10 months. Among these 10 patients, 5 lived for more than a year, 2 for more than 2 years, and 1 for more than 3 years and was still alive at the time of the review. Approximately 9% of enrolled patients discontinued treatment because of toxicity.

Based on these promising results, a pivotal multicenter phase III clinical trial was initiated at 50 centers in the USA to compare rubitecan to either the most appropriate treatment or to gemcitabine in patients with refractory PDAC. In November 2003, SuperGen announced that long term follow-up and independent review of results from the phase III clinical program of rubitecan supports the drug's activity in patients with PDAC who have failed prior treatments. This data was reviewed by an independent third-party expert radiology review panel and was presented by Howard A. Burris, III, MD, at the "Chemotherapy Foundation Symposium XXI- Innovative Cancer Therapy for Tomorrow", in New York City, in November 2003.

This phase III clinical trial randomized 409 patients, most of whom had previously failed two or more chemotherapies, to rubitecan or 'best choice' treatment. Approximately 90% of patients in the 'best choice' group

were treated with a chemotherapeutic agent such as gemcitabine, 5-FU, mitomycin C, capecitabine, or docetaxel. The primary study endpoint was overall survival with secondary endpoints of tumor response and time-to-disease progression (TTP). Statistical significance of survival outcomes was confounded by the high percentage of patients failing 'best care' and then treated with rubitecan. However, both secondary endpoints were statistically achieved.

Response rate (7% versus 1%), TTP (58 days versus 48 days) and MST (109 days versus 94 days) were all improved with rubitecan compared to 'best choice', despite the fact that patients who failed best alternative therapy were allowed to crossover to rubitecan at disease progression. As a result of this 'rescue therapy', 302/409 (74%) patients were treated with rubitecan. Although median TTP results were statistically significant, MST results were not. Among 196 patients randomized to rubitecan, there were 13 (7%) responses (CR+PR), compared to 1 (<1%) among 211 patients treated with other 'best choice' chemotherapeutics. This finding is statistically significant and independently verified. Among these 13 responders, TTP and MST were 269 and 338 days, respectively. Additionally, disease stabilized in 44/198 (22%) patients randomized to rubitecan versus 27/211 (13%) patients treated with 'best choice'. This finding is also statistically significant and independently verified. The total number of patients on rubitecan achieving 'disease control', defined as CR+PR+SD, was 28% (56/198) versus 13% (28/211) for 'best choice'.

Toxicities were generally manageable with fewer than 5% of patients in either arm needing to discontinue therapy for drug-related toxicity. Severe or most frequent adverse events with an incidence >5% in patients treated with rubitecan, compared to 'best choice' (rubitecan%/best choice%) included asthenia (20/18), abdominal pain (17/12), pain (5/6), sepsis (5/7), deep thrombophlebitis (5/5), nausea (14/9), anorexia (6/10), diarrhea (9/5), vomiting (12/8), leucopenia (22/13), anemia (16/9), thrombocytopenia (9/10), dehydration (15/12), bilirubinemia (7/2) and dyspnea (8/6).

Other studies also compared the effects of rubitecan with those of 5-FU in patients refractory to gemcitabine. Preclinical studies have also suggested that rubitecan, followed by 5-FU, may have beneficial effects, a hypothesis being investigated in clinical trials.

S-CKD602, under development by Alza (Mountain View, CA), is a Stealth liposome formulation of CKD-602, a camptothecin derivative topo I inhibitor marketed in certain Asian countries by Chong Kun Dang Pharmaceutical (Seoul, South Korea). CKD-602 has demonstrated antitumor activity in a broad spectrum of tumor types. Alza has encapsulated CKD-602 in a long circulating Stealth liposome formulation to prolong drug half-life in plasma, increase drug exposure in tumor tissue, and potentially improve antitumor efficacy. In the Stealth liposomal formulation, drug-loaded liposomes are coated with polyethylene glycol (PEG) molecules. This disguise allows Stealth liposomes to remain in circulation longer than their conventional counterparts because PEG is not recognized as a foreign substance by the body's immune system.

Antitumor activity was observed in human xenografts of Capan-2 pancreas. In tumor-bearing athymic nude mice treatment with S-CKD602 significantly enhanced antitumor efficacy compared to free CKD-602 (Yu NY, etal, AACR04, Abs. 3069). A phase I clinical trial of S-CDK602 is being conducted at the University of Pittsburgh Cancer Institute (UPCI; Pittsburgh, PA), under PI Ramesh Ramanathan, MD, in patients with advanced, refractory solid tumors. According to the protocol, S-CKD602 is administered on day 1 of each 21-day cycle. Trial goals are to determine MDT of S-CKD602 when administered every 3 weeks, the incidence and severity of toxicity of this regimen, and the pharmacokinetics of CKD-602 following administration of S-CKD602.

Antimitotics/Spindle Poisons/Cell-Cycle Modulators

Use of commercially available spindle poisons, alone or in combination regimens in the treatment of PDAC was described in Part II of this report on PDAC. In this segment, novel agents as well as novel cytotoxic drug targeting approaches are described that may prove applicable in the treatment of PDAC.

Cantuzumab mertansine (huC242-DM1), a targeted cytotoxic under development by ImmunoGen (Cambridge, MA), is an immunoconjugate of the potent spindle poison maytansine derivative (DM1), and huC242, a humanized monoclonal antibody (MAb) directed to a tumor-associated antigen (CanAg). Targeting approaches involving immunoconjugates comprising a targeting agent such as a MAb conjugated to a drug or toxin, although conceptually appealing, are faced by several limitations, including physiologic barriers to MAb extravasation and intratumoral penetration, host immune reactions to the conjugate, nonspecific conjugate uptake, low drug potency, and inefficient release of active drug, that have hampered the effective use of this technique as drug therapy. The antitumor activity of C242-DM1 was evaluated in various tumors including human xenograft models of pancreatic cancer.

ImmunoGen initiated a phase I clinical trial with huC242-DM1 in the treatment of patients with refractory solid tumors, in December 1999, at the Institute for Drug Development of CTRC, under PI Anthony Tolcher, MD, and at Brooke Army Medical Center (San Antonio, TX), designed to establish the safety of huC242-DM1 as a single dose treatment, and to characterize the agent's pharmacokinetic parameters, the presence of plasma-shed CanAg, and the development of both human antihuman antibody (HAHA) and human anti-DM1 conjugate antibody reactions. The starting dose, 22 mg/m^2 IV administred every 3 weeks, represented 1/10th of the lethal dose (LD10) in

mice. Dose-limiting (Grade 3) hepatic transaminase elevations occurred in 2/3 patients treated at 295 mg/m^2. Cumulative neurosensory changes (Grade 1) were observed in 6 patients at doses ≥132 mg/m^2. Other toxicities included ≤Grade 2 nausea and vomiting (n=9), dose-related Grade 1 myalgia/arthralgia (n=6), and Grade 1 neutropenia (n=2). A single Grade 2 hypersensitivity reaction occurred at 22 mg/m^2 but premedication permitted rechallenge. There was no evidence of HAHA (Rowinsky EK, etal, ASCO02, Abs. 118:30a).

Eventually, in this first clinical trial, 37 patients (median prior regimens=3) with solid tumors with documented CanAg expression, including colorectal (n=32), pancreatic (n=4), and lung (n=1) cancer, were treated with 110 doses of cantuzumab mertansine ranging from 22-295 mg/m^2 every 3 weeks. Dose-limiting transaminitis, thought to be related to Cmax and the extent of hepatic metastases, precluded tolerance of doses >235 mg/m^2. Hepatic, heme and neurosensory effects occurred, but were rarely severe with repetitive treatment at doses ≤235 mg/m^2. Strong expression (3+) of CanAg was documented in 68% of patients. Post-treatment tumor biopsies revealed intracellular immunoreactivity for DM1, concurrent with free CanAg immunoreactivity, suggesting SB-408075 penetration and DM1 release in the face of unsaturated CanAg. These findings, plus a strong relationship between hepatic toxicity and Cmax, and shed CanAg levels decreasing by 90% (median) after the 1st dose, provided the rationale for the 3-times weekly schedule, repeated 3 times every 4 weeks. The recommended dose for cantuzumab mertansine is 235 mg/m^2 IV every 3 weeks. Highlights of the treatment with this agent are absence of severe hematologic toxicity, preliminary evidence of MAb tumor localization, and encouraging biologic activity in chemotherapy-refractory patients (Tolcher AW, etal, J Clin Oncol, 15 Jan 2003;21(2):211-22).

In January 2003, ImmunoGen regained development and commercialization rights for cantuzumab mertansine from GlaxoSmithKline. The two companies had entered into a collaboration to develop this agent in February 1999. No payments were made by either company for the return of the product rights to ImmunoGen, which now holds the IND for cantuzumab mertansine and has rights to all clinical data generated in the phase I clinical trials. ImmunoGen expects to initiate phase II proof-of-concept clinical trials of cantuzumab mertansine during the first half of 2005.

CRx-026, under development by CombinatoRx (Boston, MA), is a novel antimitotic anticancer agent that contains two molecules, the antipsychotic drug chlorpromazine and the antiprotozoal drug pentamidine, acting in a coordinated manner to cause multiphase cell-cycle arrest by attacking multiple points in the network controlling tumor cell division and growth, leading to apoptosis.

CRx-026 is referred to as a 'syncretic' drug because it comprises multiple biologically active compounds that interact synergistically to provide an optimal therapeutic effect with the potential to minimize undesired side effects. Chlorpromazine potently and selectively inhibits the activity of hsEg5/KSP, a mitotic kinesin with an essential role in the mechanics of centrosome separation, while pentamidine has recently been reported to inhibit PRL phosphatases, which play an important role in regulating mitotic progression and proper chromosome separation. By modulating the activity of these two targets in a concerted fashion, CRx-026 achieves a synergistic anticancer effect greater than either component alone.

In a cell-free assay of kinesin function, chlorpromazine selectively inhibited the motor activity of hsEg5/KSP. Consistent with inhibition of this target, treatment with chlorpromazine led to incomplete centrosome separation and formation of monopolar spindles in mitosis. Also consistent with inhibition of kinesin activity, cell-cycle analysis revealed that treatment with chlorpromazine produced mitotic arrest. Combination of an hsEg5/KSP inhibitor and a PRL phosphatase inhibitor resulted in a substantially greater antiproliferative and cytotoxic effect than the individual components alone, across a broad spectrum of human cancer cell lines, including Panc1 (pancreas), that represent a range of different genotypic profiles, including MDR expression levels, p53 status, hormone sensitivity, retinoblastoma (Rb) status, and K-ras mutation status, indicating the broad antineoplastic potential of CRx-026. In additional studies, CRx-026 synergized with several approved classes of antineoplastic agents and exhibited potent antitumor activity in xenograft models (Zhang Y, etal, AACR04, Abs. 3999).

DHA-paclitaxel (Taxoprexin) is a docosahexaenoic acid (DHA)-linked paclitaxel conjugate based on the Targaceutical technology originally developed by Protarga whose assets were purchased in October 2003 by Luitpold Pharmaceutical (Shirley, NY), a subsidiary of Sankyo Pharma (Tokyo, Japan) for $7.5 million, after Protarga filed for Chapter 11 bankruptcy in August 2003. Targaceutical technology links drugs to natural fatty acids to improve their uptake by target tissues. In Taxoprexin, DHA is covalently conjugated via ester linkage to the 2'-OH position of paclitaxel; DHA-paclitaxel is only activated when the chemical bond between the DHA and paclitaxel is cleaved.

DHA is an omega-3 fatty acid that can be used by the body as an energy source or a component of cellular structure, and is necessary for the correct functioning of certain cell types. DHA is essential for infant nutrition and has been approved as an additive to infant formula. DHA appears to be a powerful targeting molecule. It crosses the blood-brain barrier being the most prevalent fatty acid in the brain, accounting for 25% of such acids that comprise 40% of the brain's weight.

In addition to targeting, DHA-paclitaxel is also designed to deliver more therapeutic agent to tumors and sustain therapeutic concentrations in tumor cells for longer periods

than possible with the unconjugated drug. In the phase I trial, 4.6 times as much taxane could be administered to patients relative to the prevailing paclitaxel dose. In addition, in animal studies, a therapeutic concentration was maintained within tumors 10 times longer with DHA-paclitaxel than paclitaxel alone.

A two-part, open label, dose-escalation, phase I clinical trial (protocol IDs: THERADEX-P01-02-12, PROTARGA-P01-02-12, VMRC-8846) of DHA-paclitaxel with or without gemcitabine in patients with advanced solid tumors or inoperable PDAC, was initiated in April 2003, to determine MDT and the recommended phase II dose, as well as toxicity, pharmacokinetics, and antitumor activity of these regimens in these patients. Approximately 24 to 36 patients are to be accrued for part I, and a maximum of 20 patients for part II of this trial. In part I, patients are assigned to 1 of 2 treatment groups. In Group I, patients are treated with IV DHA-paclitaxel, over 2 hours on days 1, 8, and 15. In Group II, patients are treated with DHA-paclitaxel as in group I, and with IV gemcitabine, over 30 minutes on days 1, 8, and 15. Courses in both groups are repeated every 28 days in the absence of disease progression or unacceptable toxicity. Patients with at least stable disease are treated with up to 6 courses. Cohorts of 3 to 6 patients in each group are treated with escalating doses of DHA-paclitaxel with and without gemcitabine until MTD is determined. In part II, patients are treated with DHA-paclitaxel and gemcitabine as in part I, group II, at the recommended phase II dose. Patients are followed for up to 30 days. Vincent J. Picozzi, MD, of the Cancer Institute at Virginia Mason Medical Center (Seattle, WA), is Protocol Chair.

A multicenter phase II clinical trial (protocol ID: THERADEX-P01-00-03; PROTARGA-P01-00-03; VMRC-8770) of DHA-paclitaxel in metastatic PDAC was initiated in June 2001, to determine tumor response rate, duration of response, and TTP, determine the overall survival and toxicity of this drug and assess quality of life (QoL). According to the protocol, patients were treated with DHA-paclitaxel IV over 2 hours on day 1, repeated every 21 days in the absence of disease progression or unacceptable toxicity. QoL was assessed at baseline, every 2 courses, and at completion of treatment. Patients are followed every 3 months. A total of 21 to 50 patients were to be accrued for this trial, which was closed in March 2003. Ross C. Donehower, MD, of Theradex (Princeton, NJ) is Study Chair.

A multinational, multicenter, phase I clinical trial of DHA-paclitaxel was conducted at Virginia Mason Medical Center, Rotterdam Cancer Center in the Netherlands, New Cross Hospital, (Wolverhampton, UK), Thomas Jefferson University (Philadelphia, PA), University of Glasgow in the UK, Kliniken-Essen-Mitte (Essen, Germany), New York University (New York, NY), Krankenhaus Nordwest (Frankfurt, Germany), and Johns Hopkins Hospital (Baltimore, MD), to evaluate DHA-paclitaxel in patients with inoperable, metastatic PDAC not previously treated with a cytotoxic agent. According to the protocol, patients were treated with Taxoprexin (1,100 mg/m^2) over 2 hours, every 21 days. As of October 31, 2002, 42 patients had enrolled and toxicity data were available for 30. A total of 91 courses (mean=2.17) were delivered. Toxicity was primarily Grade 4 neutropenia (n=16). Anemia was generally mild, <Grade 1 in 16 and ≤Grade 3 in 2. Other toxicities included Grade 1 (n=10), Grade 2 (n=1) and Grade 3 (n=1) thrombocytopenia. There were 2 hypersensitivity reactions, one Grade 1 and 1 Grade 2. Other toxicities included Grade 1 neuropathy (n=6), Grade 1 (n=6) and Grade 2 (n=2) stomatitis, and Grade 2 alopecia (n=2); 4 patients died during treatment from progressive disease. There was 1 objective response and disease stabilized in 18. At the time of this analysis, MST was 230 days (7.6 months), which compares favorably to the survival observed with single agent gemcitabine in several trials (Jacobs A, etal, ASCO03, Abs. 1090:272). This trial was closed to accrual in February 2003, after enrolling 51 patients. Among 45 evaluable patients, there was 1 PR (2.2%), and disease stabilized in 19 (42.2%) and progressed in 16 (35.6%). Median TTP was 46 days and overall survival was 158 days (5.2 months).

Disorazol E1, under development by Zentaris (Frankfurt am Main, Germany), in collaboration with Gesellschaft für Biotechnologische Forschung (Braunschweig, Germany), is a natural compound isolated from the myxobacterium *Sorangium cellulosum* (strain So ce12) that is potently cytotoxic in a panel of different tumor cell lines. Disorazol E1, a specific 1-phenyl-4-piperazinyl-carbonyl-substituted heterocyclic compound, was identified in a cell-based, high throughput screening (HTS) assay on five human tumor cell lines. Disorazol E1 exerted potent *in vitro* inhibition of cell proliferation in various human tumor-cell lines including the PDAC cell line ASPC1. Subsequently, a series of more than 30 analogs were synthesized and structure-activity-relationships (SAR) were determined for further optimization. These compounds are chemically stable in both basic and acidic media, and are easily accessible (Guenther EG Sr, etal, AACR02, Abs. 3654).

Disorazol E1 acts as a tubulin interacting agent inhibiting tubulin polymerization. Further studies have shown that disorazol E1 is a competitor of 3H-paclitaxel in tubulin binding assays. Upon treatment with disorazol E1, dividing KB/HeLa cells were efficiently arrested in the G2/M phase of the cell cycle with an IC_{50} value of 1.6 nM, while paclitaxel was found to have an IC_{50} value of 54 nM under the same assay conditions. Cells arrested in their division cycle were affected far less by disorazol E1 whose IC_{50} values for such cells are typically in the range of >4000 nM. In contrast to paclitaxel or vincristine, disorazol E1 was not a substrate of P-gp, and, consequently, retained antiproliferative efficacy in cell lines with MDR phenotypes (Baasner S, etal, AACR03, Abs. 2717).

DJ-927, an orally active novel semisynthetic taxane with higher solubility than paclitaxel, is under development by Daiichi Pharmaceutical (Tokyo, Japan). DJ-927 is also a poor substrate for P-gp and related resistance mechanisms.

A multicenter phase I clinical trial (protocol ID: 2447) was conducted at the Institute for Drug Development, University of Texas Health Science Center (San Antonio, TX), Karmanos Cancer Institute, Wayne State Medical Center (Detroit, MI), under PI Pat LoRusso, MD, and Brooke Army Medical Center, to determine MTD, dose-limiting toxicity (DLT) and pharmacokinetic behavior of DJ-927, administered orally every 3 weeks to patients with advanced malignancies. DJ-927 dose was escalated from the starting dose of 1.5 mg/m^2, in increments of 100% until Grade 2 toxicity was seen in course 1, and then by 50% until consistent DLT was noted. Among 22 patients (colorectal cancer=11, renal cell cancer=3, PDAC=2, hepatocellular carcinoma=1, and others=5) treated with 53 courses (median=2; range=1-8) at dose levels 1.5 mg/m^2 (n=3), 3 mg/m^2 (n=3), 6 mg/m^2 (n=3), 12 mg/m^2 (n=3), 18 mg/m^2 (n=4), 27 mg/m^2 (n=3) and 40 mg/m^2 (n=3), minimal drug-related toxicities occurred at dose levels <18 mg/m^2. At DJ 927 dose levels ≥18 mg/m^2, transient Grade 4 neutropenia (n=1), Grade 2 leucopenia, and Grade 1 thrombocytopenia were noted. Grade 2 diarrhea and Grade 1 fatigue also occurred at ≥18 mg/m^2 dose. A single patient treated with 3 courses of DJ 927 at 27 mg/m^2, experienced reversible Grade 3 peripheral sensory neurotoxicity. Stable disease for >6 months was observed in a patient with hepatocellular carcinoma. Preliminary PK results indicate dose-proportional absorption over the dose range evaluated to date. According to preliminary data, DJ-927 has favorable toxicologic, pharmacologic, and absorptive characteristics when administered orally and further dose escalation is ongoing (Beeram M, etal, ASCO03, Abs. 524:131).

Flavopiridol, under development by Aventis Pharmaceuticals, is a semisynthetic analog of rohitukine, isolated from the bark of the Indian tree *Dysoxylum binectariferum*. It is a potent CDK1 inhibitor, arresting cell-cycle progression in either G1 or G2. Flavopiridol was also shown to be a potent enhancer of tumor response to RT.

When evaluating the antitumor efficacy of flavopiridol in combination with RT in animal models, investigators at M. D. Anderson Cancer Center discovered that flavopiridol modestly delayed tumor growth as a single agent, but strongly potentiated tumor response to RT, with the radiopotentiating effect of flavopiridol being greater when drug treatment was started after RT. Flavopiridol acted through multiple mechanisms. *In vitro*, flavopiridol directly increased intrinsic radiosensitivity of tumor cells, which at the molecular level was associated with drug-induced downregulation in the expression of Ku70 and Ku80 proteins that play a role in DNA repair processes. *In vivo*, flavopiridol inhibited tumor cell-induced neoangiogenesis as demonstrated by a decrease in newly formed blood vessels at the site of tumor cell implantation (Mason AK, etal, ASCO03, Abs. 3478).

In addition to its potential synergism with RT, flavopiridol also enhances the effectiveness of gemcitabine. For instance, enhanced apoptosis was observed in human breast cancer cells treated with gemcitabine and flavopiridol at relatively low and clinically achievable drug concentrations, when flavopiridol followed gemcitabine. Similar effects were noted in other cell lines, including those from pancreatic, gastric, and colon cancer (Philip PA, etal, AACR01, Abs. 4877:908, and Jung CP, etal, AACR00, Abs. 209:32).

Based on this findings, a dose-escalation phase I clinical trial (protocol IDs: MSKCC-02057, NCI-5764) of flavopiridol, in combination with RT followed by gemcitabine, was initiated in October 2002 at Memorial Sloan-Kettering Cancer Center, under Protocol Chair Gary K. Schwartz, MD, in patients with locally advanced, inoperable PDAC. Trial objectives are to determine MDT, toxicity, pharmacokinetics and, preliminarily, the therapeutic activity of this regimen in this setting. Approximately 3 to 46 patients are to be accrued in this trial to be treated with flavopiridol IV over 1 hour, twice weekly, on days 1 and 4, or days 2 and 5, for 6 weeks. Concurrently, patients undergo RT once daily for 5 days each week for 5.5 weeks. Treatment continues in the absence of disease progression or unacceptable toxicity. One month after completion of RT, patients are treated with gemcitabine IV over 30 minutes weekly for 3 weeks. Treatment repeats every 4 weeks for up to 3 courses in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are treated with escalating doses of flavopiridol until MTD is determined. Then 10 additional patients are treated at the recommended phase II dose. Patients are followed at 4 weeks and then every 8 weeks thereafter.

MBT-0206, under development by Munich Biotech, is an antineovascular agent that combines the company's EndoTAG technology and paclitaxel, both acting synergistically to selectively target and destroy activated and reproducing tumor blood vessel endothelial cells, resulting in tumor growth inhibition or regression. MBT-0206 has been shown to exert strong antitumor efficacy in several tumor models (Kunstfeld R, etal, J Invest Dermatol, Mar 2003;120(3):476-82, and Schmitt-Sody M, etal, Clin Cancer Res, Jun 2003;9(6):2335-41), and to reduce the mitotic rate of endothelial cells in the tumor periphery. MBT-0206 was also shown to preferentially accumulate in endothelial cells of pancreatic tumor vessels in transgenic RIP-Tag2 mice.

MBT-0206 is suitable for chronic treatment as no induced tolerance nor immune reactions are expected. It could be used in tumors resistant to conventional treatment because the drug targets tumor blood vessel cells that do not develop resistance. Because of its design, MBT-0206 is expected to be exceptionally well tolerated and to show low organ and neurologic toxicity.

Investigators at the University of Munich-Großhadern, in Germany and at Munich Biotech, assessed the anticancer potential of a combination therapy of MBT-0206 with gemcitabine against highly metastatic human pancreatic cancer (L3.6pl) growing orthotopically in nude mice. Administration of MBT-0206 resulted in a strong growth inhibition of primary pancreatic tumors with a tumor size of 46% of the control size at the end of the treatment, which was similar to the efficacy of gemcitabine (47%). Combination therapy led to a strong enhancement of antitumor activity, resulting in a tumor size of only 21% of untreated control tumors. In addition, MBT-0206 alone significantly inhibited formation of liver metastases, and reduced both liver and lymph node metastases when combined with gemcitabine. Interestingly, a dose reduction of gemcitabine by 50% resulted in a comparable therapeutic efficacy and better tolerability of the combination therapy (Papyan A, etal, AACR04, Abs. 4104).

In December 2003, Munich Biotech initiated a clinical trial program of MBT-0206 as first line therapy in PDAC in combination with gemcitabine. A phase I clinical trial of this combination therapy is ongoing to evaluate its safety, tolerability and possible drug-drug interactions of both active ingredients. The trial is designed in a way so that the phase I portion will lead into a phase II trial, in which up to 70 patients will be enrolled.

Mebendazole (MZ), under development by Introgen Therapeutics (Austin, TX), is a derivative of benzimidazole. It binds to microtubules at a different site than the vinca alkaloids or taxanes; unlike colchicine, mebendazole blocked tumor cells at the G2/M phase and then induced apoptosis without inducing tetraploidy.

Mebendazole elicits a potent antitumor effect on human cancer cell lines both *in vitro* and *in vivo*. It arrested cells at the G2/M phase before the onset of apoptosis. Mebendazole treatment also resulted in mitochondrial cytochrome c release, followed by apoptosis. Additionally, mebendazole appeared to be a potent inhibitor of tumor-cell growth with little toxicity to normal WI38 and human umbilical vein endothelial cells. When administered PO to nu/nu mice, mebendazole strongly inhibited growth of human tumor xenografts and significantly reduced the number and size of tumors in an experimental model of lung metastasis. In assessing angiogenesis, significantly reduced vessel densities were found in mebendazole-treated mice compared to controls (Mukhopadhyay T, etal, Clin Cancer Res, Sep 2002;8(9):2963-9).

In a preclinical study, mebendazole inhibited the growth of cell lines including melanoma, lung, pancreatic, liver, breast, prostate, and ovarian cancer but had no affect on normal fibroblast, HUVEC, or bronchial epithelial cells. Growth inhibition and apoptosis occurred in cancer cells resistant to paclitaxel or vinorelbine, treated with mebendazole. Therefore, mebendazole is a potent antitumor agent against a wide range of cancer lines (Sasaki J, etal, AACR02, Abs. 1317).

Antimetabolites/Nucleoside Analogs

Antimetabolites such as nucleoside analogs kill cancer cells by becoming incorporated in the cell's deoxyribonucleic acid (DNA) and interfering with nucleic acid synthesis. Endogenous purine and pyrimidine nucleosides, derived from diet or produced in the body, are involved in RNA and DNA synthesis and in various physiologic functions. As anticancer agents, nucleoside analogs compete with physiologic nucleosides, interacting with a large number of intracellular targets to induce cytotoxicity. Antimetabolites such as pyrimidine nucleoside analogs are one of the first class of cytotoxic agents to be used in the treatment of both hematologic malignancies and solid tumors.

Among commercialized pyrimidine nucleoside analogs are 5´aza-cytidine, capecitabine, cytarabine, cytarabine ocfosphate, decitabine, depocyt, fludarabine, gemcitabine, tezacitabine, and troxacitabine. Use of gemcitabine in the management of advanced pancreatic cancer has spurred developers to attempt to identify novel nucleoside analogs with more favorable therapeutic profiles.

Nucleoside analogs require specific nucleoside transporters, present in the plasma membranes of most cell types, for permeation across biological membranes. Nucleoside transporters represent the route of uptake for cytotoxic nucleoside analogs used for cancer chemotherapy. There are five known, functionally characterized nucleoside transporters with varying substrate specificities for nucleosides, concentrative nucleoside transporters CNT1-CNT3, and solute carrier (SLC) transporters 28A1-28A3, which mediate the intracellular flux of nucleosides, and equilibrative nucleoside transporters ENT1-ENT2, SLC29A1, and SLC29A2, which mediate bidirectional facilitated diffusion of nucleosides (Mangravite LM, etal, Eur J Pharmacol, 31 Oct 2003;479(1-3):269-81).

Because different transporters are used by the various cytotoxic nucleoside analogs and because of variations in intracellular metabolism, these compounds differ in regards to their preferential interaction with certain targets, which may explain why some compounds are more effective against rapidly proliferating tumors and others against slow growing ones. Recent progress in the identification and characterization of nucleoside transporters and enzymes involved in nucleoside metabolism, as well as an understanding of the molecular mechanisms of anticancer nucleoside activity, provides opportunities for the development of new pyrimidine nucleoside analogs (Galmarini CM, etal, Expert Rev Anticancer Ther 2003;3(5):717–728).

Understanding of transport mechanisms and drug-target interactions may also provide solutions to drug resistance that is the limiting factor in the effectiveness of these drugs. For instance, over representation or under representation of certain nucleoside transporters in certain cells may contribute to their drug-resistance phenotype.

Exhibit 1
Novel Cytotoxics, Hormone Modulators, Prodrugs/Formulations and Related Agents 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)
Adventrx Pharmaceuticals ❑ U Southern California (USC), Sahlgrenska U Hospital	CoFactor	Vitamin analog (5, 10- methylenetetrahydrofolate) used as adjuvant therapy with 5-FU ❑ IV, PO	Phase II (begin 4/04)➢USA ❑ metastatic colorectal cancer
Alfacell ❑ Scientific Protein Laboratories	Ranpirnase ❑ Formerly P-30 protein ❑ Onconase	Cytotoxic ribonuclease with antitumor properties, originally isolated from the ova and early embryos of the Northern leopard frog (Rana pipiens); has a high degree of homology with the digestive enzyme pancreatic ribonuclease A ❑ IV	Phase III (completed 1/99)➢USA (combination) ❑ pancreatic cancer
Allergan	AGN193198	Novel retinoid-related ligand that inhibits cell-cycle progression and induces caspase-dependent apoptosis in human pancreatic cancer cells ❑ IV, PO	Preclin (ongoing 3/04)➢USA ❑ pancreatic cancer
Alza ❑ Chong Kun Dang Pharmaceutical	S-CKD-602, S-CDK602, AP-30	Stealth liposome formulation of CKD-602, a camptothecin derivative topo I inhibitor marketed abroad ❑ IV	Phase I (ongoing 4/04)➢USA ❑ advanced, refractory solid tumors
AmpliMed ❑ U Arizona	Imexon ❑ Amplimexon	Iminopyrolidone aziridine, a cyanoaziridine derivative that targets glutathione (GSH) ❑ IV	Phase I (begin 9/03)➢USA ❑ solid tumors
Austrianova ❑ U Veterinary Sciences (Austria), U Heidelberg	CapCell	Allogeneic cells encapsulated in cellulose sulphate, genetically modified to overexpress the cytochrome P450 enzyme, delivered by supraselective angiography to the tumor vasculature to locally activate systemically administered, low dose ifosfamide ❑ intratumoral, intra-arterial	Phase I/II (begin 7/98, completed 4/99)➢Europe (Germany) ❑ inoperable pancreatic cancer
Aventis Pharma ❑ National Cancer Institute (NCI)	Flavopiridol ❑ NSC 649890, L86-8275, HMR-1275, HMR1275	Semisynthetic analog of rohitukine, isolated from the bark of the Indian tree *Dysoxylum binectariferum*, that is a potent CDK1 inhibitor; arrests cell-cycle progression in either G1 or G2 ❑ continuous IV	Phase II (begin 10/02, ongoing 3/04)➢USA (combination) ❑ locally advanced, inoperable PDAC
Baxter Oncology ❑ German Cancer Research Centre	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 ❑ metastatic or inoperable, locally advanced PDAC, first line
Bioenvision ❑ Southern Research Institute (SRI), Ilex Oncology, Ferro Pfanstiehl Laboratories	Clofarabine ❑ Clofarex	2-fluoro-2-chloro substituted purine nucleoside analog that inhibits DNA synthesis ❑ PO, parenteral	Phase I (begin 7/02, ongoing 6/03) ➢USA, phase I (completed 6/03)➢USA ❑ solid tumors
BTG ❑ Cancer Research Campaign (CRC)	BGC 9331 (formerly ZD9331	Direct-acting, thymidylate synthase (TS) inhibitor ❑ IV, PO	Phase II/III (begin 11/99, completed 6/01)➢Europe (UK) (IV, combination), phase II/III (closed 02) Europe ❑ locally advanced or metastatic PDAC, first line

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Chiron ❑ Aventis Pharma, Kyowa Hakko Kogyo	Tezacitabine ❑ FMdC	Nucleoside analog with a chemical structure that is an intermediate between ara-C and gemcitabine ❑ PO, IV	Phase I (begin 3/00, completed 02, discontinued 3/04)➢USA (combination) ❑ advanced malignancies
CombinatoRx	CRx-026	Anticancer agent comprising two molecules, the antipsychotic drug chlorpromazine and the antiprotozoal drug pentamidine, that act in a coordinated way to cause multiphase cell-cycle arrest by attacking multiple points in the network controlling tumor-cell division and growth, leading to apoptosis ❑ PO	Preclin (ongoing 4/04)➢USA ❑ cancer
Daiichi Pharmaceutical	DJ-927	Orally active novel semisynthetic taxane, with higher solubility, that is a poor substrate for P-gp and related resistance mechanisms ❑ PO	Phase I (ongoing 6/03)➢USA ❑ advanced solid tumors
Daiichi Pharmaceutical	Exatecan mesylate ❑ DX-8951f	Synthetic, water-soluble camptothecin analog that exhibits more potent antitumor activity than other topo I inhibitors and a broader spectrum of activity ❑ IV	Phase II (begin 4/99, completed 4/00)➢USA; phase III (begin 8/01, completed 11/02)➢Europe (UK), phase III (begin 9/01, closed 1/03) ➢USA, Canada (combination) ❑ advanced or metastatic PDAC
Daiichi Pharmaceutical	Exatecan mesylate ❑ DE-310	Water-soluble conjugate of DX-8951 (exatecan), linked by a peptidyl spacer to a carboxymethyldextran polyalcohol polymer ❑ IV	Phase I (completed 03)➢USA ❑ advanced solid tumors
Eli Lilly ❑ Princeton U	Pemetrexed disodium ❑ LY 231514 ❑ Alimta	Multitargeted antifolate (MTA) that inhibits at least three enzymes, thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) involved in folate metabolism and DNA synthesis ❑ IV	Approved (2/04)➢USA ❑ inoperable malignant pleural mesothelioma; phase II (completed 1/00)➢USA, phase II (begin 9/99, closed 11/00)➢USA (combination), phase III (begin 5/02, closed 3/03) ➢USA, Europe, Australia, Taiwan, South America (combination) ❑ inoperable advanced or metastatic (Stage II, III, or IV) pancreatic cancer
Enzon	PEG-[gamma]-camptothecin ❑ Prothecan	Polyethylene glycol (PEG)-conjugated camptothecin-20-alanate, a water-soluble prodrug of camptothecin ❑ IV	Phase II (begin 01, discontinued 7/03)➢USA ❑ PDAC
Eximias Pharmaceutical ❑ Pfizer Global Research and Development	Nolatrexed dihydrochloride ❑ AG337 ❑ Thymitaq	Specific, membrane-permeable, lipophilic, non-polyglutamated inhibitor of thymidylate synthase (TS) ❑ continuous IV, PO, intraperitoneal, IM	Phase II (completed 9/95)➢USA, phase II (completed 6/03) ➢USA ❑ PDAC
ImmunoGen ❑ Pharmacia, Takeda Chemical Industries, Industrial Research	Cantuzumab mertansine ❑ SB-408075, huC242-DM1	Humanized MAb huC242 directed against CanAg antigen, a mucin-type tumor-associated glycoprotein, conjugated to a maytansinoid prodrug DM1; tumor-activated prodrug (TAP) ❑ IV	Phase I/II (begin 12/99, closed 02) ➢USA, phase I/II (begin 9/00, closed 02)➢USA, phase I/II (begin 5/01, closed 11/02)➢USA (dose intensification) ❑ advanced, refractory solid tumors
Insert Therapeutics ❑ Caltech	Camptothecin ❑ Cyclosert	Linear cyclodextrin-containing polymer incorporating camptothecin ❑ IV	Preclin (ongoing 3/04)➢USA ❑ solid tumors
Introgen Therapeutics	Mebendazole (MZ)	Binds to microtubules at a different site than the Vinca alkaloids or taxanes; unlike ochicine, MZ blocks tumor cells at the G2/M phase and induces apoptosis without inducing tetraploidy ❑ injection	Preclin (ongoing 3/04)➢USA ❑ solid tumors

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Keryx Biopharmaceuticals ❑ Pennsylvania State U	O(6)-benzylguanine (BG) ❑ O6BG, O6-BG ❑ Alkylade	Suicide substrate inactivator for O(6)-methylguanine-DNA methyltransferase (MGMT), a DNA repair	Phase II (begin 8/99, completed 2/02)➢USA ❑ resectable solid tumors
MacroMed ❑ Samyang Genex	Paclitaxel ❑ OncoGel	Formulation of Genexol paclitaxel based on the ReGel drug delivery system ❑ intratumoral	Phase II (planned 4/04) ➢USA ❑ PDAC
MGI Pharma ❑ Dainippon Pharmaceutical, U California	Irofulven [acylfulvene (6-HMAF)] ❑ MGI 114, MGI-114, NSC 683863	Semisynthetic analog of illudin S, a sesquiterpene isolated from the Jack o' lantern mushroom, *Omphalotus illudens* ❑ IV	Phase II (begin 4/99, closed 7/00) ➢USA, phase III (begin 2/01, discontinued 6/02)➢USA, Europe (UK) ❑ advanced, refractory PDAC
Munich Biotech	MBT-0206	Antineovascular agent that combines EndoTAG and paclitaxel both acting synergistically to selectively target and destroy activated and reproducing tumor blood vessel endothelial cells, resulting in tumor growth inhibition or regression ❑ IV	Phase I/II (begin 12/03)➢Europe (combination) ❑ PDAC
Munich Biotech	MBT-0312	Antineovascular cancer agent that combines EndoTAG and camptothecin both acting synergistically to selectively target and destroy activated and reproducing tumor blood vessel endothelial cells, resulting in tumor growth inhibition or regression ❑ IV	Phase I (begin 12/03)➢Europe ❑ advanced or metastatic solid tumors
Myriad Genetics ❑ Maxim Pharmaceuticals	MPC-6827	Lead agent within the MX128495 series of apoptosis inducing compounds ❑ IV	Preclin (ongoing 4/04)➢USA ❑ solid tumors
NeoPharm	LE-SN38 (7-ethyl-10-hydroxy-camptothecin)	Active metabolite of CPT-11, encapsulated in cardiolipin liposomes ❑ IV	Phase I (begin 10/02, ongoing 3/04) ➢USA ❑ advanced solid tumors
NewBiotics ❑ Salk Institute	NB1011 ❑ Thymectacin	Mimic of deoxyuridylic acid (dUMP), the normal substrate for thymidylate synthase (TS), which is overexpressed in many malignancies, especially those treated with 5-FU, leading to drug resistance and treatment failure ❑ IV	Phase I/II (begin 1/02)➢USA ❑ metastatic or relapsed colon cancer, refractory to 5-FU
OSI Pharmaceuticals ❑ Southern Research Institute, (SRI), Gilead Sciences	OSI-7836 (GS 7836, GS7836), T-araC	Nucleoside analog of araC in development as a next generation gemcitabine ❑ IV	Phase I (begin 11/01, ongoing 12/03) ➢Canada ❑ refractory solid tumors
Pharmacyclics ❑ Abbott Laboratories, U Texas, Celanese	Motexafin gadolinium ❑ PCI-0120 ❑ Xcytrin	Gadolinium texaphyrin (Gd-Tex) that selectively accumulates in cancer cells sensitizing them to radiation ❑ IV	Phase I (begin 9/98, closed 4/02) ➢USA ❑ locally advanced, inoperable PDAC
Pro-Pharmaceuticals	Galactomycin ❑ Davanat-1 ❑ Pro-5FU	Reformulation of 5-FU with a non-toxic carbohydrate-based compound that recognizes and adheres to specific binding sites on the surface of cancer cells; enhances the effectiveness of 5-FU while reducing its toxicity ❑ IV	Phase I (begin 2/03)➢USA ❑ advanced, refractory, solid tumors
Protarga (Luitpold) ❑ Abbott Laboratories, Bryn Mawr College, Martek Biosciences	DHA-paclitaxel ❑ Taxoprexin	Docosahexaenoic acid (DHA)-linked paclitaxel conjugate ❑ PO	Phase I (begin 4/03, ongoing 4/04) ➢USA (combination), phase I (completed 2/03)➢USA, Europe (Germany, Netherlands, UK); phase II (begin 6/01, closed 3/03)➢USA ❑ recurrent or metastatic PDAC

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Salmedix ❑ University of California San Diego (UCSD)	SDX-102	Amino acid analog (l-alanosine), a small molecule that inhibits a key enzyme in the *de novo* synthesis pathway for adenosine, the precursor for ATP ❑ continuous IV	Phase II (begin 4/03, ongoing 4/04) ➢USA ❑ solid tumors
Sanofi-Synthelabo	Thioxanthone ❑ SR271425, SR-271425, BCN326862, WIN71425	Third-generation thioxanthone with broad preclinical activity ❑ IV, PO	Phase I (ongoing 4/04)➢USA ❑ refractory solid tumors
Sankyo ❑ Cyclacel	CYC682 (formerly CS-682)	Antimetabolite prodrug of the novel nucleoside CNDAC, a lipophilic deoxycytosine analog; DNA polymerase a inhibitor ❑ PO	Phase I (begin 2/97, completed 02) ➢USA ❑ refractory solid tumors
Schering (Berlex Biosciences)	4'-thio-FAC [1-(2-deoxy-2-fluoro-4-thio-β-D-arabinofuranosyl) cytosine]	Nucleoside analog 100-fold more potent than gemcitabine in inhibiting DNA polymerase α ❑ IV	Preclin (ongoing 4/04)➢USA, Europe (Germany)
Shire Pharmaceuticals	Troxacitabine ❑ BCH-4556 ❑ Troxatyl	Stereochemically unnatural L-nucleoside analog that inhibits DNA polymerases; complete DNA chain terminator ❑ IV, PO	Phase II (begin 00, closed 2/02) ➢USA, Europe (UK), phase II (begin 8/00, completed 9/01)➢UK, Canada ❑ PDAC
SuperGen ❑ Stehlin Foundation for Cancer Research, Clayton Foundation for Research, RTP Pharma	Rubitecan ❑ RFS 2000, 9-nitrocamptothecin, 9NC, nitrocamptothecin ❑ Orathecin	Third-generation, water-insoluble camptothecin analog; topo I inhibitor, causing single strand breaks in the DNA of rapidly dividing tumor cells ❑ PO, inhaled, IM, IV, catheter-delivered, stent-delivered	NDA (filed 1/04)➢USA; phase III (closed 5/01)➢USA, phase III (closed 12/01)➢USA, phase II (begin 9/98, completed 4/00) ➢USA; phase I (ongoing 5/01) ➢USA (combination) ❑ refractory, advanced or metastatic PDAC; phase III (begin 11/98, closed 2/02) ➢USA ❑ chemotherapy-naïve locally advanced or metastatic PDAC
Telik ❑ Taiho Pharmaceutical	TLK286 (formerly TER286) ❑ Telcyta	Latent drug (prodrug) activated by human glutathione S-transferase (GST) isoforms P1-1 and A1-1 to produce an alkylating agent ❑ IV	Phase I (begin 1/00, complete 2/01) ➢USA ❑ refractory solid tumors
Vion Pharmaceuticals ❑ Yale University, Beijing Pason Pharmaceuticals	3-AP, OCX-191 ❑ Triapine	Heterocyclic carboxaldehyde thiosemicarbazone, potent ribonucleotide reductase (RR) inhibitor ❑ IV, PO	Phase II (begin 6/03, ongoing 4/04)➢Europe (Belgium, The Netherlands, UK), USA (combination), phase II (begin 2/04) Canada (combination) ❑ advanced or metastatic PDAC
Zentaris ❑ Gesellschaft für Biotechnologische Forschung	Disorazol E1	Natural compound isolated from the myxobacterium *Sorangium cellulosum* (strain So ce12), that is potently cytotoxic in a panel of different tumor cell lines ❑ IV	Research (ongoing 9/03)➢Europe (Germany) ❑ solid tumors

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

4'-Thio-FAC [1-(2-deoxy-2-fluoro-4-thio-β-D-arabinofuranosyl) cytosine], a pyrimidine (deoxycytidine) nucleoside analog, is under investigation by Berlex Biosciences (Richmond, CA) and Schering (Berlin, Germany), in collaboration with Yamasa (Choshi, Chiba, Japan). 4'-Thio-FAC inhibits cellular DNA, but not RNA and protein synthesis. This agent was shown to be 100-fold more potent than gemcitabine in inhibiting DNA polymerase α (Miura S, etal, Jpn J Cancer Res, May 2001;92(5):562-7) but showed moderate inhibition of DNA polymerase β and little inhibition of DNA polymerase γ. Gemcitabine does not show potent inhibition of these three DNA polymerases, implying that the main target enzymes of 4'-thio-FAC and gemcitabine are different. This hypothesis is supported by the fact that there was a synergistic effect of the two drugs in an *in vitro* model.

When the effectiveness of 4'-thio-FAC against human pancreatic and ovarian tumors was assessed, potent *in vitro*

antiproliferative effects were observed against pancreatic (Capan-1, MiaPaca-2, BxPC-3) and ovarian (SK-OV-3, OVCAR-3, ES-2) cell lines. Synergistic activities were also observed in combination with gemcitabine in BxPC-3 pancreatic cancer cells, and with paclitaxel or carboplatin in ES-2 and SK-OV-3 ovarian cancer cells. *In vivo*, orally administered 4'-thio-FAC significantly inhibited growth of gemcitabine-resistant BxPC-3 pancreatic tumors and induced regression of gemcitabine-refractory Capan-1 tumors. It was also a highly effective inhibitor of ovarian cancer dissemination in the peritoneal cavity. In both the SK-OV-3 and ES-2 ovarian cancer models, 4'-thio-FAC prolonged survival to a greater extent than that observed with gemcitabine or paclitaxel. In view of the superior therapeutic profile of 4'-thio-FAC to that of gemcitabine, advancement to clinical trials is justified (Zajchowski D, etal, AACR04, Abs. 3087).

Clofarabine (Clofarex), under development by Bioenvision (London, UK), in collaboration with Ilex Oncology (San Antonio, TX), is a 2-fluoro-2-chloro substituted purine nucleoside analog. Clofarabine, a halogenated nucleoside analog, is in the same chemical class as fludarabine, cladribine and gemcitabine.

A phase I clinical trial to define the MTD and DLT of clofarabine administered IV on days 1, 8, and 15 of a 28 day cycle in treating refractory solid tumors was initiated in June 2002 at Mary Crowley Medical Research Center (Dallas, TX). Among 12 patients enrolled in 4 cohorts of 3 patients each, tumor types were colon, pancreatic, prostate, bladder, head and neck, and laryngeal cancer, nsclc, and cholangiocarcinoma. The starting dose of clofarabine was 4 mg/m^2 escalated to 6, 10, and 14 mg/m^2 in subsequent cohorts, with planned escalations to 18 and 22 mg/m^2. Clofarabine was well tolerated. Grade 2 lymphopenia was noted in at least 5 patients. One patient with advanced PDAC achieved a best response of stable disease after 4 cycles of treatment and 2 patients were taken off study for progressive disease (Cunningham CC, etal, ASCO03, Abs. 605:151).

CYC682 (1-(2-C-cyano-2-deoxy-β-D-arabino-pentofuranosyl)-N4-palmitoylcytosine) is a novel orally administered 2'deoxycytidine-type antimetabolite with a unique mechanism of action. It incorporates its active metabolite CNDAC into DNA causing DNA strand breakage. It is also a potent inhibitor of DNA polymerase.

CYC682 was originally developed by Sankyo as CS-682. In December 2003, Cyclacel (Dundee, UK) acquired exclusive rights in nearly all world territories to CYC682. Sankyo received an upfront payment and will receive milestones and royalties from Cyclacel. Sankyo retained a right of first negotiation to market the compound in Japan. Specific financial terms were not disclosed.

Investigators at the University of California San Diego and AntiCancer (San Diego, CA) demonstrated that CYC682 is an effective adjuvant therapy in orthotopic models of aggressive human pancreatic cancer. Treatment with CYC682 resulted in a significant increase in survival in an orthotopic mouse model of aggressive human pancreatic cancer. To evaluate CYC682 as an adjuvant approach, treatment was initiated 7 days after surgical orthotopic implantation of red fluorescent protein (RFP)-expressing MIA-PaCa-2 tumors onto the pancreas of nude mice. Total tumor burden negatively correlated with survival. Untreated mice died of disseminated disease with an MST of 26 days. Surgical resection alone conferred a small (MST=28 days) but significant survival. Primary CS-682 treatment at all doses also significantly prolonged survival (MST=34-38 days) compared to untreated animals, and was more effective than surgery alone. Maximal survival (MST=48 days) with 30% of animals surviving >60 days, was achieved by adjuvant CS-682, administered after surgical resection of the primary pancreatic tumor. CS-682 also decreased development of malignant ascites and formation of metastases, which were reduced significantly in number in the diaphragm, lymph nodes, liver, and kidney. These results demonstrate that adjuvant oral administration of CYC682 for PDAC is highly effective with acceptable toxicity suggesting it may be used in this disease in appropriate combinations (Katz MH, AACR04, Abs. 3045, and Katz MH, Cancer Res 2003:63, 5521-5525).

CYC682 completed two phase I clinical trials in the USA involving 88 patients with a variety of tumors. According to results, CYC682 is well tolerated in man with myelosuppression as the DLT. Stable disease was observed in 17 patients after CYC682 treatment, including one patient with ovarian cancer who experienced a minor response and one patient with a gastrointestinal stromal tumor (GIST) who was treated for over 90 weeks with stable disease after failing multiple therapies. Cyclacel plans to begin phase I/II clinical studies with CYC682 in 2004.

OSI-7836 (GS 7836, GS7836), or 4'-thio-ara-C, under development by OSI Pharmaceuticals (Melville, NY), is a nucleoside analog of araC (ß-D-arabinosylcytosine) positioned as a next-generation gemcitabine. The drug was originally developed by Southern Research Institute (SRI; Birmingham, AL), and licensed exclusively to Gilead Sciences (Foster City, CA) in December 2000.

OSI acquired GS7836 from Gilead Sciences in December 2001. OSI-7836 (T-araC) and araC are structurally similar analogs with vastly different antitumor activities. Unlike araC that has limited activity against solid tumors, in preclinical studies OSI-7836 demonstrated activity against a variety of solid tumors including, nsclc, and colon, prostate, renal, breast, and pancreatic cancer (Waud WR, etal, Cancer Chemother Pharmacol, May 2003;51(5):422-6; epub 1 Apr 2003). Also, studies showed that OSI-7836 has a different mechanism of tumor growth inhibition, blocking the cell division cycle at a different point (the G2 phase) than gemcitabine. OSI-7836 is more active than gemcitabine in various xenografts and also appears to be less schedule dependent than gemcitabine.

As with other nucleoside analogs, the proposed mechanism of action of OSI-7836 involves phosphorylation to the triphosphate form followed by incorporation into cellular DNA, leading to cell death. Apoptosis induced by OSI-7836 in xenografted tumors was significantly greater in cycling cells compared to confluent non-cycling cells, despite only a modest increase in intracellular OSI-7836 triphosphate concentration. Cell-cycling studies supported the requirement of DNA incorporation in the toxicity of OSI-7836. OSI-7836 is incorporated in internal linkages in tumor DNA in a manner that appears to be dose independent, at the doses tested, and does not appear to accumulate over repeated dosing, indicating that if DNA incorporation is a toxic event, the relationships between administered dose, DNA incorporation, and toxicity are complex (Richardson F, etal, AACR03, Abs. 2632).

In November 2001, Gilead initiated a dose-escalation phase I clinical trial (protocol ID: NCIC CTG IND.147) of OSI-7836, in Canada, after the Canadian Therapeutics Products Directorate (TPD) approved Gilead's IND application in September 2001. The study, to enroll up to 40 patients with refractory solid tumors at two sites, was conducted in collaboration with the National Cancer Institute Canada Clinical Trials Group (NCIC-CTG). Glenwood Goss, MD, at Ottawa Regional Cancer Centre and Lillian Siu, MD at University Health Network-OCI/Princess Margaret Hospital are Protocol Chairs. An accelerated dose-escalation design was used in this trial with a starting dose of 100 mg/m^2. Among 9 patients (colorectal cancer=3, nsclc=2, and other primaries=4) entered at 4 dose levels (100, 200, 400 and 600 mg/m^2), Grade 1 or 2 emesis necessitated introduction of prophylactic 5-HT3 antiemetics at the 400 mg/m^2 dose level. No hematologic toxicity was seen other than lymphopenia seen in all dose levels. Both patients treated at the 600 mg/m^2 dose level experienced protocol-defined DLT, including Grade 3 rash, fever, seizure, and Grade 3 fatigue, respectively; both patients also had lymphopenia and herpes simplex. The 5th dose level opened at 500 mg/m^2. One patient with lymphoepithelioma of the thymus showed evidence of tumor shrinkage in pulmonary lesions (Goss G, etal, EORTC-NCI-AACR02, Abs. 66, and Eur J Cancer, Nov 2002; 38 (Suppl 7):25). This trial was closed to enrollment in February 2004. If toxicity issues, such as high grade fatigue, are not resolved with scheduling adjustments, OSI plans to suspend the development of OSI-7836 as a proprietary program and will look to outlicense the candidate for further development.

Tezacitabine (FMdC) is a pyrimidine nucleoside analog with a chemical structure that is an intermediate between ara-C and gemcitabine. In March 2004, Chiron discontinued further development of tezacitabine based on an data analysis from a phase II clinical trial in patients with gastroesophageal cancer. The compound did not demonstrate sufficient antitumor activity in the trial to satisfy Chiron's predetermined criteria to advance the program. The company had previously announced that it had concluded phase II development of tezacitabine in patients with colorectal cancer. No safety issues were identified in treatment with tezacitabine.

Chiron acquired tezacitabine in March 2002 with its purchase of Matrix Pharmaceutical, which had, in turn, licensed the drug from Aventis Pharmaceuticals in September 1998. Kyowa Hakko Kogyo (Tokyo, Japan), which owned licensing rights of FMdC in Japan, discontinued development of the drug during phase I clinical trials in May 2000.

Troxacitabine (Troxatyl) is a dioxolane nucleoside analog of cytidine, under development by Shire Pharmaceuticals (Laval, Quebec, Canada). Shire exited the oncology field in 2003 and plans to outlicense Troxatyl.

Incorporation of troxacitabine during DNA replication results in termination of DNA synthesis and inhibition of DNA polymerase. Troxacitabine is a complete DNA chain terminator. Troxacitabine is an L-stereoisomer cytosine analog while gemcitabine is a natural D-isomer. These isomeric configurations confer to these two drugs different properties in regards to membrane transport, deamination and DNA repair. Troxacitabine is unique among nucleoside analogs because of its structure, pharmacokinetics, cellular transport mechanisms, and insensitivity to cancer cell mechanisms of resistance. In contrast to other cytidine analogs used in cancer therapy, troxacitabine cannot be degraded by cellular cytidine deaminases.

In preclinical studies, Troxatyl has shown a broad-spectrum cytotoxic activity *in vitro* and potent antitumor effects *in vivo* against a number of wild type and MDR cell lines, suggesting a potential for multiple indications. Moreover, synergistic antitumor effects were observed when Troxatyl was combined with other cytotoxic drugs, including gemcitabine, cytarabine and cisplatin, as well as the targeted therapeutic imatinib mesylate (Gleevec/Glivec; Novartis). Phase I clinical trials have also been designed to investigate if continuous infusion further enhances the antitumor activity of Troxatyl.

After favorable preclinical data in pancreatic human tumor xenograft models, and encouraging results from a phase I trial, a phase II clinical trial involving about 250 patients with a number of different types of solid tumors, including PDAC, was initiated and is ongoing. An extensive clinical program was undertaken to determine the safety and activity of Troxatyl in a number of solid tumors using an every-three-week administration schedule. Subsequently, a daily, 5-day monthly schedule was also investigated in the clinic, in pancreatic and renal cancer. Additional Troxatyl trials were also conducted in advanced solid tumors in combination with cisplatin and paclitaxel. Across all studies, the adverse event profile for Troxatyl have been manageable and transient, with no CNS adverse events or hepatotoxicity observed. Troxacitabine is the first dioxolane nucleoside analog to be investigated as an anticancer agent in clinical trials.

A multicenter phase II clinical trial of toxacitabine in PDAC was initiated at Christie Hospital NHS Trust (Withington, Machester, UK) under PI R. E. Hawkins, MD, in August 2000, and at the University of Leicester, in the UK, in September 2000, under PI W. P. Steward, MD. The trial was conducted by Ilex Oncology in Canada and the UK. In this trial, troxacitabine (1.5 mg/m^2) was administered as first line therapy, daily, for 5 days, every 4 weeks, to patients with advanced PDAC. Between August 2000 and September 2001, 55 patients were enrolled, with 54 being evaluable for toxicity and 51 for efficacy. A total of 158 cycles of therapy were administered with 5 patients still on the trial, including 1 patient who remains stable following 12 cycles of therapy. Toxicities included fatigue, anorexia, nausea, skin rash, neutropenia and hand-foot syndrome. Disease stabilized in 24 patients and progressed in 27 with no major responses noted. Reductions in CA 19.9 tumor markers were noted in patients with stable disease. Progression-free survival (PFS) was 24/51 (47%) at 2 months with 25/54 patients still alive at the time of this report (Lapointe R, etal, ASCO02, Abs. 565:142a).

A dose-escalation phase I clinical trial of troxacitabine and gemcitabine in advanced solid malignancies, represents a new paradigm in oncology by combining two cytosine analogs. In this trial, conducted at Arizona Cancer Center (Tucson, AZ) and Leicester Royal Infirmary in the UK, gemcitabine was administered first as a 30-minute infusion followed by a 30-minute infusion of troxacitabine on day 1. Gemcitabine was then administered alone on day 8 and treatment cycles were repeated every 3 weeks. Dose levels (troxacitabine/gemcitabine) were 6 mg/m^2/1000 mg/m^2 in level 0, 8 mg/m^2/1000 mg/m^2 in level 1, and 10 mg/m^2/1000 mg/m^2 in level 2. A total of 30 cycles (range=1-5) were delivered to 11 patients (3 at level 0, 7 at level 1, and 1 in level 2). There was no DLT except one case of Grade 3 hypoalbuminemia and Grade 4 hypocalcemia at level 1, which was considered likely to be a laboratory error. Cycle 1 hematologic toxicities included Grade 3 neutropenia (n=4), thrombocytopenia (n=2) and anemia (n=1) with no Grade 4 events. In all cycles, Grade 3 and 4 neutropenia were seen in 4 and 2 patients, respectively; one patient each had Grade 3 and 4 anemia and 3 patients had Grade 3 thrombocytopenia; there were no Grade 4 events. Other reported Grade 3 toxicities were pneumonia (n=2), fatigue (n=1) and hand-foot syndrome (n=1). Best responses in six evaluable patients were 5 cases of stable disease; disease progressed in 1 patient. The troxacitabine gemcitabine combination appears to be well tolerated with doses currently approaching single agent MTD levels (Von Hoff DD, etal, ASCO03, Abs. 566).

Antimetabolites/Thymidylate Synthase (TS) Inhibitors/Antifolates

Another class of antimetabolites, the thymidylate synthase (TS) inhibitors, have also demonstrated activity in pancreatic cancer. The leading drug in the TS group, 5-FU, has been used extensively alone and in combination and multimodality approaches in the treatment of advanced PDAC. The broad anticancer activity of 5-FU has encouraged development of other TS inhibitors and, in February 2004, the FDA approved pemetrexed disodium (Alimta; Lilly) in combination with cisplatin, for the treatment of inoperable mesothelioma.

AG2037, under development by Pfizer Global Research & Development-La Jolla, is a potent glycinamide ribonucleotide formyl transferase (GARFT) enzyme inhibitor. AG2037 was engineered using structure-based design to maintain affinity toward the reduced folate carrier (RFC) as well as folypolyglutamate synthetase (FPGS), and to exhibit increased inhibitory activity toward GARFT, while avoiding binding to membrane folate-binding protein (mFBP), a protein believed to be important in the side effects of earlier GARFT inhibitors.

Generally, one of the limitations of GARFT inhibitors is their unfavorable side effects profile. As a guide for phase I clinical testing, allometric scaling has been used to prospectively estimate the human MTD and no adverse effect dose level for AG2037, based on preclinical pharmacokinetic and toxicologic findings (Bloom LA, etal, AACR01, Abs. 1582:294). Allometric interspecies scaling has been shown to successfully predict pharmacokinetic parameter values in humans from data for classical antifolates, and when applied to preclinical data for AG2037, an MTD of 4.5 mg/m^2 was predicted, supporting a 1 mg/m^2 dose level as a safe starting dose in humans.

One strategy to reduce the toxic potential of AG2037 is to employ it against tumors with genomic instability resulting in silencing of certain genes. Absence of specific proteins in tumors may result in a unique opportunity to develop novel treatment strategies. Along these lines, selective prevention of AG2037-induced toxicity may be achieved through the methylthioadenosine phosphorylase (MTAP) purine salvage pathway. In this approach, AG2037 is administered in combination with methylthioadenosine in MTAP-deficient tumors. The genomic difference in MTAP status between MTAP-deficient tumors and normal tissue allows the combination of an agent that inhibits *de novo* purine synthesis with an agent such as MTA, the natural substrate for MTAP, to potentially rescue normal cells from toxic side effects and increase the therapeutic window of the inhibitor.

In one experiment in mice bearing the BxPC-3 human pancreatic xenograft, adding MTA to the administration of AG2037 decreased its toxicity by 16-fold without affecting the antitumor activity at each dose level of AG2037, increasing the therapeutic window of AG2037 by 16-fold. In another experiment mice bearing the Panc1 human pancreatic xenograft, AG2037-induced weight loss in the mice was reduced 2-fold by administration of MTA while the antitumor activity of AG2037 was not affected. Thus MTA selectively reduced the toxicity of AG2037 without affecting its activity against MTAP-deficient tumors, increasing the therapeutic window (Kingtree JH, etal, AACR04, Abs. 4628, and Bloom LA, etal, AACR04, Abs. 4000).

The safety and pharmacokinetics of AG2037 as a single bolus IV infusion were evaluated in two phase I clinical trials in advanced cancer. In one trial (protocol ID: AG2037-002) AG2037 was administered every 3 weeks at a dose range of 1-256 mg/m^2, and in another (protocol ID: UAB-0052; AG-2037-003-A2; UAB-F001227008; NCI-G01-1954) it was administered every week for 3 weeks followed by a week off, at a dose range of 3-60 mg/m^2. The multicenter AG-2037-003 clinical trial, initiated in June 2001 at H. Lee Moffitt Cancer Center (Tampa, FL), University of Alabama (UAB) Comprehensive Cancer Center (Birmingham, AL), and at Arizona Cancer Center (Tucson, AZ), under Protocol Chair Francisco Robert, MD of UAB, was closed in September 2003. There were no responses in 29 patients treated in the AG2037-002 trial. Most AG2037-related adverse events were ≤Grade 2 nausea/vomiting, fatigue and diarrhea that were not related to dose level. No DLT was observed. In the AG2037-003 trial, among 23 patients treated, ≤Grade 2 adverse events were nausea, fatigue, stomatitis, and anemia. DLT in 2 patients was Grade 3 hyperbilirubinemia, anemia, and thrombocytopenia in 1 patient at the 12 mg/m^2 cohort, and Grade 3 fatigue, mucositis, and thrombocytopenia in 1 patient at the 60 mg/m^2 cohort. In both trials, AG2037 revealed linear PK, while total plasma clearance correlated with creatinine clearance. One PR was observed in a patient with refractory colorectal cancer at the 6 mg/m^2 cohort. AG2037 was generally well tolerated in the doses administered in both protocols, and demonstrated antitumor activity (Garrett C, etal, ASCO02, Abs. 429:108a).

BGC 9331, formerly ZD9331, is a novel, direct acting antifolate in phase II clinical development, primarily in gastric cancer and other GI malignancies. Currently, development is funded by BTG (London, UK), after AstraZeneca returned exclusive rights to the drug to BTG in June 2002. BTG is currently sponsoring clinical trials with BGC 9331 before seeking a new partner.

A randomized, phase II/III clinical trial, conducted at Clatterbridge Centre for Oncology (Bebington, UK), compared the efficacy and tolerability of ZD9331 with gemcitabine in chemonaive patients with locally advanced or metastatic PDAC. In this trial IV ZD9331 (130 mg/m^2) was administered on days 1 and 8 of a 3-week cycle (n=30) or IV gemcitabine (1 g/m^2), once weekly, for 7 weeks, followed by 1-week rest and then on days 1, 8 and 15 of a 28-day cycle (n=25). Preliminary results demonstrated that ZD9331 was at least as effective as gemcitabine, producing similar efficacy and palliation outcomes. More patients (13%) treated with ZD9331 were alive at the data cut-off point compared with gemcitabine (8%). MST of 152 days with ZD9331 was longer than 109 days with gemcitabine. Also, TTP of 70 days was longer with ZD9331 compared to 58 days with gemcitabine. Grade 1/2 nausea and vomiting were the most common toxicities in both groups. Withdrawal from adverse events occurred in 10 (33.3%) ZD9331 patients and 5 (20.0%) gemcitabine patients. There were 2 drug-related deaths in the ZD9331-treated group. These results suggest that in PDAC, ZD9331 is equivalent to gemcitabine and may offer a promising alternative to current therapies. Although the trial was terminated early, the data show that ZD9331 showed promising efficacy compared to gemcitabine (Smith DB, etal, ASCO02, Abs. 574:144a, and Smith DB and Gallagher N, Eur J Cancer, Jul 2003;39(10):1377-83).

Davanat-1, under development by Pro-Pharmaceuticals (Newton, MA), is a reformulation of 5-FU with a nontoxic carbohydrate-based compound. Davanat-1 enhances entry of 5-FU into tumor cells and its antineoplastic effects while it competes with 5-FU in the liver reducing entry of 5-FU into liver cells, thus reducing its toxicity. The Davanat technology reformulates cytotoxic drugs with nontoxic carbohydrate-based compounds that recognize and adhere to specific binding sites on the surface of cancer cells. Davanat combines a pharmaceutical compound, such as a chemotherapeutic agent, with a spacer and a galactose. The first site on the spacer is covalently linked to the therapeutic agent and a second site on the spacer is covalently linked to the galactose by an ether bond to form a conjugate. This conjugate targets sugar-specific binding sites (lectins) found on cancer cells with the goal of improving efficacy and reducing toxicity of proven, commonly used chemotherapy drugs.

A multicenter, open label, phase I clinical trial of Davanat-1 was initiated in February 2003 in patients with advanced solid tumors refractory to standard surgical, RT, and chemotherapeutic regimens. Among participating centers are the Norris Cotton Cancer Center at Dartmouth-Hitchcock Medical Center in Lebanon, NH, under Raymond Perez, MD, as the PI, the Comprehensive Cancer Center at the University of Michigan (Ann Arbor, MI), the Ochsner Cancer Institute (New Orleans, LA), and Florida Oncology Associates (Jacksonville, FL) under PI Yousif Abubakr, MD. A phase II clinical trial of Davanat-1 as third line treatment of refractory metastatic colorectal cancer was initiated in January 2004.

Nolatrexed dihydrochloride (Thymitaq), under development by Eximias Pharmaceutical (Berwyn, PA), is a specific, membrane-permeable, lipophilic, non-polyglutamated inhibitor of TS. Nolatrexed was initially developed as AG337. This drug is in a multicenter phase III clinical trial being compared to doxorubicin therapy in patients with unresectable liver cancer. This trial is designed to confirm the results of three completed phase II trials, which demonstrated a survival benefit for Thymitaq-treated patients with unresectable liver cancer.

In a phase II clinical trial, 36 patients with metastatic PDAC were treated with nolatrexed (795 mg/m^2) as a continuous infusion over 5 days, every 3-4 weeks, at Queen's Medical Center (Honolulu, HI), Scripps Clinic (La Jolla, CA), and Weill Medical College of Cornell University (NY, NY). Dose adjustments (15-20%) were made according to toxicity. Tumor profiles included 26 (72%) liver, 25% lymph

node, 11% lung, and 17% other metastatic sites. The protocol did not allow initial use of prophylactic agents. Nonhematologic toxicities were nausea or vomiting in 86% and 67% of patients, respectively. Nausea was >Grade 3 in only 4 (11%) patients and stomatitis in 30 (83%) with >Grade 3 toxicity seen in 8 (22%) of these patients. Hematologic toxicities >Grade 3 included leukopenia in 13 (36%), neutropenia in 19 (53%), and thrombocytopenia in 11 (31%) patients. Among 28 patients who completed >2 cycles of therapy and were evaluable for response, there was 1 PR, and disease stabilized in 16/28 (57%). Of these 28 patients, 11 (39%) remained stable for >3 months with tolerable toxicity. MST was 5.1 months (22 weeks); 7 (19%) patients survived 9.2 months (40 weeks) and 13 (36%) survived 6.3 months (27 weeks). Nolatrexed has activity in metastatic PDAC that contributes to disease stabilization. Further evaluation should include a randomized comparison with gemcitabine in this patient population (Loh K, etal, ASCO03, Abs. 1172:292).

Pemetrexed disodium (Alimta; Eli Lilly) is a novel antimetabolite that is metabolized by folylpolyglutamate synthase to highly polyglutamated species, able to inhibit multiple folate-dependent enzymes, such as TS, dihydrofolate reductase and GARFT, thus providing a mechanistic explanation for its broad antitumor activity against many human tumor types. Toxicities of Alimta include neutropenia, thrombocytopenia and fatigue. In February 2004, Alimta was approved by the FDA, in combination with cisplatin, for the treatment of inoperable mesothelioma. The drug has also shown promise in the treatment of PDAC.

In a phase II clinical trial, conducted to determine the safety and activity of LY231514, chemonaive patients with inoperable advanced or metastatic PDAC, were treated with LY231514 (600 mg/m^2) as a 10-minute infusion, every 3 weeks. Among 42 patients enrolled in the trial, 79% had metastatic disease. Neutropenia was common, with ≥Grade 3 seen in 40% of patients, but infectious complications were rare. Significant anemia or thrombocytopenia occurred in <20% of patients. Nonhematologic toxicities included Grade 2/3 skin reaction, which was ameliorated by dexamethasone. Among 35 patients evaluable for response, treated with 2 cycles of therapy, there was 1 CR lasting 16.2 months and 1 PR lasting 6.9 months, resulting in an objective response rate of 5.7%. In addition, disease stabilized in 17 patients (40%), lasting ≥6 months in 5 patients. MST was 6.5 months, with 28% of patients alive at one year (Miller KD, etal, Ann Oncol, Jan 2000;11(1):101-3).

A multicenter phase II clinical trial evaluated pemetrexed and gemcitabine combination therapy in previously untreated patients with histologically proven advanced PDAC. The study was conducted at the University of Chicago, Indiana Oncology Hematology Center (Indianapolis, IN), New York University, Memphis Cancer Center, and Virginia Mason Medical Center. Gemcitabine (1250 mg/m^2) was administered over 30 minutes on days 1, 8 of a 21-day cycle and pemetrexed (500 mg/m^2) over 10 minutes on day 8. Among 42 enrolled from September 1999 to November 2000, 40 were evaluable for response. A total of 212 cycles were completed (range=1-17, median=3). Among 33 eligible patients, there were 6 PR for an overall response rate of 15%. MST was 6.5 months and 1-year survival was 29%. Median TTP was 3.6 months. Grade 3/4 hematologic toxicities included neutropenia (81%), leukopenia (74%), anemia (14%), thrombocytopenia (26%), and neutropenic fever (14%). Grade 3/4 nonhematologic toxicities were diarrhea (5%), and fatigue (14%) (Kindler HL, etal, ASCO02, Abs. 499:125a).

Based on these results, Eli Lilly initiated a phase III clinical trial (protocol ID: CWRU-010224M, LILLY-H3E-MC-JMES, LILLY-LILY-1201, NCI-G02-2125), in May 2002, to compare gemcitabine alone to a combination of gemcitabine plus pemetrexed for the treatment of inoperable Stage II, III, or IV PDAC. Approximately 520 patients were to be enrolled in this trial, which was closed in March 2003. Patients were randomized to 1 of 2 treatment arms. Arm I treatment consisted of IV gemcitabine infused over 30-60 minutes on days 1 and 8, and IV pemetrexed infused over 10 minutes on day 1. Treatment was repeated every 3 weeks for up to 8 courses in the absence of disease progression or unacceptable toxicity. Patients were also treated with oral folic acid and cyanocobalamin intramuscularly every 9 weeks, beginning 1 to 2 weeks before day 1 and continuing until 3 weeks after end of trial therapy. Arm II treatment consisted of IV gemcitabine infused over 30-60 minutes on days 1, 8, and 15. Treatment was repeated every 4 weeks for up to 6 courses in the absence of disease progression or unacceptable toxicity. QoL is assessed at baseline, at the end of each course, and every 3 months thereafter. Patients are followed every 3 months. Joanna M. Brell of Ireland Cancer Center is Study Chair.

Alkylating Agents/Toxins

Alkylating agents exert their cytotoxic effects primarily by attaching alkyl groups to DNA bases, or cross-linking two DNA bases, preventing DNA from being separated for synthesis or transcription. Alkylating agents also react chemically with many cellular constituents. Replicating cells are most susceptible to these agents but alkylating agents are not cell-cycle specific. To date, commercially available alkylating agents have not been used successfully in the treatment of PDAC, both for lack of activity and severe toxicity.

Glufosfamide (D-19575), comprises isophosphoramide mustard, the alkylating metabolite of ifosfamide, glycosidically linked to β-D-glucose. It is a cytostatic substance with a mechanism of drug targeting and selective accumulation in tumor cells via the glucose transporter. It appears that DNA crosslinks are the most critical cytotoxic lesions induced by glufosfamide (Seker H, etal, Br J Cancer, Feb 2000;82(3):629-34).

The effectiveness of glufosfamide with or without hydration in treating patients with advanced PDAC was evaluated in a multicenter phase II clinical trial (protocol IDs: EORTC-16994P, ASTA-D-19575-3166). Trial objectives were to determine the activity of glufosfamide in terms of response rates, and duration of response, assess the drug's toxic effects, and the impact of hydration on the toxicity profile of this treatment in this setting. Patients are randomized to one of two treatment arms. In arm I, IV glufosfamide is administered over 1 hour on day 1. In arm II patients are treated with glufosfamide as in arm I and hydrated with excess physiological saline solution 4 hours before and for 3 hours after treatment with glufosfamide. Treatment is repeated every 3 weeks in the absence of disease progression or unacceptable toxicity. Patients with an objective CR continue treatment for a maximum of 2 courses beyond confirmation of response. Patients are followed every 6 weeks until disease progression. This trial was reported closed to patient recruitment in May 2001. Nicholas Pavlidis, MD, of EORTC New Drug Development Group is Protocol Chair.

In this trial, patients were treated with glufosfamide (5 g/m^2) using a 1-hour IV infusion, every 3 weeks. A total of 35 patients were enrolled over a 13-month period, with 114 treatment cycles (median=3, range=1-8) administered to 34 patients; 18 patients were allocated to the hydration arm. Treatment was discontinued in patients experiencing >0.4 mg/dl increase in serum creatinine compared with baseline values for safety reasons. 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; Grade 4 serum creatinine levels increase was noted in 1 patient, concomitantly to disease progression. Active hydration did not show a nephroprotective effect and plasma pharmacokinetics of glufosfamide were 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 TTP was 1.4 months. Glufosfamide had modest activity in this setting. Although hematologic toxicity was particularly mild, regular monitoring of renal function is recommended (Briasoulis E, etal, Eur J Cancer, Nov 2003;39(16):2334-40).

Ifosfamide, activated at the tumor site, is a novel approach pursued by Austrianova (Vienna, Austria). Ifosfamide, like all alkylating agents, indiscriminately kills both replicating malignant and normal cells, giving rise to severe toxicities. Ifosfamide is metabolized by liver enzymes, such as cytochrome P450 2B1 (CYP2B1), into 4-hydroxyifosfamide. This metabolite spontaneously decays to phosphoramide mustard and acrolein, which respectively alkylate DNA and proteins. Phosphoramide mustard and acrolein have a short half-life in plasma, however, and the dose of ifosfamide needed to systemically treat PDAC is high, associated with severe toxicity.

In pancreatic cancer, adding to the challenges related to all cytotoxic treatments, such as efficacy, drug resistance and host toxicity, is the fact that the pancreas is the last organ on the circulatory system reached by toxic metabolites. Thus, effective chemotherapy of pancreatic cancer requires high systemic concentrations of activated metabolites leading to unacceptably high levels of nonspecific toxicity. Approaches to circumvent this problem include tumor site-activated prodrugs of cytotoxic agents. Locating ifosfamide's site of conversion to the vicinity of the tumor should limit these side effects, and improve the effectiveness of ifosfamide in the treatment of PDAC.

To achieve this objective the cDNA coding for the CYP2B1 enzyme was cloned into plasmid pcDNA3. The resulting expression plasmid pC3/2B1 was amplified using noninvasive, nonpathogenic *Escherichia coli* clones. The CYP2B1 expressing plasmid, which carries a neomycin (G418) resistance gene, was transferred into qualified 293 cells. The expression of biologically active CYP2B1 in the transfectants was determined using a biochemical assay specific for the cytochrome P450 isoforms 1A1 and 2B1 (Lohr M, etal, J Mol Med, Apr 1999;77(4):393-8). These irradiated allogeneic CYP2B1-producing cells were then encapsulated in cellulose sulphate polymers to protect them from possible immune responses.

Efficacy of these implanted genetically modified cells, followed by ifosfamide treatment, was demonstrated in nude mice grafted with human pancreatic tumor cells. All mice treated responded with 55% of the mice showing a tumor reduction of >50%, and 18% showing complete tumor remission. Furthermore, this effect extends to other cells in the vicinity as a result of the bystander effect (Chen L and Waxman DJ, Cancer Res 1995;55:581-589, and Löhr M, etal, Gene Therapy 1998;5:1070-1078).

Based upon this data, this approach was used in an open, single arm phase I/II clinical trial of local intratumoral activation of ifosfamide. Treatment involved angiographic implantation of these encapsulated allogeneic cells into the vasculature leading to the tumor. Among 14 patients treated, tumor regressed in 4 and disease stabilized in the other 10 patients. MST of this patient cohort was twice that of a historic control group. The 1-year survival was 36%, threefold that of the control group, and twice that reported for gemcitabine. Regarding clinical benefit, 4/13 evaluable patients reported improvements in pain assessment at the last observation, with 6 remaining unchanged (4 of these experienced no pain) and 3 patients experiencing slightly greater pain. Using a worst case scenario, 50% of patients experienced a clinical benefit, which rose to 71% of patients if a best case scenario was applied. The best predictor of clinical benefit was lack of pain, whereas significant weight loss possibly predicts a poor clinical benefit (Salmons B, etal, J Gastroenterol, Mar 2003;38 Suppl 15:78-84).

Treatment-related toxicity attributed to the implantation or the drug was manageable. Follow-up angiography of target vessels 20 weeks after instillation showed no or only

minor changes to tumor vessels, such as reduction of diameter or raised compression, compared with baseline. Occluded vessels were seen in 2 patients and, in one individual, the effects of the tumor on blood vessels were visible. The finding that the vessels of the remaining patients were not appreciably affected by this process suggests that, in most patients, further instillation would be possible if required. None of the 12 serious adverse events recorded were related to treatment. There was no evidence of pancreatitis or allergic response during the trial. Recorded adverse events were, in most cases, a result of pain, underlying disease, or deteriorating general health. Only one adverse event, increased lipase activity on day 15 after instillation, might have been related to this treatment. The chemotherapy regimen was well tolerated and no toxicity beyond Grade 2 was detected (Löhr M, etal, The Lancet, 19 May 2001;357:1591-2).

A multicenter, international phase II clinical trial planning to treat 60 patients with PDAC, approved in Europe in 1999, has been delayed because of problems with scaling up production of the construct to meet demand. Initiation of the trial is now being planned for 2005. In July 2003, the European Medicinal Evaluation Agency (EMEA) awarded orphan medicinal product status to Austrianova's cell therapy product for targeted treatment of PDAC. The developers have also had a favorable pre-IND-meeting with the FDA in their attempts to broaden their clinical program in the USA.

Currently, Austrianova and its collaborators at the University of Veterinary Sciences (Vienna, Austria) are developing viruses (ProCon vectors) that can carry tissue-specific regulatory elements that allow expression of the therapeutic gene only in specific tissues or in tumors.

Irofulven, an acylfulvene under development by MGI Pharma (Bloomington, MN), is a chemically modified version of naturally produced toxin by the sesquiterpene mushroom *Omphalotus illudens*. Irofulven is a potent antiproliferative agent affecting many cellular targets. It alkylates cellular nucleophiles to form adducts with DNA and RNA. It induces single-strand DNA breaks causing cells to arrest at the S-phase of the cell cycle, resulting in caspase-dependent apoptosis. Irofulven's antitumor activity is specific and not dependent on the tumor's p53 status.

Irofulven has shown efficacy against a number of different xenografts, even those refractory to other antitumor agents. Also, despite its broad mechanism of action, effective irofulven dose levels could be attained in phase I clinical trials without untoward toxicities. The primary DLT is bone marrow suppression.

Originally, irofulven showed promise against PDAC. After encouraging results from two separate phase II clinical trials, in patients with PDAC refractory to gemcitabine, or chemotherapy-naïve, a pivotal phase III clinical trial (protocol IDs: MGI-IROF-003, UARIZ-HSC-00305) in patients with gemcitabine-refractory PDAC was initiated in February 2001, under PI Daniel Von Hoff, MD, at the Arizona Cancer Center. In this phase III trial, irofulven was compared to 5-FU, a commonly prescribed salvage chemotherapy for PDAC. MST was the primary endpoint, with objective tumor response and other clinical benefit measures as secondary endpoints. Also, the FDA granted 'fast track' designation to irofulven in gemcitabine-refractory PDAC.

However, in April 2002, MGI Pharma announced it would discontinue the phase III clinical trial. Although irofulven activity was observed in the trial, 5-FU demonstrated a greater than expected survival benefit, making it statistically improbable that the trial objectives would eventually be achieved.

OTHER NOVEL ANTICANCER AGENTS

MPC-6827, under development by Myriad Genetics (Salt Lake City, UT), is the lead compound within the MX90745 series of apoptosis inducing compounds, originally identified by Maxim Pharmaceuticals (San Diego, CA). The MX90745 series is one of more than 40 families identified by Maxim through its proprietary caspase-based high throughput screening system that targets the identification of compounds that modulate apoptosis. Maxim's screening technology can identify compounds that selectively induce apoptosis through a wide variety of underlying mechanisms of action, both known and unknown. The portfolio of potential drug candidates identified through this screening technology includes a number of compounds that induce apoptosis through molecular targets not previously described for the purpose of treating cancer.

The ability of MPC-6827 to inhibit tumor cell growth was investigated in MCF-7, HT-29, MiaPACA, OVCAR-3 and B16 tumor lines subcutaneously implanted into athymic nude mice. In all of these xenografts, treatment with MPC-6827 resulted in statistically significant inhibition of tumor growth. In the B16, OVCAR-3, and MiaPACA xenografts, MPC-6827 was equally or more active than paclitaxel, carboplatin, and gemcitabine, respectively, tested at MDT (Pleiman C, etal, AACR04, Abs. LB-252).

Natural Products/Vitamin Analogs

Isoprenoids produced by fruits and vegetables have antitumor activities in rodent models of pancreatic, liver, colon, and breast cancer as well as leukemia. Dietary administration of either farnesol or geraniol strongly suppresses hamster pancreatic tumor growth with low toxicity.

Investigators at Indiana University, Purdue University (Indianapolis, IN) and Rutgers University (Piscataway, NJ) tested the hypothesis that isoprenoids would inhibit cell proliferation and/or induce apoptosis in human pancreatic cancer cell lines. Human MIA PaCa2 and BxPC3 PDAC cells treated with farnesol for 48 hours displayed a dose-dependent increase in the percent of cells in G1 and a concomitant decrease in the percent of cells in S phase of the cell cycle. When the potency of farnesol, geraniol, and their acid and aldehyde derivatives were compared using

cell proliferation as an endpoint, geranial (citral) was more potent than geraniol, and farnesal was less potent than farnesol. In each case, the acidic derivative was far less potent than the isoprenoid alcohol. When the effects of farnesol and geraniol on apoptosis were investigated in hamster B12/13 PDAC cells, and in nonmalignant parental D27 pancreatic ductal epithelial cells, farnesol increased apoptosis 4 fold, and geraniol increased apoptosis 2.5 fold in the B12/13 pancreatic cancer cells, while no significant increase in apoptosis was seen in the nonmalignant D27 cells at the same concentrations. Farnesol treatment also significantly increased the percentage of MIAPaCa2 and BxPC3 human pancreatic cancer cells undergoing apoptosis. In summary, the antitumor activities of the isoprenoids farnesol and geraniol may be attributable to dual effects of cell-cycle arrest and induction of apoptosis, and these two agents may prove effective for the prevention and treatment of human pancreatic cancer (Wiseman DA, etal, AACR02, Abs. 2377).

Perillyl alcohol is a monoterpene found in lavender, spearmint, and several plants. In phase I clinical trials, this agent exhibited a favorable toxicity profile with preliminary data indicating some chemotherapeutic efficacy in advanced malignancies. Animal studies demonstrated the ability of perillyl alcohol to inhibit tumorigenesis in organs such as the mammary gland, colon, and pancreas. Although the precise mechanism of action is unclear, perillyl alcohol has been shown to inhibit the farnesylation of small G-proteins including ras, upregulate the mannose-6-phosphate receptor, and induce apoptosis (Liston BW, etal, AACR02, Abs. 1543).

A phase II clinical trial (protocol ID: IUMC-9710-07, NCI-T98-0046) of perillyl alcohol in treating resectable, Stage II or Stage III PDAC was closed in October 2001. The trial was conducted at Indiana University Cancer Center under the direction of Patrick J. Loehrer, MD. A total of 10 patients were administered oral perillyl alcohol, 4 times daily, on days 1-14. Surgical resection was performed on day 15 with no continuation of perillyl alcohol postoperatively. Patients were followed at a minimum of 2 and 4 months following surgery.

SR271425, a third-generation thioxanthone with broad preclinical activity, is under development by Sanofi-Sythelabo (Paris, France). In preclinical testing the drug was active in pancreatic cell lines Panc-03 and Panc-02. The agent was equally active both by the IV and oral routes of administration, although approximately a 30% higher dose was required by the oral route (Corbett TH, etal, Invest New Drugs, Feb 1999:17(1):17-27).

SR271425 is being evaluated in a dose-escalation phase I clinical trial (Sanofi-Synthelabo protocol: DFI4072) as a single dose 1-hour IV infusion, in patients with refractory solid tumors at Karmanos Cancer Institute, under PI Pat LoRusso, MD. In another dose-escalation phase I clinical trial, being conducted at CTRC Institute for Drug Development and Brooke Army Medical Center, SR271425 is being administered IV, weekly, for two weeks followed by a week of rest.

Vitamin K_2 (menaquinone 4) refers to a family of homologs of vitamin K with 2-methyl-1,4-naphthoquinone substituted in position 3 with isoprenyl side chains containing from 4 to 13 isoprene units called menaquinones. The menaquinones are synthesized by bacteria in the intestinal tract and can supply part of the vitamin K requirement. Vitamin K_2 selectively induced apoptosis in ovarian TYK-nu and pancreatic MIA PaCa-2 cells through a mechanism different from geranylgeraniol (Shibayama-Imazu T, Cancer Res Clin Oncol, Jan 2003 Jan;129(1):1-11. Epub 17 Dec 2002).

Retinoids

The retinoids, a class of compounds structurally related to vitamin A, play a key role in cell differentiation, proliferation and apoptosis. Retinoids participate in signal transduction mediated through two families of receptors, 3 types of retinoic acid receptors (RAr) and 3 types of retinoid X receptors (RXr). These receptors belong to the superfamily of nuclear receptors (Nr), comprising such diverse receptors as those for steroids and thyroid hormones, retinoids and vitamin D3.

AGN193198, under development by Allergan (Irvine, CA), causes cell death independent of the retinoid receptors, and may be useful in the treatment of PDAC. Investigators at Case Western Reserve University (Cleveland, OH) discovered that treatment of AsPC-1, MIA PaCa-2 and BxPC-3 pancreatic cancer cells with AGN193198 inhibited proliferation of each of these cell lines. AGN193198 was more potent than the RAr-selective retinoid, TTNBP or the RXr-selective retinoid, AGN194204. In addition, neither RAr nor RXr-selective antagonists inhibited the AGN193198-dependent suppression of proliferation, suggesting that the inhibition is not mediated via retinoid receptors. AGN193198 treatment caused tetraploidy in BxPC-3 cells, associated with a significant reduction in cyclin D1, cyclin E, cdk2, cdk4 and cdk6 levels, and a significant increase in p21 and p27 levels, indicating an effect on the cell cycle. Cell-cycle analyses of AGN193198-treated cells further revealed presence of sub-G1 cells, suggesting apoptosis associated with enhanced cleavage of procaspases 3, 8 and 9, and poly (ADP-ribose) polymerase (PARP), indicating that the cell death is caspase-dependent. Z-VAD-FMK, the pan-caspase inhibitor, inhibits these responses and attenuates cell death (Eckert RL, AACR04, Abs. 730).

SDX-102

SDX-102 (l-alanosine), an amino acid analog under development by Salmedix (San Diego, CA), is a small molecule that inhibits the synthesis of adenosine monophosphate (AMP), a key enzyme in the *de novo* synthesis pathway for adenosine, the precursor for adenosine triphosphate (ATP), a principal energy source in cells. Depletion

of ATP leads to tumor cell death. Of the two different pathways that exist for AMP synthesis, *de novo* and salvage, SDX-102 inhibits the former while the latter is frequently deleted in selected common cancers. SDX-102 exhibits selectivity in tumors in which the salvage pathway is deleted rendering cells deficient of methylthioadenosine phosphorylase (MTAP). Absence of MTAP identifies those tumors that are potentially sensitive to SDX-102.

Laboratory studies have shown that tumor sensitivity to SDX-102 depends highly on MTAP status. Homozygous deletions of the MTAP gene have been reported in multiple neoplasias including hematologic malignancies and solid tumors. An approximate 30% frequency of MTAP deletions has been detected in a variety of common cancers, including nsclc, pancreatic cancer, mesothelioma, soft tissue sarcoma, osteosarcoma, brain tumors and some hematologic malignancies. Use of such inhibitors as SDX-102 relies on the ability to detect the absence of MTAP expression in tumors in order to select appropriate target patient populations as participants in clinical trials. Therefore, the development of SDX-102 is a paradigm of the unique relationship between certain individual attributes of malignancy and the effectiveness of antitumor agents.

SDX-102 was previously investigated in clinical trials sponsored by the National Cancer Institute (NCI) and at the University of California, San Diego (UCSD). These trials, completed in 2000, established the safety and tolerability of SDX-102. However, these trials were conducted before knowledge of the tumor selectivity of SDX-102 was available, in tumors now known to have a low frequency of MTAP deletions. Based on recently discovered differences in metabolic pathways in normal and certain types of cancer cells, scientists at UCSD proposed and patented a new approach to evaluate SDX-102 in patients whose tumors lack MTAP expression.

Salmedix, in collaboration with the University of California San Diego and GeneTex (San Antonio, TX), developed a unique and simple laboratory test, which can be performed on tumor biopsy specimens, to identify patients predicted to be sensitive to SDX-102 and, therefore, most likely to respond to the drug. This immunohistochemical (IHC) assay detects the absence of MTAP expression in formalin-fixed paraffin-embedded (FFPE) tissues using a novel anti-MTAP MAb.

This approach was evaluated in 120 solid tumor samples of nsclc, pancreatic carcinoma, soft tissue sarcoma and astrocytoma. Tumors with completely negative MTAP staining were found in all these samples, and in most cases, normal tissue elements showed immunoreactivity for MTAP, which further served as internal positive control for MTAP staining. Lack of immunoreactivity was observed in various tumors and in 5/17 (29%) cases of pancreatic cancer (Huang W, etal, AACR03, Abs. 509).

In April 2003, a new multicenter (n=23), open label, phase II clinical trial (protocol IDs: MSKCC-03029, SALMEDIX-SDX-102-01) was initiated with SDX-102, in patients with MTAP-deficient cancer, including nsclc, mesothelioma, pancreatic cancer, osteosarcoma and soft tissue sarcoma. Endpoints of the trial are to determine response rates, time-to-response, duration of response, PFS, safety and tolerability, and pharmacokinetics. Between 50 and 145 patients (10 to 29 per cancer type) are to be enrolled and administered SDX-102 as a continuous infusion on days 1 to 5. Treatment repeats every 21 days for up to 9 courses in the absence of disease progression or unacceptable toxicity. Lee Krug, MD, of Memorial Sloan-Kettering Cancer Center, is Protocol Chair.

Triapine

Triapine, a heterocyclic carboxaldehyde thiosemicarbazone in development by Vion Pharmaceuticals (New Haven, CT), under a license from Yale University (New Haven, CT), is a potent inhibitor of the enzyme ribonucleotide reductase (RR). Triapine is a an injectable formulation of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) with improved aqueous solubility than 3-AP.

RR is composed of 2 subunits, M1, the nucleotide binding site, that is the target of nucleoside analogs, and M2 that contains iron and a tyrosine free radical responsible for enzymatic conversion of ribonucleotides to deoxyribonucleotides. Triapine, like hydroxyurea (HU) binds the M2 subunit of RR. RR catalyzes the reductive conversion of ribonucleoside diphosphates to their deoxyribonucleotide counterparts, a particularly critical step in DNA synthesis. RR is essential for the generation of the cytosine, adenine, and guanine deoxyribonucleotide 5'-triphosphate building blocks of DNA. Inhibitors of RR arrest DNA synthesis and cellular replication, making them of interest as anticancer agents, particularly against rapidly proliferating tumor cells (Finch RA, etal, Adv Enzyme Regul 1999;39:3-12). Triapine binds to and destroys the M2 subunit of the RR enzyme, with >1000-fold potency relative to HU and with activity in HU-resistant cell lines.

In October 2003, Beijing Pason Pharmaceuticals in China, obtained exclusive rights to develop, manufacture and market Triapine for anticancer and antiviral uses in the mainland of the People's Republic of China, Taiwan, Hong Kong and Macao. This agreement was finalized in November 2003. Terms of the agreement include an initial payment of $500,000, $4.75 million in additional milestone payments, and a royalty of 11% of any Triapine revenues in the countries covered by the agreement. Pason will fund the preclinical and clinical development necessary for regulatory approval of Triapine in the covered territory.

In May 2003, Vion initiated a multicenter (n=8), multinational phase II clinical trial (protocol ID: VION-CLI-031) of Triapine in combination with gemcitabine in patients with inoperable metastatic or advanced PDAC. Triapine is administered IV over 4 hours and gemcitabine IV over 30 minutes on days 1, 8, and 15. Treatment repeats every 4 weeks for up to 12 courses in the absence of disease progression or unacceptable toxicity. Trial objectives are to

determine the objective response rate (PR + CR), PFS interval and survival, and the safety and feasibility of this regimen in these patients. A total of 22 to 40 patients will be accrued for this trial. This trial is being co-sponsored by Eli Lilly.

In February 2004, an NCI-sponsored multicenter (n=4) phase II clinical trial (protocol IDs: PMH-PHL-023, NCI-6271) of Triapine was initiated in Canada, in combination with gemcitabine, in patients with inoperable, advanced or metastatic PDAC who failed 5-FU plus RT. Primary objectives are to determine the antitumor activity of this regimen, in this setting, in terms of response (CR+PR) and 6-month PFS. Secondary objectives include objective response rates, MST, 1-year survival rate, duration of response or stable disease, and PFS as well as safety and tolerability of this regimen in these patients. A total of 28 to 50 patients will be accrued for this trial and treated with Triapine IV over 2 hours and gemcitabine IV over 30 minutes on days 1, 8, and 15. Treatment repeats every 28 days for up to 12 courses in the absence of disease progression or unacceptable toxicity. Patients achieving a CR or PR are treated with an additional 2 courses of therapy beyond response. Patients are followed every 3 months.

A 96-hour continuous IV infusion (CIV) schedule of Triapine was investigated in a phase I clinical trial, conducted at Weill Medical College of Cornell University, to determine DLT, MDT, and pharmacokinetics of this schedule in patients with advanced cancer. Initially, courses were administered every 3 weeks, using an accelerated titration design. Subsequently, courses were administered every 2 weeks. Among 21 patients enrolled, 7 were treated by the every-3-week schedule, and 14 by the every-other-week schedule. DLT, including neutropenia, hyperbilirubinemia, and nausea or vomiting, developed in 3/6 patients at 160 mg/m^2/day level administered every 3 weeks. Based on these results, dose was re-escalated beginning at 80 mg/m^2/day, administered every 2 weeks. At 120 mg/m^2/day, 3/7 patients experienced dose-limiting but reversible asthenia, hyperbilirubinemia, and azotemia or acidosis; however, renal and hepatic adverse events were related to pre-existing borderline abnormal organ function. Therefore, the recommended phase II dose, administered by 96-hour CIV, is 120 mg/m^2/day every 2 weeks. There were no objective responses, but disease stabilized for a prolonged period; serum tumor markers associated with stable disease decreased in 4 patients (Wadler S, etal, J Clin Oncol. 2004 May 1;22(9):1553-63). To facilitate clinical development, Vion is producing an oral formulation of Triapine that should generate a pharmacokinetic profile similar to the CIV schedule, but will be easier to administer to patients.

DIGESTIVE ENZYMES

Treatment of pancreatic cancer with digestive enzymes was revived when, in the early 1980s, a retrospective review of the records of 1306 patients with pancreatic cancer treated with these enzymes over a 20-year period, revealed that some of them survived more than 5 years. This led to a phase I clinical trial in which patients with inoperable, biopsy-proven PDAC were treated on an outpatient basis. As of January 1999, among 11 patients entered into the trial, 9 (81%) survived one year, 5 (45%) survived two years, and 4 have survived at least 3 years, well above the usual 25% survival at 1 year and 10% survival at 2 years for all stages of PDAC. The average survival of the treated patients was 17.5 months. This pilot study suggests that an aggressive nutritional therapy with large doses of pancreatic enzymes may increase survival significantly over what would normally be expected for patients with inoperable PDAC. (Gonzalez NJ and Isaacs LL, Nutr Cancer 1999;33(2):117-24). The regimen used in this trial was subsequently named after New York immunologist Nicholas Gonzalez, MD.

The Gonzalez regimen is a complex daily process combining nutrition, freeze-dried pancreatic enzymes derived from pigs, plus vitamins, minerals, amino acids, trace elements, and glandular extracts, and daily enemas. The diet requires ingestion of copious amounts of raw fruits, raw and lightly steamed vegetables and juices, and plant-based proteins, such as cereals and nuts. No red meat or chicken is allowed, except some fish and no refined grain products or white sugar. Soy is also not allowed because it interacts with the pancreatic enzymes. Patients must take up to 150 pills a day.

The National Institute of Health's National Center for Complementary and Alternative Medicine (NCCAM), initiated in August 1999, an open label phase III clinical trial (protocol IDs: CPMC-IRB-8544, NCCAM, NCI-V99-1538) of gemcitabine versus intensive pancreatic proteolytic enzyme therapy based on the Gonzalez regimen, in patients with Stage II, III, or IV PDAC. Trial objectives were to compare survival and QoL of patients treated with these regimens. Originally the trial was to be randomized between the Gonzalez and a standard gemcitabine-based regimen. However, the trial was eventually converted into a single arm evaluation of the Gonzalez regimen.

According to the Gonzalez regimen patients are treated with pancreatic enzymes orally every 4 hours, and at meals daily on days 1-16, followed by 5 days of rest. Patients take magnesium citrate and Papaya Plus with the pancreatic enzymes, as well as supplementation with vitamins, minerals, trace elements, and animal glandular products 4 times per day on days 1-16, followed by 5 days of rest. Courses repeat every 21 days despite relapse until death. Patients consume a moderate vegetarian diet during the course of therapy. Coffee enemas are performed twice a day, along with skin brushing daily, skin cleansing once a week with castor oil during the first 6 months of therapy, and a salt and soda bath each week. Patients also undergo a complete liver flush and a clean sweep and purge on a rotating basis each month during the 5 days of rest. QoL is assessed at 0, 2, 6, and 12 months and then yearly thereafter. Patients are followed at 1, 3, 7, and 12 months and then yearly thereafter. John Chabot, MD, of

the Herbert Irving Comprehensive Cancer Center at Columbia University (New York, NY) is the PI.

HORMONE MODULATORS

Pancreatic tumors in both males and females express receptors for sex steroid hormones. There is experimental and clinical evidence that endocrine therapy may play a role in the management of PDAC.

Hormone modulation was tried using tamoxifen, a drug commonly used to block the effects of estrogen on breast cancer. A phase II clinical trial was performed in Italy to assess whether the combination of gemcitabine and tamoxifen would be an active and safe schedule for the treatment of locally advanced, unresectable or metastatic PDAC in terms of response rate and clinical benefits. A total of 27 evaluable patients were treated with IV gemcitabine (1000 mg/m^2) as a short infusion once weekly for 3 consecutive weeks out of every 4 weeks, and daily tamoxifen (20 mg) starting the second day after gemcitabine. Treatment was continued until progression or unacceptable toxicity. Evaluation of efficacy included response rate, TTP, survival and clinical benefit, an integrated measurement of pain parameters, weight and performance status. PR was achieved in 11% of patients while disease stabilized in 48%, lasting at least 8 weeks, and progressed in 41%. Median TTP was 4.5 months, MST was 8 months and 1-year survival was 31%. Clinical benefit was documented in 59% of patients with a median duration of 13 weeks. No gastrointestinal or hematologic Grade 4 toxicity was observed. In general, treatment was associated with a satisfactory safety profile without any tamoxifen-related toxicity (Tomao S, etal, Anticancer Res, Jul-Aug 2002;22(4):2361-4). This approach offered some benefit, albeit minimal.

Monotherapy with goserelin, an inhibitor of luteinizing hormone-releasing hormone (LH-RH), did not improve survival. Another agent, octreotide (Sandostatin Lar Depot; Novartis), a potent long-acting synthetic analog of the hormone somatostatin with affinity for two of the five somatostatin receptors, also did not affect survival in two clinical trials involving a total of 66 patients with metastatic PDAC.

It is theorized that failure of somatostatin in PDAC may be attributed to loss of somatostatin receptors by cancer cells. Scientists at Baylor College of Medicine (Houston, TX) have shown that somatostatin receptor gene transfer inhibits established pancreatic cancer xenografts. Somatostatin inhibits growth of pancreatic tumors expressing somatostatin receptor subtype-2 (SSTR2), but not receptor-negative cancers. SSTR2 expression may be an important tumor-suppressor pathway that is lost in human pancreatic cancer. Systemic delivery of SSTR2-expressing adenovirus by intraperitoneal injection resulted in expression of SSTR2 protein in the subcutaneous human pancreatic cancers in nude mice models. Final tumor weight was significantly decreased in the groups expressing SSTR2 receptors compared to controls. Interestingly, treatment with Sandostatin LAR increased plasma octreotide levels but had no significant effect on tumor growth. Western blot analysis revealed an upregulation of the cyclin-dependent kinase inhibitors p27 and p16 in the SSTR2-transfected tumors. Therefore, expression of SSTR2 by PDAC causes significant slowing of tumor growth by a mechanism independent of exogenous somatostatin. The mechanism may involve upregulation of known tumor-suppressor genes. Restoration of SSTR2 gene expression deserves further study as a potential gene-therapy strategy in human pancreatic cancer (Celinski SA, etal, J Surg Res, Nov 2003;115(1):41-7).

In addition to PDAC, hormone modulators are used in the treatment of neuroendocrine tumors of the pancreas. Lanreotide (Somatuline; Ipsen), a commercialized somatostatin analog, was investigated either alone, or in combination with interferon α (IFN-α), in a randomized, multicenter trial conducted by the International Lanreotide and Interferon Alfa Study Group, to investigate their effect on the treatment of hormone-active/functional neuroendocrine gastroenteropancreatic tumors. The trial enrolled 80 therapy-naive patients with histologically verified neuroendocrine tumors who were randomly treated either with subcutaneous (SC) lanreotide (1 mg), three times a day or SC IFN-α (5 x 10^6 U), three times a week, or both. Disease had progressed all patients in the 3 months before trial entry, verified with imaging procedures. Of 80 patients, 25 were treated with lanreotide, 27 with IFN-α, and 28 were treated with the combination. There were 4 PR, in one patient treated with lanreotide, one patient treated with IFN-α, and two patients treated with the combination. During the 12 months of therapy, disease stabilized in 19 patients, 7 treated with lanreotide, 7 with IFN-α, and 5 with the combination, and disease progressed in 14/25 patients treated with lanreotide, 15/27 treated with IFN-α, and 14/28 treated with the combination. Side effects leading to an interruption of therapy were more frequent in the combination group than in the monotherapy arms. According to the trial's results somatostatin analogs, IFN-α, or the combination of the two have comparable antiproliferative effects in the treatment of metastatic neuroendocrine gastroenteropancreatic tumors. Response rates in this trial were lower compared with those published in previous, nonrandomized studies. (Faiss S, etal, Clin Oncol. 2003 Jul 15;21(14):2689-96).

RADIOSENSITIZERS/CHEMOSENSITIZERS

Because RT is an integral part of the management of PDAC, means of enhancing the effects of RT play a particularly significant role in the treatment of this disease. Equally important is a way to enhance the effects of gemcitabine and other cytotoxics that currently have minimal effects on survival in patients with PDAC.

CoFactor

CoFactor (5, 10-methylenetetrahydrofolate), under development by Adventrx (San Diego, CA), is a vitamin

analog used as adjuvant therapy with 5-FU for the treatment of breast, and colorectal cancer and other gastrointestinal malignancies. CoFactor was developed by researchers at the University of Southern California (USC; Los Angeles, CA) and the Sahlgrenska University Hospital (Göteborg, Sweden) and licensed to Adventrx.

CoFactor, a form of folic acid, acts by enhancing the antitumor effects of 5-FU while reducing side effects compared with current therapies. Unlike leucovorin, the currently used adjunctive agent, which has to be metabolized in the body to be effective, CoFactor bypasses the biochemical pathway to deliver the correct form of folate to cancer cells allowing 5-FU to work more effectively. The CoFactor and 5-FU combination creates more stable drug-enzyme complexes to better inhibit TS to block cancer growth, and may improve the overall clinical benefit of 5-FU-based combination chemotherapies.

In a phase I/II clinical trial conducted at Sahlgrenska University Hospital, CoFactor (100-200 mg/m^2) was administered to 62 cancer patients 20 minutes before 5-FU-based therapy. PR in the range of 21%-55% were noted in patients with colorectal, pancreatic, stomach, gallbladder and breast cancer. Average duration of remissions was 9 to 15 months, which is at least a two-fold greater than with 5- FU/leucovorin therapy. MST was 21.5 months. Toxicity was milder than expected for 5-FU or 5-FU/leucovorin, and no toxicities, per se, of CoFactor were observed. A phase II clinical trial with CoFactor was initiated in April 2004, in colorectal cancer.

Motexafin Gadolineum (MGd)

Motexafin gadolineum (Xcytrin), under development by Pharmacyclics (Sunnyvale, CA), is a redox-active drug that selectively accumulates in cancer cells and reacts with intracellular reducing metabolites, such as ascorbate, producing reactive oxygen species. MGd sensitizes cells to ionizing radiation (Magda D, etal, Int J Radiat Oncol Biol Phys, 15 Nov 2001;51(4):1025-36). MGd also increases the antitumor activity of certain chemotherapy agents. This effect is believed to be related to its ability to stabilize cytotoxic free radicals produced by certain chemotherapy agents, such as bleomycin and doxorubicin. In an *in vivo* model, MGd enhanced the antitumor effect of doxorubicin without altering pharmacokinetics or increasing hematologic toxicity (Miller RA, etal, Clin Cancer Res, Oct 2001;7(10):3215-21). MGd's selective uptake in tumors potentiates the activity of cancer chemotherapy agents in tumor cells but not in normal tissues, thereby increasing the therapeutic margin.

In a phase I clinical trial, initiated at the University of Wisconsin (Madison, WI) and the University of Pittsburgh, a combination of MGd and doxorubicin is being evaluated in patients with refractory solid tumors. Doxorubicin is administered on day 1 of a 21-day cycle, followed by 3 daily doses of MGd, with the day 1 dose of MGd administered 4 hours after doxorubicin. A paired course design was employed to assess the effect of MGd on toxicity and oxidative stress. Patients in group A are treated with both doxorubicin and MGd during cycle 1 and doxorubicin alone during cycle 2, while patients in Group B are treated with doxorubicin alone during cycle 1 and both doxorubicin and MGd during cycle 2. All patients are treated with both doxorubicin and MGd for all subsequent cycles (3-6). Dose levels evaluated to date were 30 mg/m^2 doxorubicin plus 1 mg/kg/day MGd and 30 mg/m^2 patients plus 2 mg/kg/day MGd. A total of 8 patients were enrolled, 1 each with pancreatic, breast, renal, prostate, gastric, liver, and esophageal cancer, and sarcoma. Grade 3/4 pulmonary embolism, neutropenia, and hypophosphatemia occurred in 1 patient each. No increased cardiac toxicity was seen. Values of plasma isoprostane 8-epi PGF2α, a marker of oxidative stress, were no different between pretreatment and post-treatment in group A patients. At these doses, there was also no difference in isoprostane levels between group A and B patients during cycle 1. Among 4 patients who completed 6 treatment cycles and were evaluable for response, disease stabilized in 2 and progressed in 2. Escalation continues to establish MTD (Thomas JP, etal, ASCO03, Abs. 909:227).

In September 1998, a dose-escalation phase I clinical trial (protocol IDs:PCI-97-108, NCI-T97-0109) of Xcytrin was initiated in patients with locally advanced, unresectable, biliary cancer or PDAC, in combination with RT. Approximately 18 to 21 patients were to be accrued in this trial to be administered Xcytrin IV over 15 minutes, 3 times weekly, with concurrent external beam radiation therapy (EBRT) over 5.5 weeks for a total of 16 doses; RT is delivered 5 days a week for a total of 28 fractions. In the absence of DLT in the first cohort of 3 patients treated, subsequent cohorts each are administered escalating doses on the same schedule, until MDT is determined. Patients are followed at 4 weeks after the end of RT. Ramesh Ramanathan, MD of Hillman Cancer Center at the University of Pittsburgh Cancer Institute is Protocol Chair. This trial was closed in April 2002.

O(6)-benzylguanine

Pancreatic tumors are resistant to DNA alkylating agents that adduct the O(6) position of guanine, because these tumors overexpress O(6)-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein that may be important in tumor resistance to alkylating agents. Inactivation of MGMT may, therefore, sensitize pancreatic tumors to alkylating agents. One agent, O(6)-benzylguanine (O6BG, Alkylade), under development by Keryx Biopharmaceuticals (NY, NY), is a suicide substrate inactivator for MGMT. O6BG inactivates tumor alkylguanine DNA alkyltransferase (AGT), and thus sensitizes a variety of human tumor cell lines to the cytotoxic effects of alkylating agents. Currently, active clinical trials are investigating the effectiveness of Alkylade in conbination with temozolomide (Temodar/Temodal; Schering-Plough) in the treatment of brain cancer.

Another agent similar to O6BG is O6-benzyl-2'-deoxyguanosine, under development by scientists at the NCI in collaboration with the University of Pittsburgh, that was shown to be significantly more effective than O6BG in enhancing the efficacy of carmustine (BCNU) against pancreatic tumor xenografts although both these agents has a similar effect on the efficacy of temozolomide. The effect of MGMT inactivation on the resistance of pancreatic tumors to BCNU and to temozolomide was examined in five human pancreatic tumor xenografts in athymic mice. O6-benzyl-2'-deoxyguanosine, a weak MGMT inactivator in vitro as compared with O6BG, was significantly more effective than the latter in enhancing the efficacy of BCNU against pancreatic tumor xenografts. Both of these drugs also enhanced the efficacy of temozolomide against pancreatic tumors. These results suggest that pancreatic tumors, which are resistant to DNA alkylating agents, may be sensitized to such agents when pretreated with MGMT inactivators (Kokkinakis DM, etal, Clin Cancer Res, 1 Sep 2003;9(10 Pt 1):3801-7).

In a phase I clinical trial (protocol IDs: CWRU-ICC-1994, NCI-T94-0022D), conducted at Case Western Reserve University/Ireland Cancer Center, 29 patients with advanced, refractory, solid tumors were treated with a 1-hour infusion of 10 mg/m^2 to 120 mg/m^2 O6BG on days 1 and 15, and on day 15 BCNU (13 mg/m^2) was infused over 1 hour, 1 hour after the completion of the O6BG infusion. O6BG, administered at a dose of 120 mg/m^2, resulted in complete AGT depletion in tumors and was the recommended dose for phase II clinical trials. O6BG was well tolerated with transient lymphopenia being the only observed drug-related side effect (Spiro T, etal, ASCO99, Abs. 820:212a).

In August 1999, a phase II clinical trial (protocol ID: UCCRC-9523, NCI-T98-0038) of O6BG was initiated at the University of Chicago Medical Center under the direction of Mark Ratain, MD. The primary objective of this trial was to confirm the minimal O6BG dose that significantly inactivates AGT in patients with a variety of surgically resected solid tumors. A single dose of IV O6BG was administered over 1 hour at one of two dose levels. Patients underwent surgery 16 to 20 hours after administration of O6BG, and were followed at 1 and 3 weeks post-surgery. This trial, completed in February 2002, was sponsored by the NCI under a CRADA.

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