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

PANCREATIC CANCER — PART IV
NOVEL TARGETED THERAPEUTICS

PANCREATIC CANCER PART V covers angiogenesis inhibitors/antimetastatic agents and targeted drug delivery.

PANCREATIC CANCER PART VI covers immunotherapy/vaccine approaches in the treatment of pancreatic cancer.

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

PANCREATIC CANCER — PART IV
NOVEL TARGETED THERAPEUTICS

Previously, novel drugs and formulations used in the treatment of pancreatic ductal adenocarcinoma (PDAC), based primarily on cytotoxic principles, were described in Volume 7 #12 issue of FUTURE ONCOLOGY (pp 1702-1727). In this issue new approaches in the treatment of PDAC are presented that involve targeted therapeutics addressing putative molecular targets shown to play an active role in the development and progression of PDAC.

To date success with almost all new approaches has been elusive in PDAC. However, growing understanding of the molecular basis of PDAC is expected to yield more effective treatment strategies. Novel combination therapies addressing the well established heterogeneity of PDAC may prove to be more the way to extend life but a cure is unlikely with approaches in the current pipeline.

Exhibit 1 lists agents in preclinical or clinical development targeting certain biomarkers that may prove effective in the treatment of PDAC. Antiangiogenesis/antimetastatic agents are covered in Part V of this series in Volume 8 issue #3, and immunotherapy approaches in development for the treatment of PDAC are described in Volume 8 issue #4 of FUTURE ONCOLOGY. The agents described in this report have been selected because they have shown activity either preclinically or clinically in PDAC. Nearly all agents covered here are also in development for many other cancer indications, which may be more appropriate for these agents, and in a more advanced state of development. The complete preclinical and/or clinical history, and the current development status of these agents is presented in NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), a subscription-based comprehensive resource in drug development in oncology and related areas, residing at www.nmok.net.

Research in new treatment approaches for the management of pancreatic cancer, a malignancy mostly neglected in the past, has come to the forefront through various initiatives. In July 2004, the Pancreatic Cancer Action Network (PanCAN; www.pancan.org) partnered with the National Cancer Institute (NCI; Bethesda, MD) to introduce a mapping model for cancer research. PanCAN is the first national patient-based advocacy organization serving patients with pancreatic cancer, as well as families, medical researchers, and physicians. PanCAN and the NCI have agreed to develop a map summarizing the nation's current pancreatic cancer research investment. PanCAN originated the mapping concept and NCI has been moving the project forward that will identify and track research and clinical trials that are primarily focused on pancreatic cancer. The map will facilitate the development of national strategies to maximize and leverage available resources in the fight against PDAC. The completed PanCAN-NCI Pancreatic Cancer Research MAP (PanC Research MAP) will chart all research focused on pancreatic cancer and make it publicly available through a web-based database. Phase I of the MAP, currently in progress, focuses on publicly funded research. Phase II will incorporate privately funded research into the database. The MAP will afford patients, families, researchers and physicians access to a comprehensive listing of ongoing research projects and clinical trials information. This information will be used to highlight research progress, identify gaps in areas of research funding, and assist in the development of collaborative research projects and clinical trials.

The PanCAN MAP can be used by researchers to identify potential partners for collaboration and to locate biological specimens and shared services; by funding agencies to develop or establish funding opportunities, and develop interactions among researchers, agencies, and advocacy groups; and by patients, families, researchers, and clinicians to identify rate-limiting steps in research, drug development and delivery. In addition, PanCAN and the NCI will collaborate with researchers and industry to establish links for community outreach programs and patient accrual for clinical trials, and optimize career development opportunities that strengthen the research field. The PanC Research MAP is expected to be up and running by the end of 2004.

CHALLENGES IN THE TREATMENT OF PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

Management of PDAC is particularly challenging because most patients are diagnosed with advanced disease at presentation, the cancer involves a vital organ, and pancreatic cancer cells are widely heterogeneous and drug resistant. Also, despite intense research efforts, issues such as the cellular origin of most PDAC, the role of each genetic lesion in carcinogenesis, disease progression/metastasis, and tumor-cell autonomy, remain unresolved.

Heterogeneity

Human cancer cell lines originating from primary PDAC tumors do not respond uniformly to the same stimulus, highlighting the great heterogeneity of these cells. Therefore, exposure of PDAC cells to the various treatment agents may result in unpredictable outcomes in clinical situations. A study of the genetics, tumor heterogeneity and molecular profile of familial pancreatic cancer is being conducted at several institutions, including Creighton University School of Medicine (Omaha, NE), under PI Henry T. Lynch, MD.

Investigators at the University Hospital Malmo, in Sweden, demonstrated that the serine proteinase inhibitor, alpha 1-antitrypsin (AAT), dependent on its molecular form, exerted diverse effects on the properties of PDAC tumor cells, confirming the complexity of cell-protein interactions. Cell lines LPC-3, -5 and -10, established from primary PDAC cultures, were exposed to AAT or its

C-terminal peptide C-36, and analyzed for cytokines and for NFκB. Native AAT lowers TGF-β 1 levels and increases NFκB activity in LPC-3 cells, while C-36 increases TGF-β 1 levels and upregulates NFκB in LPC-5 cells. In LPC-10 cells AAT lowers TGF-β1. However, both AAT and C-36 did not change NFκB expression. IL-6 and TNF-α levels also increased in LPC-10 cells treated with C-36 (Zelvyte I, etal, Anticancer Res, May-Jun 2003;23(3B):2267-73).

Heterogeneity of PDAC is particularly challenging when treatment involves anticancer agents targeting specific biomarkers in cancer cells. One of the hypothesis for failure of targeted agents in pancreatic cancer and other solid tumors, centers on tumor heterogeneity and presence of various signaling events other than the one being targeted. Disappointing results of the phase III trials of the antimetastatic matrix metalloproteinase inhibitors (MMPI) and the antiproliferative ras pathway blockers farnesyltransferase inhibitors (FTI), in advanced PDAC, that parallel those of gefitinib trials in non-small cell lung cancer, illustrate this problem. Despite their failure in advanced/metastatic disease, these and other targeted agents may prove effective when used in earlier stages of rapidly progressing diseases such as PDAC. However, before these agents become applicable in the treatment of PDAC, methods must be devised to diagnose the disease early enough to justify their use.

To date, targeted therapeutics were mostly evaluated clinically as monotherapies in advanced/metastatic disease to establish their safety profile in active doses. During these trials, it became apparent that in most cases of advanced disease, such agents alone were insufficient to cause significant tumor regression, although disease stabilization was seen in some cases. A new approach, combining these agents with cytotoxic drugs produced better results but somewhat compromised the targeted advantage of these agents. Currently, a new approach involves the combination of several targeted therapeutics with or without cytotoxics, in the treatment of advanced disease. These approaches either combine therapeutics addressing different targets, or address the same target but differ in the way they modulate the target. For instance, a randomized, multicenter, phase II clinical trial (protocol ID: UCCRC-NCI-6580, NCI-6580, UCCRC-13200A) of bevacizumab (Avastin; Genentech) and gemcitabine (Gemzar; Lilly) with cetuximab (Erbitux; ImClone Systems) versus erlotinib (Tarceva; Genentech) in patients with advanced PDAC, was initiated in August 2004 that combines the angiogenesis inhibitor bevacizumab either with the MAb-based cetuximab, that targets the surface portion of the epidermal growth factor receptor (EGFr), or with erlotinib, a small molecule intracellular inhibitor of EGFr.

A preclinical study performed by investigators at the University of Wisconsin School of Medicine (Madison, WI) examined the combination of cetuximab with either gefitinib (Iressa; AstraZeneca) or erlotinib in a variety of human cancer cells. The combination of cetuximab with gefitinib or erlotinib increased growth inhibition compared to that observed with either agent alone. Moreover, although animal studies confirmed that single EGFr inhibitor treatment caused partial and transient tumor regression in human lung cancer xenografts, more profound tumor regression and regrowth delay were observed in mice exposed to the combination of cetuximab with gefitinib or erlotinib (Huang S, etal, Cancer Res, 1 Aug 2004;64(15):5355-62).

Resistance

Among malignancies, PDAC is particularly resistant to cytotoxic chemotherapy. Resistance diminishes the ability of drugs to destroy all malignant cells, giving rise to metastasis. Inhibition of the activity of targets promoting resistance may provide a two-pronged anticancer approach by also preventing metastasis.

Investigators at Brigham and Women's Hospital, Harvard Medical School (Boston, MA) tested the hypotheses that Src tyrosine kinase overactivity represents a chemoresistance mechanism, and that Src inhibition may enhance gemcitabine cytotoxicity in PDAC cells. PDAC cells PANC1, MiaPaCa2, Capan2, BxPC3, and PANC1GemRes, a stably gemcitabine-resistant subline of PANC1, were exposed to combinations of gemcitabine and Src tyrosine kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). The study analyzed Src expression, phosphorylation (Tyr-416), and activity; quantified the expression of the M2 subunit of ribonucleotide reductase (RRM2), a putative chemoresistance enzyme; and characterized cellular proliferation and apoptosis. Also, the effects of constitutively active and dominant-negative Src were determined, and the therapeutic efficacy of PP2, in combination with gemcitabine, was tested in nude mice orthotopically xenografted with PANC1GemRes. Greater gemcitabine resistance was associated with higher Src phosphorylation and activity, both of which were higher in PANC1GemRes, relative to PANC1; total Src levels were alike. PANC1GemRes overexpressed RRM2. PP2 enhanced inherent gemcitabine chemosensitivity and attenuated gemcitabine resistance in PANC1GemRes. Constitutively active Src increased gemcitabine chemoresistance, which was impaired by dominant negative Src. In PANC1GemRes cells, PP2 augmented gemcitabine-induced caspase-mediated apoptosis, suppressed RRM2 expression, and decreased activity of the RRM2-regulating transcription factor E2F1. In combination, PP2 and gemcitabine substantially decreased tumor growth and inhibited metastasis *in vivo*. Therefore, increased Src tyrosine kinase activity represents a potential chemoresistance mechanism and a promising therapeutic target warranting further investigation in gemcitabine-resistant PDAC (Duxbury MS, etal, Clin Cancer Res, Apr 2004;10:2307-2318).

The same investigators examined the role for carcinoembryonic antigen (CEA)-related cell adhesion molecule 6 (CEACAM6) as a determinant of gemcitabine chemore-

sistance in PDAC cells. CEACAM6 is a glycosylphosphatidylinositol-linked cell-surface oncoprotein of importance in the anchorage-independent survival and metastasis of PDAC cells. Overexpression of CEACAM6 in PDAC is associated with resistance to anoikis and increased metastasis. CEACAM6 was stably overexpressed in Capan2 cells, which inherently express very low levels of this protein. Suppression of CEACAM6 expression was achieved in BxPC3 cells, which inherently overexpress CEACAM6, by stable transfection of a CEACAM6 small interfering RNA (siRNA)-generating vector. In addition to the effects of modulating CEACAM6 expression on gemcitabine-induced cytotoxicity, the role of Akt and c-Src kinases as downstream targets of CEACAM6 signaling were also examined. Stable overexpression of CEACAM6 in Capan2 increased gemcitabine chemoresistance, whereas CEACAM6 gene silencing in BxPC3 significantly increased the sensitivity of these cells to gemcitabine. Therefore, differential expression of CEACAM6 modulates Akt activity in a c-Src-dependent manner, and CEACAM6 overexpression appears to protect cells from cytochrome c-induced caspase 3 activation and apoptosis (Duxbury MS, etal, Cancer Res, 1 Jun 2004;64:3987-3993).

In addition, antibody-mediated CEACAM6 cross-linking induces Caveolin-1-dependent, Src-mediated focal adhesion kinase phosphorylation (FAK) in BxPC3 PDAC cells. CEACAM6 cross-linking increased c-Src activation and induced tyrosine phosphorylation of p125FAK focal adhesion kinase. FAK phosphorylation was dependent on c-Src kinase activation, for which caveolin-1 was required. CEACAM6 cross-linking induced a significant increase in cellular resistance to anoikis. These observations represent the first characterization of the mechanism through which CEACAM6 influences intracellular signaling events and, hence, malignant cell behavior (Duxbury MS, etal, J Biol Chem, 28 May 2004;279(22):23176-23182).

Animal Models

Development of new therapies and diagnostics relies on the availability of reliable animal models. Drug development in PDAC has been hindered by the paucity of mouse models of this disease, limited access to early stages of disease development, and a lack of syngeneic primary pancreatic epithelial cells and adenocarcinoma cell lines. Also, across the board, despite great efforts to improve the use of murine models for the preclinical evaluation of novel agents, rarely has the work in the lab translated in humans. The problem with animal models is particularly challenging in PDAC. Although researchers have made animal models of PDAC in the past, these models lacked the necessary sophistication. In the last couple of years, however, investigators came up with several new versions that advance the state-of-the-art in this area.

Two researchers, David Tuveson of the University of Pennsylvania (Philadelphia, PA) and Ron DePinho at the Dana-Farber Cancer Institute (Boston, MA) and their colleagues, created the first mice that mimic human PDAC. Researchers at the University of Pennsylvania developed mice with human pancreatic intraepithelial neoplasia (PanIN) by directing endogenous expression of a mutant Kras allele (KrasG12D) to progenitor cells of the mouse pancreas. Physiologic levels of KrasG12D induce ductal lesions that recapitulate the full spectrum of human PanIN, a putative precursor to invasive PDAC. PanIN are highly proliferative, show evidence of histologic progression, and activate signaling pathways normally quiescent in ductal epithelium, suggesting that they represent potential therapeutic and chemopreventive targets. At low frequency, these lesions also progress spontaneously to invasive and metastatic PDAC, establishing PanIN as definitive precursors to the invasive disease. Also, mice with PanIN have an identifiable serum proteomic signature, suggesting a means of detecting the preinvasive state in patients (Hingorani SR, etal, Cancer Cell, Dec 2003;4(6):437-50).

Investigators at Dana-Farber Cancer Institute and Harvard Medical School discovered that, in mice, KrasG12D expression and Ink4a/Arf deficiency results in an earlier appearance of PanIN lesions that progress rapidly to highly invasive and metastatic tumors, resulting in death in all cases by 11 weeks, indicating that activated Kras and Ink4a/Arf deficiency cooperated to produce metastatic PDAC. The phenotypic impact of KrasG12D alone was limited, primarily to the development of PanIN, whereas inactivation of Ink4a/Arf alone failed to produce any neoplastic pancreatic lesions. Evolution of tumors in mice bears striking resemblance to human PDAC, possessing a proliferative stromal component and ductal lesions with a propensity to advance to a poorly differentiated state. These findings in the mouse provide experimental support for the human PDAC model in which activated Kras serves to initiate PanIN lesions, and the INK4A/ARF tumor suppressors function to constrain the malignant conversion of these PanIN lesions into lethal PDAC. This faithful mouse model may permit the systematic analysis of genetic lesions implicated in human PDAC and serve as a platform for the identification of early disease markers and for the efficient testing of novel therapies (Aguirre AJ, etal, Genes Dev, 15 Dec 2003;17(24):3112-26).

Investigators at University of California San Francisco (UCSF) are also developing *in vivo* and *ex vivo* mouse models of PDAC to allow the systematic investigation of the genetic requirements of pancreatic carcinogenesis. To this end, several transgenic mouse lines were derived harboring the avian retroviral receptor (TVA), and the tetracycline-controlled trans-activator (tTA) molecule under the transcriptional control of the cytokeratin 19 (CK19) promoter-enhancer. CK19-driven transgenes were expressed in a number of simple epithelia of the pancreas, prostate and breast. PDAC epithelial cells were isolated from these mice in order to introduce avian retroviruses carrying a number of genes commonly found mutated in PDAC, to assess their roles in pancreatic carcinogenesis. While a number of genetic lesions have been described for

late stage PDAC, the unparalleled incidence of p53 and p16INK4A inactivation, coupled with activating Kras mutations, stands out as a genetic fingerprint of this cancer (Dankort DL and McMahon M, AACR03, Abs. 4165).

TECHNOLOGIES EMPLOYED IN NOVEL TARGETED THERAPEUTICS IN PDAC

Almost every approach has been employed to develop targeted therapeutics. Currently, one of the most popular therapeutic options is the orally available small molecule drug. Many of the novel targeted therapeutics on the market today, such as anticancer drugs Gleevec (Novartis) and Iressa, are small molecule agents conveniently dosed orally.

However, although oral drugs seem to have the edge on convenience, injectables may in the long run provide a more effective anticancer treatment. Naked monoclonal antibodies (MAb) are employed as immunotherapies and inhibitors of cell-surface markers. Incorporating a payload, MAb are used to target drugs, toxins, radioisotopes, or other substances to tumors. MAb-based agents have been approved and are already used in the clinic. Trastuzumab (Herceptin; Genentech) to treat HER-2-expressing breast cancer, and rituximab (Rituxan; Biogen Idec) to treat CD20-expressing non-Hodgkin's lymphoma (NHL), have gone on to create large markets for their developers. Bevacizumab (Avastin; Genentech), recently approved for the treatment of colorectal cancer, is expected to also evolve into a major drug.

Another approach is use of synthetic nucleic acid sequences (SNAS), such as antisense constructs. A new SNAS technology, RNA interference (RNAi) may prove a novel way to silence genes involved in disease, including genes involved in the establishment of PDAC.

MAJOR MOLECULAR TARGETS/PATHWAYS IMPLICATED IN PDAC

Various strategies are employed in the development of novel approaches to controlling the initiation and spread of malignancy without indiscriminate killing of normal cells. These include modulation/inhibition of key signal transduction pathways by targeting major participants, and prevention of the release of proteins/growth factors promoting malignancy.

Significant defects in 'molecular circuitry' in PDAC cells have been identified. A long list of molecular markers whose aberrant behavior is associated with PDAC was presented in Volume 7 issue of FUTURE ONCOLOGY (pp 1648-1659). Frequent genetic modifications in PDAC include activation of the Kras oncogene (~85 to 95%) and inactivation of the p16/RB1 (>90%), p53 (75%) and DPC4 (55%) tumor suppressor genes. Many of these biomarkers have evolved into well characterized anticancer drug targets, some are being used as prognostic and/or disease monitoring tools, while the utility of others remains to be elucidated. For the purposes of this article, drugs described in this section are classified arbitrarily in groups sharing a similar target and/or mechanism of action (Exhibit 2). In many cases, however, inhibition of the immediate target of a drug may exert important influence on downstream effectors resulting in a completely different outcome than the one anticipated against the original target. For instance, a drug may have both antiproliferative and antiangiogenic effects or, by modulating the intended target, have profound effects on others sharing the pathway.

The incredible complexity of interactions between proteins found to play a role in PDAC has rendered simple one-marker-at-a-time targeting approaches inadequate to treat advanced disease. Currently, the trend is to combine various anticancer agents and other modalities in a multi-targeted approach. Trials are ongoing using combinations of cytotoxic agents and regulatory drugs and or of multiple drugs targeting different pathways implicated in cancer. For instance, a randomized, multicenter, phase II clinical trial is ongoing evaluating bevacizumab, gemcitabine and cetuximab versus bevacizumab, gemcitabine and erlotinib in patients with advanced PDAC (see below).

Identifying useful drug targets in PDAC is a tedious and challenging job. Like with other malignancies, genesis and progression of PDAC arises from its genome that harbors amplifications and deletions in numerous oncogenes and tumor suppressor genes. Drug developers have put in place a variety of methodologies to sort out the significance of all the aberrant aspects of the PDAC genome. The existence of large scale public and proprietary databases on gene expression, protein interactions, and genetics, have further complicated the search for meaningful actionable data to identify relevant pathways and promising novel druggable targets. Bioinformatics methodologies that blend together data covering protein-protein interactions, gene expression, and genetic variation, in a systems biology approach, are used to sort out the incredible volume of fragmented information to enable researchers to channel it into a useable drug development resource.

Investigators at Dana-Farber Cancer Institute, using high-resolution genomic analysis to define the copy number alterations in a panel of 24 PDAC cell lines, and 13 primary tumor specimens, identified all known regional gains and losses as well as many previously uncharacterized highly recurrent copy number alterations. A systematic prioritization scheme selected 64 focal minimal common regions (MCR) of recurrent copy number change. These MCR possess a median size of 2.7 megabases (Mb), with 21 (33%) MCR spanning ≤1 Mb, and an average of 15 annotated genes. Furthermore, complementary expression profile analysis of a significant fraction of the genes residing within these 64 prioritized MCR, identified a subset of candidates with statistically significant association between gene dose and mRNA expression (Aguirre AJ, etal, PNAS USA, 15 Jun 2004;101(24):9067-72).

Investigators at the University of Sydney (St. Leonards, Australia) performed comparative studies of protein expression profiles of PDAC tissue (n=10) versus mucinous

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

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Development Status ❑ Indication(s)
Abgenix ❑ Japan Tobacco, Amgen	Panitumumab ❑ ABX-EGF (formerly known as clone E7.6.3)	Fully human anti-epidermal growth factor receptor (EGFr) monoclonal antibody (MAb) ❑ IV	Phase I (begin 3/03, ongoing 10/04)➢USA, phase I (begin 9/04)➢USA, phase I (ongoing 10/04)➢USA ❑ advanced solid tumors
Aegera Therapeutics ❑ Canadian Genetic Diseases Network, Hybridon	AEG35156/GEM640	Antisense oligonucleotide against the gene sequence responsible for regulating the production of X-linked inhibitor of apoptosis, protein (XIAP); a potent anti-apoptotic protein; acts by inhibiting key proteases involved in the cellular apoptotic pathway ❑ IV	Phase I (begin 3/04)➢Canada ❑ solid tumors
Æterna Zentaris ❑ Keryx Biopharmaceuticals	Perifosine ❑ KRX-0401, D-21266	Novel heterocyclic alkylphospholipid (alkylphosphocholine derivative) analog of hexadecylphosphocholine (miltefosine), oral Akt inhibitor that activates the MAP kinase (MEK/ERK) pathway leading to the activation of p21 independently of p53 ❑ PO	Phase II (begin 7/03, ongoing 8/04) ➢USA, phase II (begin 8/03, temporarily closed 4/04)➢Canada ❑ locally advanced or metastatic pancreatic cancer
Alfacell ❑ Scientific Protein Laboratories	Ranpirnase ❑ Formerly P-30 protein ❑ Onconase	Cytotoxic ribonuclease with anti-tumor properties; originally a molecule isolated from the ova and early embryos of the Northern leopard frog (*Rana pipiens*), it was identified by amino acid sequencing to be a small single-chain protein composed of 104 amino acid residues; has a high degree of homology with the digestive enzyme pancreatic ribonuclease A ❑ IV	Phase III (completed 1/99)➢USA (combination) ❑ pancreatic cancer
AmpliMed	Imexon ❑ Amplimexon	Cyanoaziridine derivative that targets glutathione (GSH) ❑ IV	Phase I (begin 9/03, ongoing 3/04)➢USA ❑ solid tumors
Antisense Pharma	AP 12009	Phosphorothioate antisense oligonucleotide acting as a specific inhibitor of the synthesis of human transforming growth factor-β 2 (TGF-β2) isoform mRNA ❑ injection, intratumoral, intracerebral	Phase I/II (planned 8/04)➢Europe (Germany) ❑ pancreatic cancer
Aptamera ❑ Archemix	AGRO100	G-rich oligonucleotide that binds to nucleolin, a protein found on tumor-cell surfaces, involved in cancer cell proliferation ❑ IV	Phase I (begin 9/03, closed 8/04) ➢USA ❑ advanced solid tumors; phase II (planned 8/04)➢USA ❑ advanced pancreatic cancer
Ariad Pharmaceuticals	AP23573	Sirolimus analog, inhibitor of protein mammalian target of rapamycin (mTOR) that blocks cancer-cell growth and proliferation ❑ PO, injection	Phase I (begin 1/03, ongoing 7/04) ➢USA ❑ solid tumors
Array BioPharma ❑ AstraZeneca	ARRY-142886	Selective, orally active inhibitor of MAPK/ERK/kinase (MEK) ❑ PO	Phase I (begin 6/04)➢USA ❑ solid tumors

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ArQule ❑ Beth Israel Deaconess Medical Center, Dana-Farber Cancer Institute, Roche	ARQ 501 (formerly CO-501)	Naturally occurring tricyclic o-naphthoquinone, a checkpoint regulator of the E2F pathway that selectively activates checkpoint-mediated apoptosis in cancer cells without causing damage to normal cells ❑ IV	Phase I (begin 9/03, ongoing 4/04) ➢USA; phase Ib (planned 11/04) ➢USA (combination) ❑ advanced, refractory solid tumors
AstraZeneca	AZM475271	Novel, orally available Src kinase inhibitor ❑ PO	Preclin (ongoing 12/03)➢Europe (France, Germany, UK) ❑ solid tumors
Bristol-Myers Squibb	BMS-354825	Small molecule dual function Src/abl tyrosine kinase inhibitor designed to overcome mechanisms giving rise to resistance to treatment with imatinib mesylate ❑ PO	Phase I (begin 6/03, ongoing 11/03)➢USA ❑ chronic myelogenous leukemia (CML)
Celgene ❑ U California, U Massachusetts	CC-401	Lead of several small molecule oral inhibitors of c-Jun N-terminal kinase (JNK) ❑ PO, IV	Phase I/II (ongoing 10/04)➢USA ❑ solid tumors
Cephalon ❑ Kyowa Hakko Kogyo	Lestaurtinib ❑ CEP-701, KT-5555, SPM-924	Novel indolocarbazole derivative, an orally active, tyrosine kinase inhibitor, that inhibits the autophosphorylation of wild-type and constitutively activated FLT3, resulting in cell death of leukemia cells harboring the mutation ❑ PO	Phase II (begin ongoing 2/04, 10/4) ➢USA ❑ pancreatic cancer
Curis ❑ Genentech	HhAntag	Small molecule drugs or MAb that inhibit the Hedgehog pathway, being developed as cancer therapeutics ❑ PO, injection	Preclin (ongoing 9/04)➢USA ❑ solid tumors
Curis ❑ Johns Hopkins U	Cyclopamine	Mimetics/derivatives of cyclopamine, a compound originally isolated from an extract of a lily (*Veratrum californicum*) found in the western USA, that is a specific inhibitor of the Hedgehog pathway ❑ PO	Preclin (ongoing 9/04)➢USA ❑ solid tumors
Eleos	EL831 (antisense JunD)	Oligonucletide-based drug for treatment of malignancies with a mutated p53 ❑ IV	Preclin (ongoing 4/04)➢USA ❑ pancreatic cancer
Eli Lilly	LY293111	Orally available small molecule with antineoplastic activity, known to be an LBT4 receptor antagonist, 5-lipoxygenase inhibitor, and a peroxisome proliferator activated receptor-γ (PPAR-γ) agonist ❑ PO	Phase II (begin 12/02, ongoing 8/04)➢Europe (Finland), USA (combination) ❑ locally advanced or metastatic pancreatic cancer
Isis Pharmaceuticals ❑ Eli Lilly	LY900003 (formerly known as ISIS-3521, ISI641A, CGP64128A) ❑ Affinitac, Affinitak	20-mer phosphorothioate antisense oligonucleotide inhibitor of protein kinase C α (PKC-α) isoform gene expression via an RNase H-mediated mechanism ❑ IV	Phase II (ongoing 12/00, completed 02)➢USA, Canada, Europe ❑ relapsed or refractory solid tumors
Isis Pharmaceuticals ❑ Eli Lilly	LY2181308, ISIS-23722, ISIS 23722	Antisense construct that inhibits survivin, a molecule that blocks apoptosis ❑ IV	Preclin (ongoing 9/04)➢USA ❑ solid tumors
Janssen Pharmaceutica ❑ Kyowa Hakko Kogyo, Ortho Biotech Products	Tipifarnib ❑ R115777 ❑ Zarnestra	Imidazole farnesyltransferase inhibitor (FTI), that targets activated p21 ras ❑ PO	Phase I (begin 1/04, ongoing 8/04)➢USA (combination); phase II (begin 5/00, closed 4/02)➢USA, phase II (begin 11/01, closed 1/04) ➢USA, Canada (combination); phase III (completed 02)➢USA, Europe (combination) ❑ metastatic pancreatic cancer

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Lorus Therapeutics ❑ AVI BioPharma, U Manitoba, Proligo	GTI 2040, GTI-2040	Phosphorothioate 20-mer antisense oligonucleotide directed against the R2 subunit of RNR ❑ continuous IV (CIV)	Phase I (begin 2/00, completed 6/03)➢USA, phase I (begin 5/04) ➢USA, phase I/II (begin 9/03, ongoing 6/04)➢Canada (combination) ❑ advanced or metastatic solid tumors
Merck ❑ Vertex Pharmaceuticals	VX-680	Aurora and FLT-3 kinase inhibitor ❑ PO	IND (filed 3/04)➢USA ❑ hematologic malignancies
Merck KGaA	Matuzumab ❑ EMD 72000, EMD72000	Humanized MAb that targets EGFr-expressing tumors ❑ infusion	Phase I (closed 6/04)➢Europe (combination) ❑ advanced pancreatic cancer
Montigen Pharmaceuticals ❑ U Arizona, Translational Genomics Research Institute	MP235	Novel small molecule that effectively inhibits Aurora-2 kinase ❑ PO	Preclin (ongoing 8/04)➢USA ❑ solid tumors
Myriad Genetics ❑ Maxim Pharmaceuticals	MPC-6827	Lead compound within the MX128495 series of apoptosis-inducing compounds ❑ IV	Preclin (ongoing 9/04)➢USA ❑ solid tumors
NS Pharma (Nippon Shinyaku) ❑ Ivax	HMN-214, HMN-176	Stilbazole derivative; oral antimicrotubular agent with polo-like- and cyclin-dependent kinase inhibitory activities; prodrug of HMN-176 ❑ PO	Phase I (completed 2/04)➢USA ❑ advanced solid tumors
OSI Pharmaceuticals ❑ Pfizer, Genentech, Roche	Erlotinib ❑ CP-358,774, OSI-774, R1415 ❑ Tarceva	Small molecule that directly and reversibly inhibits EGFr tyrosine kinase; CP-373,420, a desmethyl metabolite of CP-358,774, is also a potent inhibitor of EGFr ❑ PO, IV	Phase I (begin 6/03, ongoing 8/04) ➢USA ❑ (combination), phase I (closed 6/04)➢USA ❑ (comination); phase Ib (begin 4/02, closed 11/03)➢USA ❑ (combination); phase III (begin 8/01, closed 1/03)➢Canada (combination) ❑ newly diagnosed, locally advanced or metastatic pancreatic cancer (Stage III or IVa/IVb)
Pfizer Global Research and Development	PD0325901 (successor to CI-1040)	A significantly more potent analog of CI-1040 with an improved pharmaceutical profile, shown to be exquisitely specific for MAPK/ERK/kinase (MEK) and, particularly MEK1 ❑ PO	Phase I/II (initiated 03, ongoing 10/04)➢USA ❑ advanced, refractory solid tumors
Raven Biotechnologies	RAV12	Chimeric form of glycotope-specific IgG1 MAb KID3, with potent antiproliferative activity against colon and gastric tumor cell lines *in vitro* ❑ injection	Preclin (ongoing 10/04)➢USA ❑ gastrointestinal cancer
RESprotect ❑ Fraunhofer Institute for Toxicology and Experimental Medicine, Australian Cancer Technology	RP101	Suppresses activation of apoptosis-antagonizing gene products induced by cytostatic drug treatment ❑ PO	Phase I/II (completed 03)➢Europe (Germany) ❑ advanced solid tumors; phase I (planned 9/04) ➢Europe (Germany) ❑ metastatic pancreatic cancer
Rigel Pharmaceuticals	R763	Potent, highly selective, small molecule inhibitor of Aurora2 kinase ❑ PO	Preclin (ongoing 8/04)➢USA ❑ solid tumors, hematologic malignancies
Salmedix ❑ U California San Diego	SDX-102	Amino acid analog (L-alanosine), a small molecule that inhibits adenosine monophosphate (AMP), a key enzyme in the *de novo* synthesis pathway for adenosine, the precursor for ATP ❑ CIV	Phase II (begin 5/03)➢USA ❑ solid tumors

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Schering-Plough	Lonafarnib ❑ SCH 66336, SCH66336 ❑ Sarasar	Orally bioavailable nonpeptide tricyclic farnesyltransferase (FTase) inhibitor (FTI) in the pyridobenzo-cycloheptene class ❑ PO	Phase II (begin 11/99, closed 3/00) ➢USA ❑ pancreatic cancer
Wyeth Pharmaceuticals	EKB-569	3-cyanoquinoline that binds covalently and irreversibly to EGFr and potently inhibits EGFr tyrosine kinase and phosphorylation of EGFr in cells ❑ PO	Phase I (ongoing 6/03)➢USA (combination) ❑ advanced pancreatic cancer
Wyeth	Temsirolimus ❑ CCI-779	Structural analog of the macrocyclic lactone sirolimus initially isolated from the soil bacteria *Streptomyces hygroscopicus*, is an immunophilin-binding antibiotic that blocks the initiation of the translation of mRNA by inhibiting mTOR ❑ IV, PO	Phase II (begin 12/03, ongoing 6/04)➢USA ❑ advanced pancreatic cancer

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

cystadenoma, a benign pancreatic disease (n=4), and adjacent normal pancreatic tissue (n=14) using Ciphergen Biosystems' (Fremont, CA) ProteinChip technology coupled with SELDI-TOF MS. Differential expression profiles were seen between invasive PDAC, benign and normal tissue. This study suggests that pancreatic cancer may differentially express a variety of protein isoforms compared to benign and normal pancreatic tissue, a characteristic that may provide an approach to early diagnosis and monitoring of this disease (Scarlett CJ, etal, AACR04, Abs. 5227).

Investigators at the University of the District of Columbia (Washington, DC), Georgetown University (Washington, DC) and the NCI, used Digital Differential Display (DDD) to compare expression profiles of 5 pooled pancreatic tumor libraries comprising 43,953 clustered expressed sequence tags (EST), with 18 libraries comprising 181,216 clustered EST representing normal pancreas and other tissues. DDD is an *in-silico* method used to compare the frequencies of EST between normal and tumor expression libraries. Among 15 cDNA overexpressed in pancreatic tumor libraries, 13 were known genes overexpressed in various malignancies and two genes, DDD1 and DDD2, were novel. DDD1 cDNA sequence is 1679 base-pairs (bp) long and contains an open reading frame (ORF) of 1191 bp coding for a putative protein of 396 amino acids (~44 kDa). Preliminary *in situ* hybridization experiments on cancer tissue microarray suggest that DDD1 is overexpressed in various tumors. The characterization of DDD1 may provide insight into the mechanism of pancreatic cancer and development of new biomarkers and therapeutic targets (Kumar D, etal, AACR04, Abs. 1716).

Investigators at Johns Hopkins University (Baltimore, MD), University of Texas Southwestern Medical Center (Dallas, TX), in collaboration with GeneLogic (Gaithersburg, MD), used GeneLogic's BioExpress platform and Affymetrix' (Santa Clara, CA) U133 GeneChip arrays to analyze genes associated with the response to ionizing radiation in normal and neoplastic pancreatic cell lines and tissues; cDNA was prepared from samples of normal pancreas (n=25), resected pancreatic cancer tissues (n=25), or pancreatic cancer cell lines (n=14), and hybridized to the complete Affymetrix Human Genome U133 GeneChip set for simultaneous analysis of 45,000 gene fragments corresponding to 33,000 known genes. Expression data corresponding to 196 genes with a putative role in the radiation response or DNA damage was determined, and analyzed. There were 50 genes highly expressed among normal pancreatic tissues, cancer tissues and cancer cell lines. A small cluster of 10 genes was also identified that was highly expressed in resected pancreatic cancer tissues or cell lines but not in normal pancreatic tissues. Genes within this cluster included Rad51, MutS homolog 6 and BRCA1. Immunohistochemical analysis of a series of pancreatic tumors confirmed the increased expression of these proteins in paraffin-embedded pancreatic cancer tissues. Some of these 60 genes that may contribe to the radiation response often observed in PDAC, may represent retention of expression from normal pancreas, whereas others, such as Rad51, are truly overexpressed. Some of these genes have not been implicated within the radiation response before, and may have implications for understanding the radioresistance observed in PDAC (Swartz M, etal, AACR03, Abs. 5133).

Investigators at Celera Genomics (Rockville, MD) have identified over 110 differentially expressed cell surface proteins implicated in pancreatic cancer, and have selected 25 proteins for validation as targets for antibodies and small molecules.

Apoptosis Induction/Enhancement

Defective regulation of apoptosis is the key mechanism that results in tumor-cell proliferation and maintenance of the malignant state. There are numerous treatment approaches to induce apoptosis in cancer cells; the ones described here have been shown clinically or preclinically to play a role in PDAC.

AEG35156/GEM640, under development by Aegera Therapeutics [Ile des Soeurs (Montreal), Canada], in collaboration with Hybridon (Cambridge, MA), is an antisense oligonucleotide against the gene sequence responsible for regulating the production of X-linked inhibitor of apoptosis protein (XIAP), a potent antiapoptotic protein that acts by inhibiting key proteases involved in the cellular apoptotic pathway. Aegera Therapeutics, formerly Apoptogen, has an exclusive license to the IAP gene family of apoptotic inhibitors, including XIAP, from the Canadian Genetic Diseases Network (Vancouver, Canada), part of the National Centres of Excellence program.

The IAP family of apoptosis control genes was elucidated by the Network's Robert Korneluk, PhD, and Alex MacKenzie, MD, PhD, Aegera's founding scientists. XIAP is a key regulator of apoptosis. Translation of XIAP is controlled by a 162-nucleotide internal ribosome entry site (IRES) element located in the 5' untranslated region of XIAP mRNA; XIAP IRES mediates efficient translation of XIAP under physiological stress, enhancing cell protection (Holcik M and Korneluk RG, Mol Cell Biol, Jul 2000;20(13):4648-57). IAP expression is elevated in multiple cancer cell lines, including pancreatic cancer.

XIAP plays a distinct role in the radiation-resistant phenotype of human cancer. Acute low dose ionizing irradiation results in the translational upregulation of XIAP that correlates with an increased resistance to radiation. Also, transient overexpression of XIAP renders human cancer cells resistant to low dose gamma radiation. In contrast, antisense targeting of XIAP, resulting in XIAP downregulation, is accompanied by increased cell death following irradiation (Holcik M, etal, Oncogene, 24 Aug 2000; 19(36):4174-7).

In September 2002, Hybridon and Aegera entered into a collaboration and licensing agreement to combine Hybridon's 2nd generation antisense technology with XIAP to develop an antisense drug for the treatment of cancer. The resulting drug candidate was licensed to Aegera on an exclusive worldwide basis.

In March 2004, Aegera Therapeutics, in collaboration with Cancer Research UK, in London, initiated a phase II clinical trial with AEG35156/GEM640 in advanced solid tumors, at Christie Hospital (Manchester, UK), under PI Malcolm Ranson, PhD, Director of the Derek Crowther Trials Unit. The trial is also being conducted at a second site at the University of Edinburgh Cancer Research Centre, in the UK, under the supervision of Duncan Jodrell, DM.

Imexon (Amplimexon), under development by AmpliMed (Tucson, AZ), is an iminopyrolidone aziridine, a cyanoaziridine derivative that targets glutathione (GSH). Imexon was developed at Arizona Cancer Center, University of Arizona (Tucson, AZ).

Imexon exhibits antitumor activity against a variety of human malignancies, including PDAC. Human pancreatic cancer cells were sensitive to imexon at concentrations achievable in mice and dogs (Dorr RT, etal, Invest New Drugs 1995;13(2):113-6). In the human MiaPaCa cell line, cytotoxicity from imexon was schedule-dependant. Cell-cycle analysis in MiaPaCa cells showed that imexon blocked cell division at the G2/M phase resulting in an accumulation of cells in G2 and late S-phase. Cell death from imexon occurred via both apoptosis (about 65%) as well as necrosis (about 35%). MiaPaCa cells were studied for morphologic features of apoptosis, which occurred in a dose-dependent fashion. Pan-caspase activation was detected following imexon treatment. Because prior mechanistic studies showed a loss of glutathione (GSH) following imexon treatment of myeloma cells, GSH was measured in MiaPaCa cells treated with imexon. There was no detectable GSH at all time points in the imexon-treated cells. Finally, imexon induced inhibition of MiaPaCa tumor growth in SCID mice without significantly decreasing white blood cells or platelets. Therefore, imexon is an active agent in PDAC *in vitro* and *in vivo*. These data, coupled with the lack of myelotoxicity makes imexon an attractive antitumor agent for combination with cytotoxics in the treatment of PDAC (Dorr R, etal, AACR04, Abs. 2064).

A phase I clinical trial of imexon was initiated in September 2003, at the Arizona Cancer Center, under the direction of Tom Dragovich, MD, jointly supported by the NCI and AmpliMed. This trial is designed to assess how well the drug is tolerated when administered alone to cancer patients with advanced solid tumors. The dosing schedule involves a 30-minute drug infusion, daily, for 5 days, repeated every 2 weeks. It is anticipated that approximately 20 patients will be enrolled in this trial.

In December 2003, imexon was granted orphan drug designation by the FDA for the treatment of pancreatic cancer.

Lestaurtinib (CEP-701), a novel indolocarbazole derivative, is under development by Cephalon (West Chester, PA), in collaboration with Kyowa Hakko Kogyo (Tokyo, Japan). CEP-701 is a synthetic analog of the indolocarbazole metabolite K252a (Ruggeri BA, etal, Curr Med Chem, Sep 1999;6(9):845-57), a low molecular weight alkaloid isolated from the actinomycete strain K-252T, originally thought to belong to the genus *Nocardiopsis* (Kase H, etal, J Antibiot (Tokyo), Aug 1986;39(8):1059-65, Nakanishi S, etal, J Biol Chem, 5 May 1988;263(13):6215-9), but subsequently proposed as a new species of the genus *Nonomuraea* (Chiba S, etal, Int J Syst Bacteriol, Oct 1999;49 Pt 4:1623-30).

CEP-701 is an orally active tyrosine kinase inhibitor, that blocks the autophosphorylation of wild-type and constitutively activated FLT3, a receptor tyrosine kinase (RTK) expressed on lymphoid and myeloid progenitors in the hematopoietic system, resulting in cell death of leukemia cells harboring the mutation. In addition, CEP-701 impedes growth of cancer in various animal models in a cell-cycle independent fashion via induction of apoptosis. CEP-701 induces apoptosis by inhibiting kinase activity of the tyrosine kinase receptor, preventing its autophosphorylation. Ligand-occupied tyrosine kinase receptors signal via several pathways, including Ras/Raf/MEKK and PI-3 kinase/Akt. This latter pathway is important in the phosphorylation of the proapoptotic protein Bad, sequestering it in the cytoplasm, and in phosphorylation-dependent inactivation of pro-caspase-9. This, in turn, prevents the activation of tyrosine kinase-associated Ras and PI-3 kinase pathways. CEP-701 also binds to calmodulin, leading to a delayed elevation in intracellular free calcium ions and subsequent activation of calcineurin, which causes the dephosphorylation of Bad, and thus its translocation to the mitochondria and downstream activation of caspases and cell death (Weeraratna AT, etal, AACR-NCI-EORTC99, Abs. 104). In addition to exhibiting potent inhibitory effects on neurotrophin receptor-linked tyrosine kinase, to a lesser extent, CEP-701 inhibits protein kinase C (PKC), and kinases linked to platelet-derived growth factor receptor (PDGFr) and vascular endothelial growth factor receptor (VEGFr) (Miknyoczki SJ, etal, AACR99, Abs. 3199, and Marshall J, etal, ASCO00, Abs. 713).

The therapeutic potential of inhibiting neurotrophin-specific tyrosine kinase receptor interactions using CEP-701 was evaluated in several preclinical models of human PDAC. CEP-701 inhibited tumor growth in a statistically significant manner in Panc-1, AsPc-1, BxPc-3, Colo 357, and MiaPaCa2 PDAC xenografts, subcutaneously implanted in athymic nude mice, reducing tumor growth volume by 50-70% relative to vehicle-treated controls. Significant reductions of *in vivo* PDAC tumor invasiveness were observed as well in 4/6 CEP-701-treated rat tracheal xenografts implanted subcutaneously in athymic nude mice. The *in vivo* antitumor efficacy of CEP-701 occurred in the absence of pronounced morbidity or toxicity (Miknyoczki SJ, etal, Ann NY Acad Sci, 30 Jun 1999;880:252-62, Miknyoczki SJ, etal, Clin Cancer Res, Aug 1999;5(8):2205-12, and Miknyoczki SJ, etal, AACR99, Abs. 3199). Also, CEP-701 in combination with gemcitabine was found to be more effective at inhibiting the growth of PDAC xenografts in murine models than either compound alone.

As of October 2004, a small phase II clinical trial of CEP-701 was ongoing for the treatment of PDAC.

LY2181308 (formerly ISIS-23722, ISIS 23722), under development by Isis Pharmaceuticals (Carlsbad, CA), in collaboration with Eli Lilly, is an antisense construct that inhibits survivin, a molecule that plays an important role in both cell division and inhibition of apoptosis. Survivin, which is commonly expressed in the majority of human malignancies, including PDAC, is a member of the IAP protein family.

LY2181308 downregulates survivin expression in various human cancer cells including pancreatic cancer cells. Specifically, inhibition of survivin expression by LY2181308 induced caspase-3-dependent apoptosis, cell-cycle arrest in the G2/M phase, and multinucleated cells. Most importantly, in an *in vivo* human xenograft tumor model, LY2181308 exhibited significant antitumor activity as compared to saline, or its sequence-specific control oligonucleotide. Furthermore, in these xenografts, antitumor activity was associated with significant inhibition of survivin expression (Carrasco RA, etal, AACR04, Abs. 5373).

MPC-6827, under development by Myriad Pharmaceuticals (Salt Lake City, UT), in collaboration with Maxim Pharmaceuticals (San Diego, CA), is the lead compound within the MX90745 series of apoptosis inducing compounds that comprises 40 families identified by Maxim through its proprietary caspase-based high-throughput screening system that targets the identification of modulators of apoptosis.

In December 2003, Maxim granted an exclusive worldwide license to Myriad for the development and commercialization of the MX90745 series of compounds covered by a composition of matter patent application. Myriad is responsible for the clinical development and commercialization of compounds from the MX90745 series, with oversight to be provided by a joint development committee comprised of Myriad and Maxim personnel. Maxim is to provide research services to Myriad for a period of at least one year. The license agreement calls for Myriad to pay license fees and research support, and make milestone payments totaling up to $27 million, assuming successful commercialization of a product from the MX90745 series. Myriad will also pay royalties to Maxim on sales of MX90745-related products.

When tested in pancreatic tumors in a xenograft mouse model, MPC-6827 was twice as effective as gemcitabine at a significantly lower dose. MPC-6827 also performed well against other tumor types in animal models.

RP101, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), under development by RESprotect (Dresden, Germany), in collaboration with the Fraunhofer Institute for Toxicology and Experimental Medicine (Hannover, Germany), suppresses the activation of apoptosis-antagonizing gene products induced by cytostatic drug treatment. BVDU (Brivudin) has a long history, having been approved and sold in Europe as an antiviral for the past 15 years.

In September 2004, Australian Cancer Technology (AustCancer; Sydney, Australia) acquired the North American license to develop RP101. Milestone payments to RESprotect are made in the form of cash or AustCancer

shares and are triggered by the achievement of key clinical developments, such as an IND application in the USA. AustCancer will also pay RESprotect an annual royalty fee based on North American sales.

When tested *in vitro*, RP101 downregulated the oncogene STAT3, and the DNA-repair gene APEX, which are overexpressed in PDAC. Combination of RP101 with chemotherapy prevented decrease of apoptotic effects during treatment, and reduced nonspecific toxicity. Amplification of chemoresistance genes Mdr1 and Dhfr and overexpression of gene products involved in proliferation (DDX1) or DNA repair (UBE2N and APEX), were inhibited whereas activity of NAD(P)H:quinone oxidoreductase 1 (NQO1) was enhanced. During recovery after BVDU monotherapy, microfilamental proteins were upregulated, and proteins involved in ATP generation or cell survival such as oncogenes STAT3, and Jun-D were downregulated. In three different rat tumor models, RP101 optimized the antitumor efficiency of chemotherapy, and reduced toxic side effects (Fahrig R, etal, Cancer Res, 15 Sep 2003;63:5745-5753).

In December 2003, results were reported from a randomized, single blind, placebo-controlled, phase I/II clinical trial of RP101, conducted at the University of Dresden in Germany, in 31 patients with five different tumor types, including metastasized PDAC. In this trial RP101 was administered in combination with standard chemotherapy. Treatment in this setting was safe and well tolerated. In addition, certain side effects of chemotherapy were considerably reduced. In this trial, results favored patients with metastatic PDAC. Usually, MST for metastasized PDAC is 3 to 6 months, with response to treatment being <10%. In this trial, 3/4 patients with metastasized PDAC, treated with RP101 plus cytostatics, went into remission and were alive over a period of up to 14 months. Of the two patients not treated with RP101, one died after one month and the other after 9 months. Although the small number of patients involved does not allow statistical analysis, treatment success in PDAC merits further investigation.

Arachidonic Acid Pathway Inhibitors

The arachidonic acid pathway is one of the main mechanisms associated with pain and inflammation. It also controls homeostatic functions. The pathway produces different classes of end products, including prostaglandins, arising from cyclooxygenase (COX) metabolism; prostacyclines; thromboxane A2; and leukotrienes, arising from lipoxygenase (LOX) metabolism.

There are two COX enzymes, COX-1 and COX-2. Inhibition of COX-2, related to inflammation, is the basis of a several anti-inflammatory/analgesic drugs on the market or in development. There is increasing evidence that COX-2 plays an important role in carcinogenesis. Cox enzymes appear to be involved in tumor cell proliferation, tumor-related angiogenesis and act as apoptosis inhibitors. COX-2 is induced in response to growth factors, tumor promoters, and carcinogens, and it may be involved in tumor proliferation and angiogenesis. Although studies have shown that antagonists of COX enzymes inhibit angiogenesis in tumor xenografts, the molecular mechanisms involved in this action have not been elucidated. COX-2 protein is expressed in 67% to 90% of pancreatic tumors.

Lipoxygenase (LOX) inhibitors are also under investigation as therapeutic targets in PDAC. LOX is a key enzyme in arachidonic acid metabolism. Investigators at Northwestern University Medical School (Chicago, IL) demonstrated that blockade of either the 5-lipoxygenase (LOX) or 12-LOX pathway of arachidonic acid metabolism inhibited pancreatic cancer cell proliferation and induced apoptosis.

The underlying mechanisms of LOX inhibitor-induced apoptosis, and the potential of LOX inhibitors as antipancreatic cancer agents, were further investigated using the athymic mice xenograft model. LOX inhibitors, including the nonselective LOX inhibitor nordihydroguaiaretic acid, the 5-LOX inhibitor Rev-5901, and the 12-LOX inhibitor baicalein, all induced apoptosis in MiaPaCa-2 and AsPC-1 human pancreatic cancer cells. Expression of antiapoptotic proteins Bcl-2 and Mcl-1 was significantly decreased after LOX inhibition while that of the proapoptotic protein Bax was increased. LOX inhibitors also induced the release of cytochrome c from mitochondria into the cytosol. Caspase-9, -7, and -3, but not -8, were activated after treatment, concomitant with cleavage of the caspase-3 substrate poly(ADP-ribose) polymerase. *In vivo* studies in the athymic mice xenograft model also confirmed that these LOX inhibitors inhibit growth and induce apoptosis in pancreatic cancer through the mitochondrial pathway both *in vivo* and *in vitro* (Tong WG, etal, Mol Cancer Ther, Sep 2002;1(11):929-35).

Celecoxib (Celebrex; Pfizer), a cyclooxigenase-2 (COX-2) inhibitor, globally marketed as an anti-inflammatory and analgesic, has also been approved as an adjunctive treatment in familial adenomatous polyposis (FAP), a colon cancer precursor.

In a pilot trial, conducted at Regina Elena National Cancer Institute (Rome, Italy), celecoxib and infusional 5-fluorouracil (5-FU) were used as second line treatment in advanced PDAC. Oral celecoxib (400 mg) twice daily, and protracted daily IV infusion of 5-FU (200 mg/m^2), were administered continuously for a maximum of 9 months, in the absence of disease progression or unacceptable toxicity. Patients were examined weekly for toxicity and were restaged every 6-8 weeks for tumor assessment. Among 17 patients enrolled in the trial, asymptomatic transaminase elevation that reached Grade 3/4 in 4/133 treatment weeks, was the most common toxicity. There was no other >Grade 2 hematologic or nonhematologic toxicity. Celecoxib was discontinued in 4 patients because of upper gastrointestinal (GI) tract toxicity. Among 16 evaluable

patients, there were 2 confirmed PR, lasting 23 weeks and 68 weeks, respectively, and disease stabilized in 2 patients for 10 weeks and 13 weeks, respectively, for an overall response rate of 12% in the intent-to-treat population. A significant decrease (≥50%) in serum CA 19.9 levels was observed in 3/9 evaluable patients. Median TTP was 8 weeks, and the median overall survival was 15 weeks. This regimen was feasible and well tolerated, and induced durable objective responses, even in patients with far advanced, gemcitabine-resistant/refractory PDAC (Milella M, etal, Cancer, 1 Jul 2004;101(1):133-8). In contrast, bolus administration of 5-FU in patients with metastatic, advanced PDAC has shown minimal activity. Also a relatively benign toxicity makes this combination attractive in an essentially palliative setting.

A phase I clinical trial was conducted at M. D. Anderson Cancer Center (Houston, TX), using a regimen of IV gemcitabine (750 mg/m^2) administered at the rate of 10 mg/m^2/minute on days 1, 8, and 15, every 4 weeks, and PO celecoxib (400 mg) administered in divided doses continuously starting two days after the first gemcitabine dose, in chemotherapy-naïve patients with advanced, inoperable PDAC, was more myelosuppressive than gemcitabine monotherapy causing Grade 3/4 granulocytopenia (n=3), and Grade 3 thrombocytopenia (n=1). Among 6 patients treated there was 1 (34%) PR after 8 weeks of treatment, and disease stabilized in 2 (66%) (Xiong HQ, etal, ASCO02, Abs. 448:113a). A phase II clinical trial of this combination (protocol ID: MDA-2003-0288, NCI-6167) is ongoing at M. D. Anderson Cancer Center.

In a phase II clinical trial, IV gemcitabine (1000 mg/m^2), administered weekly for 7 weeks, was combined with concurrent daily oral celecoxib (400 mg), administered in divided doses, and daily oral low dose aspirin (81 mg), administered throughout the trial as a precaution for increased risk of thrombotic events. Responders, or those with stable disease, continued treatment for 3 weeks. Toxicities included neutropenia (35%) and anemia (20%); there were no unexpected drug-related adverse events. Among 18 evaluable patients from the 20 patients (locally advanced disease=6, metastatic disease=14) with advanced PDAC enrolled in the trial, there were 3/18 (17%) PR and disease stabilized in 4/18 (22%). The trial was conducted at the University of Arizona Health Sciences Center (Phoenix, AZ), Sarah Cannon Cancer Center (Nashville, TN), Indiana University Cancer Center (Indianapolis, IN), and Arizona Cancer Center (Smith SE, etal, ASCO03, Abs. 1502:374).

A trial combining gemcitabine and irinotecan with celecoxib is ongoing at Karmanos Cancer Institute (Detroit, MI) in advanced PDAC (Lipton A, etal, ASCO-GI04, Abs. 153).

LY293111, under development by Eli Lilly, is an orally available small molecule drug with antineoplastic activity, known to be an LBT4 receptor antagonist, 5-LOX inhibitor, and peroxisome proliferator activated receptor-γ (PPAr-γ) agonist. LY293111 inhibits tumor cell growth through its PPAR-γ agonist activity. In pancreatic tumor cells, this agent caused lipid vesicle formation correlating with the inhibition of DNA synthesis.

The effectiveness of LY293111, alone and in combination with gemcitabine, was investigated in a heterotopic xenograft model in athymic mice using HT29 and LoVo human colonic cancer cells. The combined therapy significantly inhibited tumor growth (Hennig R, etal, Cancer Lett, 8 Jul 2004;210 (1):41-6). In a preclinical study, LY293111 demonstrated PPAR-γ agonist properties *in vitro* and *in vivo* at concentrations that inhibit tumor cell growth. At an IC50 dose, LY293111 caused lipid vesicle formation in pancreatic tumor cells, indicating PPAR-γ agonist activity and inhibition of DNA synthesis (Marshall M, etal, AACR02, Abs. 4741:957).

A multinational, multicenter, randomized, placebo-controlled, double blind, phase II clinical trial (protocol ID: 4840) of gemcitabine plus LY29311, compared to gemcitabine plus placebo, in patients with locally advanced or metastatic pancreatic cancer, was initiated in December 2002, in two locations in the USA and in Finland. The purpose of this trial is to determine if treatment with LY293111 plus gemcitabine, compared with gemcitabine alone, improves survival, and to evaluate the safety of this regimen as well as response rate and progression-free survival (PFS). Approximately 130 patients are expected to be accrued for this trial.

Aurora Kinase Modulators

The Aurora family of protein serine/threonine kinases has been implicated in chromosome segregation and cytokinesis. In addition, Aurora A (Aurora2) and Aurora B are frequently overexpressed in human tumors, often correlating with poor prognosis. Aurora kinases have been also shown to downregulate p53.

Aurora kinases regulate cell proliferation by serving as a checkpoint in the process of cell division. Thus, expression and activity of Aurora2 is strictly regulated during cell cycle to maintain chromosome stability in human cells (Marumoto T, etal, AACR03, Abs. 1178). Aberrant expression of Aurora kinases can result directly in the transformation of a normal cell into a tumor cell. Both overexpression and inactivation of wild type Aurora2 contribute to chromosome instability, which results in development and progression of malignancy. Because Aurora2 kinase is upregulated and amplified in virtually all human tumors, it is a ubiquitous and attractive target for anticancer drug development. This activity is supported by clinical evidence, which demonstrates that amplification of Aurora genes is associated with progression and/or poor prognosis in certain types of cancer including leukemia, and colon and breast cancer. Therefore, inhibitors of Aurora kinases offer considerable potential for targeted intervention in a variety of tumors.

Exhibit 2
Selected Putative Targets of Novel Anticancer Agents in Development for Pancreatic Cancer

Primary Developer	Generic Name ❑ Number ❑ Brand Name	Target
Montigen Pharmaceuticals	MP235	Aurora2 kinase
Rigel Pharmaceuticals	R763	Aurora2 kinase
AstraZeneca	ZM447439	Aurora2 kinase; aurora B kinase
Merck	VX-680	Aurora2 kinase; FLT3
Celgene	CC-401	c-Jun N-terminal kinase (JNK)
ArQule	ARQ 501	E2F
Abgenix	Panitumumab ❑ ABX-EGF	Epidermal growth factor (EGF) receptor (EGFr)
Merck KGaA	Matuzumab ❑ EMD 72000, EMD72000	Epidermal growth factor (EGF) receptor (EGFr)
OSI Pharmaceuticals	Erlotinib ❑ CP-358,774, OSI-774, R1415 ❑ Tarceva	Epidermal growth factor (EGF) receptor (EGFr)
AstraZeneca	AZD3409	Farnesyltransferase (FTase); ras
Janssen Pharmaceutica	Tipifarnib ❑ R115777 ❑ Zarnestra	Farnesyltransferase (FTase); ras
Schering-Plough	Lonafarnib ❑ SCH 66336, SCH66336 ❑ Sarasar	Farnesyltransferase (FTase); ras; H-ras
AmpliMed	Imexon ❑ Amplimexon	Glutathione (GSH)
Curis	Cyclopamine.	Hedgehog pathway
Curis	HhAntag	Hedgehog pathway
ImClone Systems	IMC-A12	Insulin-like growth factor (IGF)-1
Eli Lilly	LY293111	Leukotriene B4 receptor; 5-lipoxygenase; peroxisome proliferator-activated receptor γ (PPAr-γ)
Wyeth	Temsirolimus ❑ CCI-779	Mammalian target of rapamycin (mTOR)
Ariad Pharmaceuticals	AP23573	Mammalian target of rapamycin (mTOR)
Array BioPharma	ARRY-142886, AZD6244	MAPK/ERK/kinase (MEK)
Pfizer Global Research and Development	PD0325901 (successor to CI-1040)	MEK 1 and MEK 2
Cephalon	Lestaurtinib ❑ CEP-701, KT-5555, SPM-924	Neurotrophin receptor-linked tyrosine kinases
Aptamera	AGRO100	Nucleolin
Eleos	EL831	p53 (mutated)
Æterna Zentaris	Perifosine ❑ KRX-0401, D-21266	Protein kinase C (PKC)
Isis Pharmaceuticals	LY900003 (ISIS-3521, ISI641A, CGP64128A) ❑ Affinitac, Affinitak	Protein kinase C (PKC), α isoform PKCα
Lorus Therapeutics	GTI 2040, GTI-2040	R2 mRNA
RESprotect	RP101	Signal transducer and activator of transcription 3 (STAT3)
Bristol-Myers Squibb	BMS-354825	Src kinase; ABL-specific tyrosine kinase
Isis Pharmaceuticals	LY2181308	Survivin
Antisense Pharma	AP 12009	Transforming growth factor-β (TGF-β)
Aegera Therapeutics	AEG35156/GEM640	X-linked inhibitor of apoptosis protein (XIAP)

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

In the mid 1990s, Dr. Von Hoff's group at Arizona Cancer Center identified Aurora2 kinase as a potential target in PDAC. Investigators at the Arizona Cancer Center have shown that Aurora2 is both amplified and overexpressed in human PDAC cell lines, with a 2- to 5-fold increase in gene copy number, and a 3- to 4-fold increase in protein levels compared with controls. In samples obtained directly from patients, overexpression of Aurora2

was detected in 26 of 28 tumors. Antisense nucleotides specifically targeting Aurora2 arrest the cell cycle in pancreatic cancer cells, indicating the potential of Aurora2 as a therapeutic target in PDAC (Rojanala S, etal, Mol Cancer Ther, Apr 2004;3(4):451-7).

Aurora kinases also play a key role in the phosphorylation of histone H3 during mitosis. Suppression of phosphohistone H3 *in vitro* and significant inhibition of histone H3 phosphorylation was achieved in an acute *in vivo* model using Aurora kinase inhibitors. This *in vivo* activity is consistent with inhibition of Aurora B, potentially providing a new approach to the targeting of cell division in proliferating tumors. Inhibition of Aurora B has been shown to result in polyploidy and apoptosis in tumor cells.

Using high throughput screening, investigators at AstraZeneca (Alderley Park, Cheshire, UK) identified a series of potent and selective ATP-competitive Aurora kinase inhibitors based upon an established quinazoline core, but structurally distinct from other kinase inhibitors; selectivity for the Aurora kinases was achieved through the introduction of a large lipophilic group into the ATP binding pocket of the molecules. The observed structure-activity relationships (SAR) for these inhibitors are readily understood in terms of the crystal structure of Aurora2. Subsequently, substitution of quinazolines with a range of 5-membered-ring amino-heterocycles was investigated. Although direct analogs of the preferred pyrimidino-quinazolines showed modest potency, the introduction of a methylene spacer led to the development of highly potent and selective inhibitors. These novel compounds, particularly the thiazolo-quinazolines, display a significant increase in *in vitro* potency (cellular proliferation and cell-cycle effects) compared with previously reported compounds (Mortlock AA, etal, AACR04, Abs. 2480).

MP235 is a novel small molecule that effectively inhibits Aurora2 kinase under development by Montigen Pharmaceuticals (Salt Lake City, UT) and the University of Arizona. Scientists at the Arizona Cancer Center at the University of Arizona and Montigen developed Aurora2 kinase-specific inhibitors through both rational-based drug design and experimental screening. To validate human Aurora2 as a druggable target in PDAC, investigators at Arizona Cancer Center used a structure-based approach to design specific inhibitors using homology modeling, affinity docking and an *in vitro* kinase assay in an iterative process (Mahadevan D, etal, Curr Med Chem Anti-Canc Agents, Jan 2003;3(1):25-34).

In December 2003, at a presentation at the International Conference on Molecular Targets and Cancer Therapeutics, scientists reported that these inhibitors arrest cancer cell division and cause tumor cell death. These agents caused a dramatic arrest of the cell cycle at the G2/M transition and induced pancreatic cell death.

MP235 is being evaluated preclinically in 3 mouse tumor models, including colon, breast, and pancreatic cancer.

R763, under development by Rigel Pharmaceuticals (South San Francisco, CA), is a potent, highly selective, small molecule inhibitor of Aurora2 kinase.

The R763 compound class was discovered using a high content cell-based phenotype assay that simultaneously measures cell proliferation, apoptosis, cellular morphology, and normal cell cycling. R763 potently inhibited proliferation and triggered apoptosis in several tumor cell lines including cervical, colon, lung, pancreatic and prostate cancer. Potency has also been demonstrated for earlier generations of molecules in animal models of human tumors. Additional studies show that R763 selectively inhibit Aurora kinase over other related kinases.

Rigel plans to file an IND application to initiate clinical trials with R763 by late 2005.

VX-680, under development by Vertex Pharmaceuticals (Cambridge, MA) in collaboration with Merck, is a potent and selective small molecule inhibitor of Aurora kinases. It blocks cell-cycle progression and induces apoptosis in a diverse range of human tumor types. VX-680 profoundly inhibits tumor growth in a variety of *in vivo* xenograft models, leading to regression of leukemia, and colon and pancreatic tumors at well tolerated doses (Harrington EA, etal, Nat Med, Mar 2004;10(3):262-7).

In June 2004, Vertex Pharmaceuticals and Merck entered into a global collaboration to develop and commercialize VX-680 for the treatment of cancer. Under the agreement, Merck is responsible for clinical development and worldwide commercialization of VX-680. The company will pay Vertex development milestones and royalties on product sales. In addition, the companies will conduct a joint research program to characterize VX-680's activity across a broad range of cancer types, as well as to identify follow-on drug candidates directed at Aurora kinases, using molecular profiling approaches and microarray technologies pioneered by Merck.

As part of the agreement, Vertex received a $20 million upfront payment, is eligible for an additional $14 million in research funding over the next two years, and could receive more than $350 million in milestone payments, including $130 million for the successful development of VX-680 in a first oncology indication. Additional milestones of $220 million may be earned by Vertex as VX-680 and follow-on compounds advance in clinical development in multiple oncology indications. Vertex could earn more than $350 million in additional royalties for development of Aurora kinase inhibitors outside the area of oncology. Beyond these substantial payments, Vertex retains significant downstream interest in its Aurora kinase program. Vertex will provide input to Merck's clinical development and will have the opportunity to negotiate a copromotion agreement with Merck prior to commercialization. In addition, Vertex will receive royalties based on product sales, with a royalty range being similar to that of other Vertex collaborations.

Epidermal Growth Factor Receptor (EGFr) Inhibitors

Cancer cells often express membrane receptors for a variety of polypeptide growth factors. Many of the features of the malignant phenotype, such as increased proliferation, angiogenesis, and evasion of apoptosis, are associated with the signaling networks that involve EGFr family members. EGFr tyrosine kinase inhibitors act on tumor-cell invasion and angiogenesis for the prevention and treatment of solid tumors controlled by oncogenes and epigenetic factors.

The epidermal growth factor (EGF) superfamily of tyrosine kinase receptors, namely EGFr (ErbB1), ErbB2 (HEr2/neu), ErbB3, and ErbB4, and their ligands, are involved in cell differentiation, proliferation, migration, and carcinogenesis. Members of the EGFr family possess intrinsic tyrosine kinase activity whereby ligand binding to the extracellular portion of the receptor leads to autophosphorylation in tyrosine residues located in intracellular domains.

EGFr, the prototypical member of the superfamily of receptors, is widely expressed on many cell types, including those of epithelial and mesenchymal lineages, and is overexpressed in a variety of human tumors (Moscatello D, etal, AACR96, Abs. 363:52). Among patients with pancreatic cancer, EGFr is detected in approximately 90% of clinical specimens. Studies analyzing paraffin-embedded PDAC specimens for EGFr overexpression using varying techniques and schemes have found expression or overexpression rates ranging from 0% to 89%.

Inhibition of autophosphorylation of EGFr tyrosine kinase and subsequent signaling events is possible with small molecules administered orally. These drugs are competitive inhibitors of adenosine triphosphate (ATP), displaying a relatively selective activity against specific receptors. Among these drugs, inhibitors of EGFr are in more advanced phases of clinical development. Two such inhibitors are gefitinib and erlotinib (OSI-774). Another class of drugs, MAb targeting the extracellular portion of the receptor, are also in development, with cetuximab having been approved for clinical use. The activity of these two different approaches to EGFr inhibition are being evaluated in randomized trials, pitting one against each other and in combination trials evaluating their potential synergistic effects.

A randomized, multicenter, phase II clinical trial (protocol ID: UCCRC-NCI-6580, NCI-6580, UCCRC-13200A) of bevacizumab and gemcitabine with cetuximab versus erlotinib in patients with advanced PDAC was initiated in August 2004 at the University of Chicago Cancer Research Center (Chicago, IL) to determine the response rate, toxicity, median progression-free survival (PFS), and overall survival for this regimen. Patients are stratified according to participating center (University of Chicago versus other) and ECOG performance status (0-1 versus 2). Patients are randomized to 1 of 2 treatment arms. In arm I, patients are administered cetuximab IV over 1-2 hours on days 1, 8, 15, and 22, gemcitabine IV over 30 minutes on days 1, 8, and 15, and bevacizumab IV over 30-90 minutes on days 1 and 15. In arm II, patients are administered gemcitabine and bevacizumab as in arm I, and are also treated with oral erlotinib once daily on days 1-5, 8-12, and 15-26. In both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months. A total of 54-126 patients (27-63 per treatment arm) are to be accrued for this trial. Hedy Kindler, MD, of the University of Chicago Cancer Research Center is the PI.

It has also been widely reported that HEr2/neu, another member of the EGFr family is also overexpressed in up to 45% of PDAC. Overexpression, defined as 2+ or 3+ scores on immunohistochemistry, was detected in 17% of 123 archival specimens. However, the rate of HEr2 overexpression in PDAC may be lower. Investigators at the University of Kiel, in Germany, evaluated the expression rate of HEr2/neu and EGFr in metastatic PDAC, to see if MAb therapies such as trastuzumab would be appropriate in treating these tumors. Immunohistochemical staining was used to measure HEr2/neu and EGFr in 28 PDAC specimens and their associated lymph node metastases. The percentage of cells in each sample that stained positive for HEr2/neu was also analyzed in a second series of 104 primaries only. HEr2/neu overexpression was seen in 28% of PDAC; 3+ overexpression was noted in 10% of primary tumors and 21% of metastatic tumors. EGFr was overexpressed in 50% of primaries and 28% of metastases. All lesions classified as PanIN (n=23) were negative for HEr2/neu and EGFr, with the exception of three PanIN3 lesions that overexpressed HEr2. This suggests that these tumors are less suitable for treatment with HEr2 antagonists such as trastuzumab. However, the high rate of EGFr overexpression indicates that this may be a promising therapeutic target in PDAC (Luettges J, etal, ASCO04, Abs. 4246).

Cetuximab (Erbitux), developed by ImClone Systems (NY, NY) and marketed by Bristol-Myers Squibb in the USA and Merck KGaA (Darmstadt, Germany) in Europe as second line treatment of metastatic colorectal cancer, is a chimeric MAb against EGFr that has shown preclinical activity in a variety of tumor models. In clinical trials, the main toxicity of cetuximab has been skin rash and occasional allergic reactions. The combination of cetuximab and gemcitabine was at least additive in preclinical models.

In *in vitro* studies, treatment of cultured PDAC cells with cetuximab resulted in dose-dependent inhibition of EGFr-specific autophosphorylation in response to exogenous ligand as well as inhibition of cell proliferation. In a murine PDAC xenograft model, IP administration of cetuximab significantly inhibited growth of established tumors, with decreased cell proliferation and massive tumor necrosis apparent; combination treatment with cetuximab and gemcitabine resulted in no macroscopic tumors and a 90%

reduction in microscopically visible lesions, compared with only a 27% reduction using gemcitabine alone (Bruns CJ, etal, AACR99, Abs. 154, and Prewett M, etal, AACR99, Abs. 4818).

Combined therapy using cetuximab, gemcitabine, and radiation caused complete tumor regression in a nude mouse model inoculated with pancreatic MiaPaCa-2 cells but only a delay in tumor growth with BxPC-3 cells. Scientists at the University of Alabama at Birmingham, investigated the effects of prolonged cetuximab treatment on the sensitivity to gemcitabine and/or radiation on the EGFr signal transduction pathway. MiaPaCa-2 and BxPC-3 cells were first cultured with or without cetuximab for 6 weeks and were then treated with gemcitabine and/or radiation. Proliferation assays indicated that prolonged exposure to cetuximab increased the sensitivities of MiaPaCa-2 to gemcitabine and radiation therapy but not of BxPC-3. Expressed EGFr levels decreased by about 42% on MiaPaCa-2, whereas no loss was seen on BxPC-3. Expression of Bax was upregulated on MiaPaCa-2. Poly (ADP-ribose) polymerase cleavage indicated the killing was mediated by apoptosis. Incubation with cetuximab blocked growth factor receptor-bound protein 2 (Grb2) binding in MiaPaCa-2 but not BxPC 3. Internalization of EGFr induced by cetuximab did not differ between MiaPaCa-2 and BxPC-3. Association of Grb2 to EGFr in BxPC-3 induced by EGF in the presence of cetuximab indicates an alternate pathway of Ras-MAPK activation, which may be related with tumor resistance to treatment. Transactivation of EGFr downstream Ras-MAPK pathway by fibroblast growth factor (FGF) contributes to treatment resistance. Downregulation of EGFr may, therefore, increase response to therapy (Huang ZQ, etal, J Surg Res, 15 May 2003;111(2):274-83).

Cetuximab was evaluated in a phase II clinical trial (protocol ID: UAB-9929, NCI-G00-1729, IMCL-CP02-9814, UAB-F990927003), at the University of Alabama at Birmingham, in combination with gemcitabine, for the treatment of patients with advanced PDAC whose tumors expressed EGFr by immunohistochemistry (Abbruzzese JL, etal, ASCO01, Abs.518:130a, and Xiong H, etal, J Clin Oncol, 1 Jul 2004;22(13):2610-6). Patients were administered cetuximab at an initial dose of 400 mg/m^2, followed by 250 mg/m^2, weekly, for 7 weeks. Gemcitabine (1,000 mg/m^2) was administered for 7 weeks, followed by 1 week of rest. In following cycles, cetuximab and gemcitabine were administered weekly for 3 weeks every 4 weeks. Among 41 patients, after 2 treatment cycles, there were 5 (12.2%) PR, and disease stabilized in 26 (63.4%); TTP was 3.8 months, 1-year overall survival (OS) was 13.7%, and median OS was 7.1 months. The most commonly observed Grade 3/4 adverse events included neutropenia (39.0%), asthenia (22.0%), abdominal pain (22.0%), and thrombocytopenia (17.1%). These results prompted the investigators to design a phase III trial of gemcitabine with or without cetuximab as well as other combinations with cetuximab (Exhibit 3).

EKB-569, under development by Wyeth, is a 3-cyanoquinoline that binds covalently and irreversibly to EGFr, and potently inhibits EGFr tyrosine kinase and phosphorylation of EGFr.

An open label, dose-escalation phase I clinical trial of EKB-569 by continuous daily oral administration, in combination with IV gemcitabine on days 1, 8, and 15 of a 28-day cycle to treat advanced PDAC, was conducted at Dana-Farber Cancer Institute and M. D. Anderson Cancer Center. Dose escalation was based on safety evaluation of patient cohorts (≥3 patients/cohort) after day 28 of treatment. Dose level 1 included EKB-569 (25mg), and gemcitabine (750 mg/m^2), in dose level 2 the EKB dose was doubled to 50 mg, at dose level 3 EKB-569 (50 mg) was combined with gemcitabine (1000 mg/m^2), and at dose level 4, EKB-569 dose was increased to 75 mg. At dose level 2, 2/7 patients experienced DLT manifested as Grade 3 diarrhea and Grade 3 elevation in liver transaminases. Dose level 1 was identified as the maximum tolerated dose (MTD), and 18 additional patients were treated at MTD, bringing the total patients treated at 29. The most frequently reported adverse events were diarrhea, nausea, and rash. There were no treatment-related deaths. One patient has remained on therapy for 8+ months (Morgan JA, etal, ASCO03, Abs. 788:197).

Erlotinib (Tarceva), under development by OSI Pharmaceuticals (Melville, NY) in collaboration with Pfizer, is a small molecule drug that directly and reversibly inhibits EGFr tyrosine kinase. Among pancreatic tumor cell lines BxPC3, HPAC, MIA-PaCa-2, AsPC1 and PANC-1, treated with erlotinib, BxPC3 and HPAC showed growth inhibition (Iwata KK and Mantis C, AACR04, Abs. 4831).

The randomized phase III clinical trial (protocol ID: CAN-NCIC-PA3, OSI-CAN-NCIC-PA3), evaluating erlotinib, in combination with gemcitabine, versus gemcitabine alone, met its primary endpoint of improving overall survival in patients with locally advanced metastatic PDAC. This multicenter, randomized, double blind, placebo-controlled, phase III clinical trial evaluated erlotinib at 100 mg/day or 150 mg/day in patients with locally advanced or metastatic pancreatic cancer. The trial randomized patients to either gemcitabine plus concurrent erlotinib, or gemcitabine plus placebo. A total of 569 patients were randomized in the trial, 521 patients in the group treated at 100 mg/day of erlotinib or placebo, and 48 patients in the group treated with 150 mg/day of erlotinib or placebo. This international trial was conducted at sites in the USA, Asia, Canada, Europe, Australia and South America. In this trial, a statistically significant 23.5% improvement (hazard ratio=0.81) was seen in overall survival in patients treated with the erlotinib combination compared to those treated with gemcitabine plus placebo. Median and 1-year survival in the erlotinib plus gemcitabine arm were 6.4 months and 25.6%, respectively, compared to 5.9 months and 19.7%, respectively, in the gemcitabine plus placebo arm. A statistically significant improvement in PFS was also seen,

although no difference in tumor response was observed. A preliminary analysis of the safety data did not reveal any unexpected safety issues beyond those encountered in prior use of erlotinib in both monotherapy and combination settings. As expected, rash and diarrhea were the principal erlotinib-related side effects seen in the trial.

A phase Ib clinical trial (protocol ID: OSI-774-155, NCI-V02-1694, UARIZ-HSC-01128) of erlotinib and gemcitabine in patients with advanced PDAC and other malignancies, performed by the Arizona Cancer Center, the University of Texas Health Science Center (Houston, TX), and the Beth Israel Deaconess Medical Center (Boston, MA), was initiated in April 2002 and closed in November 2003. The purpose of this trial was to evaluate the tolerability and preliminary activity of escalating doses of erlotinib when administered in combination with standard-dose gemcitabine. Eligible patients included those with advanced PDAC or other malignancies potentially responsive to gemcitabine. Patients were stratified into 2 groups, based on performance status, organ function and exposure to prior chemotherapy regimens. A single oral daily dose of erlotinib of either 100 mg or 150 mg was administered in combination with a weekly dose of IV gemcitabine (1000 mg/m^2), initially for 7 weeks, and weekly for 3 weeks repeated every 4 weeks in subsequent cycles. For treatment group I, 6 patients evaluable for toxicity were entered at the 100 mg dose level of OSI-774. Of these, 3 patients developed Grade 3 elevations of transaminases and 1 patient with lung cancer developed fatal pulmonary toxicity, likely related to gemcitabine and prior radiation therapy. There was 1 PR in a patient with urinary bladder adenocarcinoma. For treatment group II, 3 patients at the 100 mg-dose level of erlotinib and 8 patients at the 150 mg-dose level were evaluable for toxicity and response. No serious toxicities or DLT were observed at the 100 mg or 150 mg dose level of erlotinib in treatment group II. The most common toxicities included skin rash, neutropenia, fatigue, nausea and diarrhea. At the 150 mg dose level in this treatment group, there was 1 PR. Disease stabilized in 4 patients with PDAC; CA 19/9 levels decreased by >90% from baseline in 2 of them (Dragovich T, etal, ASCO03, Abs. 895).

Subsequently, another 14 gemcitabine-naïve patients with inoperable PDAC were enrolled in this trial, 2 patients at the 100 mg dose level and 12 patients at the 150 mg dose level. Among 14 patients evaluable for response, there was 1 (7%) PR, 3 (21%) MR, and disease stabilized in 6 (43%) and progressed in 4 (29%). This outcome corresponds to overall disease control rate of 71 % (PR+SD). Median length of treatment was 4 months (range=2-12+) with 4 patients remaining on the trial. The most frequently observed toxicities included skin rash, neutropenia, fatigue, nausea and diarrhea (Porterfield B, etal, ASCO04, Abs. 4110).

A nonrandomized, open label, dose-escalation, phase I clinical trial (protocol ID: MSKCC-03031, NCI-5441) of erlotinib, gemcitabine, and radiotherapy in patients with locally advanced inoperable PDAC was initiated at Memorial Sloan-Kettering Cancer Center (MSKCC; NY, NY) in June 2003. This trial was designed to determine MTD, toxicity, antitumor efficacy, TTP and overall survival for this regimen. Patients undergo radiotherapy 5 days-a-week for 5.5 weeks. Beginning on day 1 and continuing concurrently with radiotherapy, patients are administered gemcitabine IV over 30 minutes, twice weekly, and oral erlotinib, once daily. Treatment continues in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are administered escalating doses of erlotinib until MTD is determined. Once MTD is determined, an additional 10 patients are administered treatment at that dose. Patients are radiologically restaged 3-4 weeks after completion of radiotherapy. Patients with stable or responsive disease are administered maintenance chemotherapy comprising gemcitabine IV over 30 minutes, on days 1 and 8, and oral erlotinib, once daily. Treatment repeats every 21 days for a total of 4 courses in the absence of disease progression or unacceptable toxicity. The trial is to accrue 19-28 patients. Jeremy Kortmansky, MD, of MSKCC is Protocol Chair.

A phase I clinical trial of erlotinib, gemcitabine, paclitaxel and radiation therapy in patients with locally advanced PDAC was performed at Brown University (Providence, RI) to determine the safety and efficacy of this regimen. Patients were administered gemcitabine (75 mg/m^2) and paclitaxel (40 mg/m^2), weekly, for 6 weeks; radiation (50.4 Gy) included the primary tumor and draining lymph nodes with a 2 to 3 cm margin. Erlotinib was administered on the first day of chemoradiation at three planned dose levels, 50 mg/day, 100 mg/day, and 150 mg/day. Three weeks following chemoradiation, all patients were administered erlotinib (150 mg/day), until progression. Among 12 patients evaluable for toxicity, 9 had locally advanced disease and 3 underwent resection but had positive margins. DLT was Grade 3 diarrhea and dehydration, experienced by 2/6 patients at dose level 2. Acneiform rash, most prominent in the face, was seen in 9/12 patients. Diarrhea and rash were generally experienced together beginning in the 2nd and 3rd week of treatment. There were 4/9 PR in patients with locally advanced disease; reduction of >75% in CA19-9 with extensive necrosis in the mass on post-treatment CT was experienced by 3 additional patients. These results indicate that diarrhea and rash are the DLT in this chemoradiation regimen. The protocol is being revised to add a 75 mg erlotinib dose level with chemotherapy and radiation (Iannitti D, etal, ASCO04, Abs. 4093).

Gefitinib (Iressa), marketed by AstraZeneca for the treatment of refractory non-small cell lung cancer (nsclc), is a quinazoline-derivative that selectively and reversibly inhibits EGFr tyrosine kinase-mediated intracellular signaling pathways.

In a preclinical study, performed by investigators at the University of Heidelberg in Germany, in PDAC cell lines,

gefitinib completely blocked EGF-induced cell proliferation, but did not significantly affect insulin-like growth factor (IGF)-induced mitogenesis. Gefitinib also completely inhibited EGF-induced phosphorylation of EGFr and MAP kinase. Additionally, gefitinib prevented basal and EGF-induced anchorage-independent cell growth and invasion. These results indicate that gefitinib inhibits pancreatic cancer cell growth through EGFr-dependent pathways. Gefitinib also prevents anchorage-independent growth and invasiveness, indicating that it may offer a new approach for the treatment of pancreatic cancer (Li J, etal, Int J Oncol, Jul 2004;25(1):203-10).

In a phase I clinical trial, performed in the USA and Brazil, gefitinib was combined with celecoxib in treating 30 patients with advanced GI malignancies. Patients were administered gefitinib (250 mg) daily and celecoxib (400 mg), twice daily. Toxicities were mild and included acne, diarrhea, and nausea; 5 patients required a dose reduction of celecoxib to 200 mg twice daily because of GI toxicity. Disease stabilized in 3 patients (colorectal cancer=2 and pancreatic cancer=1) and progressed in 27.

Matuzumab (EMD72000), under development by Merck KGaA, is a humanized MAb that targets EGFr-expressing tumors. In preclinical studies, the efficacy of EMD72000 was investigated on pancreatic (adenosquamous carcinoma PAXF546) tumors transplanted subcutaneously into nude mice. Treatment with EMD72000 was well tolerated and resulted in significant antitumor effects. A strong reduction in tumor growth was observed in the PDAC model with a mean tumor volume of 31% compared with the vehicle control. These results are striking considering that the xenograft used in this study previously exhibited almost complete resistance to clinically available chemotherapeutic drugs (Burger AM, etal, AACR03, Abs. 5719).

However, PDAC xenografts responded less well to single agent EMD72000 than xenografts from gastric and lung tumors. Therefore, the efficacy of EMD72000 was investigated in combination with gemcitabine in the L3.6pl pancreatic cancer cell line. Whereas exposure to EMD72000 or gemcitabine alone resulted in detectable tumor shrinkage and reduced lymph node and liver metastases, these effects were enhanced by the combination regimen. Furthermore, a strong effect of the combined treatment was evident as judged by significantly reduced microvessel density, and proliferative index, and significantly increased apoptosis. These effects were also observed in the single agent EMD72000 study. In this model, treatment was most effective when administered shortly after tumor-cell injection, whereas treatment duration appeared less important (Amendt C, etal, AACR03, Abs. 6180).

Using *in vivo* contrast enhanced MRI, investigators monitored the antiangiogenic effect of EMD72000 following blockade of EGFr signaling pathways in primary pancreatic tumors. A significantly reduced vascular permeability was observed in primary pancreatic tumors following therapy with EMD72000 as compared to controls and animals treated with low doses of the drug. EMD72000 effectively reduced primary pancreatic tumor growth and lymph node metastasis growing orthotopically in nude rats in a dose-dependent manner (Bruns CJ, etal, AACR02, Abs. 2880).

An open label, nonrandomized, dose escalation, phase I clinical trial of EMD 72000, in combination with gemcitabine in patients with advanced PDAC, was performed at Ruhr University Bochum (Germany), University Hospital of Schleswig-Holstein (Kiel, Germany), and Vall D'Hebron University Hospital (Barcelona, Spain), to determine the safety and tolerability of this regimen. Patients were administered escalating doses of EMD 72000 (I=400 mg/week, II=1000 mg/week, or III=800 mg/wk) followed by a weekly dose of gemcitabine (1000 mg/m^2). Following two 4-week cycles, patients with stable or responding disease continued treatment until disease progression or unacceptable toxicity. A total of 17 patients (I=5, II=4, III=8) were enrolled. EGFr expression was confirmed in 16 tumors. Treatment-related toxicities included skin effects (n=15), fever (n=4), neutropenia (n=3), and leukopenia (n=3). Grade 3 toxicities included neutropenia (n=3), leukopenia (n=2), fever (n=2), cholangitis (n=2), hypokalemia (n=2), increased liver enzymes (n=11), and LDH (n=2). Nontreatment-related Grade 4 toxicities were observed in 3/5 patients in dose-group I (cachexia, increased GGT and bilirubin) and 1/8 patients in dose-group III (quadriplegia). Grade 4 Guillain-Barré syndrome in a patient with quadriplegia was reported as possibly treatment-related. There was 1 death (400 mg) as a result of nontreatment-related respiratory failure. After continuous treatment for up to 50 weeks, disease stabilized in 3/5 (60%) patients in group I, 3/4 (75%) in II, and 5/8 (63%) in III. Skin biopsies demonstrated that EMD 72000 inhibited EGFr activation and affected receptor-dependent signaling and transduction *in vivo*, effects observed even in the lowest dose group. Pharmacokinetic data was comparable to results from other studies of EMD 72000 monotherapy (Graeven U, etal, ASCO04, Abs. 3061).

Panitumumab (ABX-EGF), under development by Abgenix (Fremont, CA) in collaboration with Amgen (Thousand Oaks, CA), is a fully human MAb targeting EGFr. ABX-EGF binds EGFr with high affinity, blocking the binding of both EGF and TGF-α in various EGFr-expressing human carcinoma cell lines, and abolishing EGF-dependent tumor cell activation and proliferation, including EGFr tyrosine phosphorylation. Upon binding to the receptor on tumor cells, ABX-EGF is internalized but not degraded, suggesting that it may be recycled to the cell surface. ABX-EGF also inhibits *in vitro* the spontaneous production of angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8) from tumor cells by 75% and 85%, respectively (Yang X-D, etal, ASCO00, Abs. 183).

Panitumumab is in phase II clinical trials in various malignancies. Also, three phase I clinical trials in advanced solid tumors, including pancreatic cancer, were ongoing as of October 2004.

In March 2003, a multicenter, dose-escalation phase I clinical trial (protocol IDs: UCLA-9906078-03B, NCI-G00-1673, ABX-EG-9901) of ABX-EGF was initiated at the Jonsson Comprehensive Cancer Center (Los Angeles, CA) and Fox Chase Cancer Center (Philadelphia, PA) in patients with renal, prostate, pancreatic, non-small cell lung, colon, rectal, esophageal, or gastroesophageal junction cancer, to determine MTD and pharmacokinetics. Arie Belldegrun, MD, of the Jonsson Comprehensive Cancer Center is Study Chair. An estimated 76 patients are being administered escalating doses of IV ABX-EGF over 1 hour weekly on weeks 0-3 for a total of 4 doses. Patients undergoing full pharmacokinetic analysis are administered a loading dose on week 0 and the subsequent 3 doses on weeks 3-5. Patients are followed every 2 weeks for 5 weeks. An open label, maintenance dosing, phase I clinical trial (protocol ID: ABX-EG-9902) of ABX-EGF as a follow-up of ABX-EG-9901, is ongoing as of October 2004 at Fox Chase Cancer Center.

An open label, phase I clinical trial (protocol ID: 20030251) of a two-dose schedule of panitumumab in patients with advanced solid tumors was initiated in September 2004 at multiple locations in the USA to determine the safety and pharmacokinetics of this treatment. Eligible patients must have tumors that are refractory to standard treatment or for which no standard therapy is available.

Farnesyltransferase Inhibitors (FTI)

Farnesyl transferase is an enzyme that activates ras, an oncogene mutated in many types of malignancies. Ras encompasses several related proteins encoded by three distinct genes, Hras, Kras (A and B), and Nras. Normal Ras is located in the inner surface of the cell membrane, where it plays an essential role in signal transduction. The protein cycles between inactive guanosine 5'-diphosphate-bound and active guanosine 5'-triphosphate (GTP)-bound configurations. Upon ligand binding to extracellular receptors, GTP-bound Ras interacts with Raf kinase and other proteins, leading to downstream activation of several intracellular pathways. In order for Ras to function, it has to be post-translationally modified by the addition of a 15-carbon farnesyl moiety to a cysteine amino acid at the C terminus of the protein. This reaction is catalyzed by the enzyme farnesyltransferase (FTase).

Kras mutations are the most common oncogene mutations in PDAC occurring in 75% to 95% of PDAC (Minamoto T, etal, Cancer Detect Prev 2000;24(1):1-12; Sakorafas GH, etal, Cancer Treat Rev, Feb 2000;26(1):29-52; Lohr M, etal, Int J Pancreatol, Apr 2000;27(2):93-103). Such mutations may lead to constitutive activation of the protein. Oncogenic forms of Ras are locked in their active state and transduce signals essential for transformation, angiogenesis, invasion and metastasis through downstream pathways. Abnormally activated Ras pathways lead to tumor growth, survival and metastasis. Activation of mutated Ras may be blocked by inhibiting FTase.

Several oral, small molecule FTase inhibitors (FTI) have been synthesized that are cytotoxic or cytostatic against a variety of solid tumors and hematologic malignancies. However, the precise mechanism of action of these drugs remains obscure because Ras may also be prenylated by other enzymes such as geranylgeranyl transferase (GGT). In addition, targeting of other farnesylated proteins may be partly responsible for the antitumor activity of FTI. Also, although FTI were developed as a specific inhibitor of Ras farnesylation and activity, it soon became clear that FTI activity is independent of Ras status.

Despite the involvement of Ras in PDAC, results of phase II clinical trials with FTI were uniformly disappointing. The failure of FTI in many settings is probably attributed to the fact that they do not block farnesylation sufficiently to prevent Ras activation. Although Hras is exclusively modified by farnesyltransferase, Kras and, to a lesser extent, Nras can also be modified by GGT that can support the biological activity of Ras. Geranylgeranylation of Kras and Nras becomes important only when farnesylation is blocked. In tumors with Kras mutations, as is the case in PDAC, inhibition of mutant Ras farnesylation is probably not responsible for any of the antitumor effects of FTI. Although GGT inhibitors (GGTI) have been developed, inhibition of Kras and Nras function by concomitant use of FTI and GGTI is not possible because of very high toxicity. The minimal toxicity associated with FTI may be actually attributed to the fact that they fail to inhibit effectively the function of all endogenous Ras proteins, which are essential for normal cell growth. FTI that target Ras also inhibit the farnesylation of a number of other proteins. Therefore, the ability of FTI to inhibit tumor-cell growth and survival may arise from an altogether different effect. Interestingly, FTI have shown more promising activity in advanced breast cancer, a disease in which Ras mutations are rare, and in several types of myeloid malignancies.

Lonafarnib (SCH66336, Sarasar), under development by Schering-Plough, is a potent, orally bioavailable nonpeptide tricyclic FTI in the pyridobenzocycloheptene class. Lonafarnib competes with the protein substrate for binding to FTase, inhibiting Hras processing in whole cells and blocking transformed growth properties of fibroblasts and human tumor cell lines expressing activated Kras.

Based on promising phase I results, lonafarnib was investigated in two phase II clinical trials, one in metastatic PDAC, refractory to gemcitabine (protocol ID: UCLA-9906030, NCI-G99-1610, SPRI-P00346), conducted at Jonsson Comprehensive Cancer Center, and in an open label, randomized trial (protocol ID: MSKCC-98115, NCI-G99-1571, SPRI-C98-545-12, CWRU-SCH-1298, NCI-

Exhibit 3
Ongoing Randomized Combination Clinical Trials of Cetuximab in PDAC

Regimen ❑ Protocol ❑ Institution ❑ Reference	Clinical Status ❑ Indication ❑ Enrollment	Protocol Description
Regimen 1 Cetuximab + docetaxel + irinotecan **Regimen 2** Docetaxel + irinotecan Protocol ID: ECOG-E8200 Yale Cancer Center (New Haven, CT)	Phase II (begin 8/02, ongoing 10/04)➢USA, Puerto Rico, Peru, South Africa, Australia ❑ metastatic pancreatic cancer Enrollment: 92 patients (46 per treatment arm)	Regimen 1: IV cetuximab is administered over 1-2 hours on days 1, 8, 15, 22, 29, and 36; IV docetaxel over 1 hour; and IV irinotecan over 30 minutes weekly on days 1, 8, 15, and 22. Regimen 2: docetaxel and irinotecan are administered as in arm 1. Courses repeat in both arms every 6 weeks in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months for 2 years, every 6 months for 1 year, and then periodically thereafter.
Regimen 1; Gemcitabine + cetuximab **Regimen 2** Gemcitabine Protocol ID: SWOG-S0205, CDR0000347414 Barbara Ann Karmanos Cancer Institute (Detroit, MI)/SWOG	Phase III (begin 12/03, ongoing 10/04)➢USA ❑ locally advanced inoperable or metastatic pancreatic cancer Enrollment: 704 patients (352 per treatment arm)	Patients are randomized to 1 of 2 treatment arms. In arm 1 patients are administered cetuximab IV over 1-2 hours on days 1, 8, 15, and 22 and gemcitabine IV over 30 minutes on days 1, 8, 15, and 22 for course 1 and days 1, 8, and 15 for all subsequent courses. In arm II patients are administered gemcitabine as in arm 1. In both arms, courses repeat every 4 weeks in the absence of disease progression or unacceptable toxicity. QoL is assessed at baseline, before each course, and at the end of the trial. Patients are followed every 6 months for 2 years and then annually for 1 year.
Regimen 1 Bevacizumab + gemcitabine + cetuximab **Regimen 2** Bevacizumab + gemcitabine + erlotinib Protocol ID: UCCRC-NCI-6580, NCI-6580, UCCRC-13200A University of Chicago Cancer Research Center (Chicago, IL)	Phase II (begin 8/04, ongoing 10/04)➢USA ❑ advanced pancreatic cancer Enrollment: 54-126 patients (27-63 per treatment arm)	Patients are stratified according to participating center (University of Chicago versus other) and ECOG performance status (0-1 versus 2). Patients are randomized to 1 of 2 treatment arms. In arm 1, patients are administered cetuximab IV over 1-2 hours on days 1, 8, 15, and 22; gemcitabine IV over 30 minutes on days 1, 8, and 15; and bevacizumab IV over 30-90 minutes on days 1 and 15. In arm II, patients are administered gemcitabine and bevacizumab as in arm 1, and oral erlotinib once daily on days 1-5, 8-12, and 15-26. In both arms, courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months.

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

G99-1534, SPRI-C98-545-18) comparing lonafarnib with gemcitabine in metastatic PDAC. This trial, being performed at multiple locations in the USA and Europe, is designed to determine PFS at 3 months, as well as safety and efficacy of this regimen. In this latter trial 63 patients, mostly chemotherapy-naïve, were randomized to lonafarnib (n=33) or gemcitabine (n=30). Patients were treated with either PO lonafarnib (200 mg), twice daily, or IV gemcitabine (1000 mg/m^2), weekly, for 7 weeks, followed by 1 week of rest. The 3-month PFS for lonafarnib was 23% and 31% for gemcitabine. The median overall survival for lonafarnib was 3.3 months and 4.4 months for gemcitabine. In the lonafarnib arm, there were 2 PR and 6 SD, while in the gemcitabine arm, there was 1 PR and 11 SD. Adverse events, including nausea, vomiting, and diarrhea, were reported equally for both drugs, but were more severe with gemcitabine. The frequency of hematologic toxicities, including thrombocytopenia (lonafarnib=0%, gemcitabine=17%), and neutropenia (lonafarnib=3%, gemcitabine=17%), was significantly lower with lonafarnib compared to gemcitabine (Lersch C, etal, ASCO01, Abs. 608).

Tipifarnib (R115777, Zarnestra), an imidazole in development by Johnson & Johnson, is a non-peptidomimetic FTI targeting activated p21 ras. Oral administration of R115777 inhibited the growth of human CAPAN-2 pancreatic xenografts, which contain mutations in KrasB. At the histologic level, R115777 significantly inhibited cell proliferation and angiogenesis. Evidence for apoptosis was also observed. In CAPAN-2 tumors, an overt increase of apoptosis led to the elimination of tumor endothelial cells. Also, a dramatic antiproliferative effect was measured within the tumors (Smets G, etal, AACR98, Abs 2170:318). However, in preclinical models, tumor-cell lines with wild-type Ras or mutated Nras or Hras were more sensitive to tipifarnib than those with Kras mutations. In addition, one study demonstrated that tipifarnib has a predominantly cytostatic effect on CAPAN-2 pancreatic cancer cell lines.

Despite promising activity of tipifarnib monotherapy in pancreatic cancer in phase I clinical trials, results were disappointing in two phase II trials of single agent tipifarnib in patients with advanced PDAC. In one multicenter phase II clinical trial (protocol ID: FCCC-00005, NCI-45), initiated in May 2000 at Fox Chase Cancer Center and M.D. Anderson Cancer Center, to determine the safety, effectiveness, and pharmacokinetics of R115777 in treating patients with metastatic PDAC, oral R115777 was administered twice daily for 21 consecutive days. Treatment continued every 28 days in the absence of disease progression or unacceptable toxicity. Patients were then followed every 3 months for 1 year. This trial was reported closed in April 2002. In this trial, 20 chemotherapy-naïve patients with metastatic PDAC were treated with R115777 (300 mg), every 12 hours, for 21 days. Grade 3/4 toxicities observed were neutropenia (n=3), liver-enzyme elevation (n=3), and rash (n=2). Grade 1/2 toxicities included fatigue (74%), anemia (63%), nausea/vomiting (58%), liver-enzyme elevation (53%), and diarrhea (47%). There were no objective responses. Median TTP was 4.9 weeks and median survival time (MST) was 19.9 weeks at a median follow-up of 19.3 weeks. Estimated 6-month survival was 28.3%, with no patients progression-free at 6 months. FTase activity was reduced by 49.8% ± 9.8% 4 hours following treatment on day 1, and 36.1% ± 24.8% before treatment on day 15. However, although treatment with tipifarnib caused partial inhibition of FTase activity in mononuclear cells, it did not demonstrate antitumor activity in patients with previously untreated metastatic PDAC (Cohen SJ, etal, ASCO02, Abs. 545:137a and Cohen SJ, etal, J Clin Oncol, 1 Apr 2003;21(7):1301-6).

Another phase II clinical trial (protocol ID: SWOG-S9924) of R115777 in patients with advanced PDAC was conducted by the Southwest Oncology Group (SWOG), chaired by Charles A. Coltman, Jr, MD. Between June 2000 and November 2001, 56 patients with PDAC were enrolled with 47 being eligible for inclusion. R115777 (300 mg) was administered PO twice daily for 3 out of every 4 weeks. Toxicities included Grade 3 in 14/47 (30%) patients, Grade 4 in 6/47 (13%), and Grade 5 in 7/47 (15%). Most frequent toxicities were fatigue and malaise in 28/47 (60%) patients, nausea in 27/47 (57%), and anemia in 24/47 (51%). Grade 5 toxicities included thromboembolism in 1 patient, infection in 3, and other effects in 3; 2 patients remained on therapy. MST was 2.7 months, 6-month survival was 17%, median time-to-treatment failure was 1.3 months. In this trial, R115777 was ineffective as monotherapy in advanced PDAC (Macdonald JS, etal, ASCO02, Abs. 548:138a).

Tipifarnib has also been studied with gemcitabine, on the basis of a phase I trial that demonstrated the safety of the combination. A multicenter (n=133) double blind, placebo-controlled, phase III clinical trial (protocol ID: JRF-R115777-INT-11) of gemcitabine plus placebo, or plus R115777, in pathologically confirmed, locally advanced, inoperable or metastatic PDAC, was closed to patient recruitment in March 2001. This trial was conducted in the USA and in Europe by the Janssen Research Foundation (Beerse, Belgium).

Patients were randomized to one of two treatment arms. In arm I, patients were administered oral R115777 (200 mg) every 12 hours, continuously, in combination with IV gemcitabine (1000 mg/m^2) over 30 minutes, weekly, for 7 weeks, followed by 1 week of rest, then weekly for 3 weeks every 4 weeks. In arm II, patients were treated with gemcitabine as in arm I. Treatment continued in the absence of disease progression or unacceptable toxicity. This trial enrolled 688 patients previously untreated with systemic therapy. Primary endpoint was MST. No statistically significant differences in survival parameters were observed between the two arms. Median overall survival for gemcitabine and R115777 was 193 days versus 182 days for controls; 6-month and 1-year survival rates for gemcitabine and R115777 were 53% and 27%, respectively, versus 49% and 24% for controls; median PFS for gemcitabine and R115777 was 112 days versus 109 days for controls. There were 10 drug-related deaths in the gemcitabine and R115777 arm, and 7 in the control arm. Neutropenia and thrombocytopenia, ≤Grade 3, were observed in 40% and 15% in gemcitabine and R115777, respectively, versus 30% and 12% in controls. With the exception of diarrhea (38% gemcitabine and R115777 versus 25% for controls) and hypokalemia (15% gemcitabine and R115777 versus 8% for controls), incidences of non-hematologic adverse events were similar in both groups. Patients on gemcitabine and R115777 may have maintained QoL longer than controls. Although the combination of gemcitabine and R115777 had an acceptable toxicity profile, it did not prolong overall survival in advanced PDAC as compared to single agent gemcitabine (Van Cutsem E, etal, ASCO02, Abs. 517:130a, and J Clin Oncol, 15 Apr 2004;22(8):1430-8).

A trial was also conducted to evaluate combining chemoradiation with tipifarnib in patients with advanced PDAC. This multicenter, randomized, phase II clinical trial (protocol ID: RTOG-PA-0020; RTOG-DEV-1003), to com-

pare 1-year survival rate and toxicity of gemcitabine, paclitaxel, and radiation therapy with or without R115777 in treating patients with locally advanced pancreatic cancer, initiated in November 2001, under Study Chair Tyvin Andrew Rich, MD, of Radiation Therapy Oncology Group (RTOG), was closed in 2003. A total of 154 patients were to be randomized to 1 of 2 treatment arms in the USA and Canada. In arm I, patients undergo radiotherapy once daily, 5 days a week, for 5.5 weeks, beginning on day 1. IV paclitaxel is administered over 1 hour and IV gemcitabine over 30 minutes on days 1, 8, 15, 22, 29, and 36. Patients in arm II undergo chemoradiotherapy as in arm I. Within 3-8 weeks after completion of chemoradiotherapy, patients without disease progression are treated with oral R115777 twice daily for 21 days. Treatment is continued every 28 days in the absence of disease progression or unacceptable toxicity. Patients are to be followed every 3 months for 2 years, every 6 months for 3 years, and then annually thereafter.

A dose-escalation, multicenter, phase I clinical trial (protocol ID: UPCC-20203, NCI-6407) of tipifarnib and radiotherapy in patients with inoperable locally advanced PDAC was initiated in January 2004 at the Abramson Cancer Center (Philadelphia, PA), under PI Stephen Michael Hahn, MD, to determine MTD, DLT, and 3-month clinical response for this regimen. Patients are administered oral tipifarnib once or twice daily on weeks 1-8. Patients also undergo concurrent radiotherapy daily, 5 days a week, on weeks 2-8. Cohorts of 3-6 patients are administered escalating doses of tipifarnib until MTD is determined. Patients are followed at 1, 3, and 6 months. A total of 8-18 patients will be accrued for this trial.

It is not clear why tipifarnib did not prove effective in the treatment of advanced PDAC. A general theory may attribute such failure to the heterogeneity of pancreatic tumors, and their dependence on multiple pathways for survival, which is also the case for many other targeted therapeutics. More specifically, failure of this FTI in advanced disease may be attributable to the fact that blocking FTase is not sufficient to abrogate Ras activity, as Ras may still undergo post-translational prenylation by other enzymes, such as GGT. It is also conceivable that tipifarnib produces insufficient inhibition of protein farnesylation at clinically tolerable concentrations. However, the activity of tipifarnib in earlier stages of PDAC warrants investigation.

Hedgehog Pathway Signaling Inhibitors

The Hedgehog (Hh) signaling pathway encompasses a family of secreted proteins that are essential in controlling normal development and growth of tissues and organs, maintaining somatic stem cells, and specifying organ size. Genetic and biochemical analyses in flies and vertebrates have identified an Hh signaling pathway conserved from flies through to humans. Sonic hedgehog (Shh) is the vertebrate homolog of Hedgehog in *Drosophila*. In contrast with the single Hh in flies, vertebrates have at least three Hh homologs, Sonic hedgehog (Shh), Desert hedgehog (Dhh) and Indian hedgehog (Ihh). Components of the Hh signaling pathway include Shh, Patched, a tumor suppressor gene, and Gli1, a transcription factor. Patched is a transmembrane receptor for Shh and functionally associated with another transmembrane protein, Smoothened, a proto-oncogene associated with neuroectodermal tumors. The multipass Patched receptor functions in association with Smoothened, to activate the transcription of target genes. Pathway activation is triggered by binding of Hh proteins to the Patched receptor. Blocking of Smoothened by Patched is released upon Hh binding, leading to the activation of *cubitus interruptus* (Ci), a zinc finger protein that is the transcription factor of the Hh signaling pathway. At present three vertebrate Ci genes, gli 1, 2 and 3, have been identified but their exact role in the activation and repression of Hh target genes remains to be elucidated (van Tuyl M and Post M, Respir Res 2000;1(1):30-5).

In the absence of Shh, Patched represses the constitutive signaling activity of Smoothened and also regulates Gli. Release of Smoothened culminates in the activation of one or more of the Gli transcription factors that regulate the transcription of downstream targets. Individuals with germline mutations of the Shh receptor gene Patched are at high risk of developmental anomalies and various malignancies. Activation of the Hh signaling pathway by sporadic mutations or in familial conditions such as Gorlin's syndrome, is associated with malignancies of the skin, the cerebellum and skeletal muscle.

Dr. Philip Beachy and his colleagues at Johns Hopkins University School of Medicine reported that the Hh pathway was overactive 20- to 5000-fold in malignancies of the esophagus, stomach, biliary tract, and pancreas. The only exception was colorectal cancer in which the Hh pathway seems not to be implicated. When tumors with overactive Hh were injected into mice without a functioning immune system, the mice developed tumors. Cyclopamine, an inhibitor of the Hedgehog pathway, suppressed tumor cell growth *in vitro*. Tumors in mice disappeared with the administration of cyclopamine, further confirming the role of the Hh pathway in these malignancies (Berman DM, etal, Nature, 23 Oct 2003;425(6960): 846-51; comment:780-2). Unlike tumors associated with Gorlin's syndrome, caused by mutations, pathway activity and cell growth in digestive tract tumors are driven by endogenous expression of Hh ligands. This is demonstrated by the presence of Shh and Ihh transcripts, by the pathway- and growth-inhibitory activity of a Hh-neutralizing antibody, and by the dramatic growth-stimulatory activity of exogenously added Hh ligands. Therefore, methods of inhibiting the Hh signaling pathway using small molecule drugs and/or Hh antibodies, may constitute a promising new therapeutic approach to the treatment of these malignancies.

Analysis of human tumors reveal mutations in various components of the Shh signaling pathway that appear to activate this pathway, as inferred by the increased expression

of Gli1. Interestingly, a proportion of the human tumors and most of those arising in mouse models continue to express the normal patched allele, suggesting the involvement of additional molecular events in tumor cells (Wetmore C, Curr Opin Genet Dev, Feb 2003;13(1):34-42).

One of the biological properties of Hh is to promote the expression of various tissue growth factors, including angiogenesis factors that support tumor growth. Recent findings point to a previously uncharacterized role for Hh signaling in vascular development, and identify Hh signaling as an important component of the molecular pathway leading to vascular tube formation (Vokes SA, etal, Development, Sep 2004;131 (17):4371-80).

Shh is abnormally expressed in PanIN and PDAC. When normal adult human pancreatic tissue was compared to specimens from patients with PDAC, no Hh protein was detected in the normal tissue, but it was found in 70% of precancerous and cancerous specimens. Furthermore, key genes in the Hh pathway were also overexpressed in PDAC.

A group, led by Sarah Thayer, MD, at Massachusetts General Hospital (Boston, MA) and Matthias Hebrok, PhD at the University of California, San Francisco, found that Hh was overactive in all 26 human PDAC cell lines screened (Thayer SP, etal, Nature, 23 Oct 2003; 425(6960):851-856). Pancreata of Pdx-Shh mice, in which Shh is misexpressed in the pancreatic endoderm, develop abnormal tubular structures, similar to those of human PanIN-1 and -2. Moreover, these PanIN-like lesions also contain mutations in Kras and overexpress HEr-2/neu, genetic mutations found early in the progression of human PDAC. Furthermore, Hh signaling remains active in cell lines established from primary and metastatic PDAC. Inhibition of Hh signaling by cyclopamine induced apoptosis and blocked proliferation in a subset of the pancreatic cancer cell lines both *in vitro* and *in vivo*. These data suggest that this pathway may have an early and critical role in the genesis of this cancer, and that maintenance of hedgehog signaling is important for aberrant proliferation and tumorigenesis. PDAC tumors in mice xenografted with PDAC were reduced by 50% to 60% in size after 7-day treatment with cyclopamine. However blocking the Hh pathway killed only half of these cells, indicating that not all PDAC require Hh activity for survival, and mutations downstream from the Hh pathway may be implicated in the disease.

Indian hedgehog (Ihh) and its two signaling receptors, Patched and Smoothened, are involved in pancreatic development and regulation of β-cell function as well as in certain human tumors. When expression, distribution and function of Ihh and its receptors in PDAC, were analyzed by investigators in at the University of Heidelberg in Germany, Ihh, Patched and Smoothened mRNA levels were increased 35-, 1.2- and 1.6-fold, respectively, in pancreatic cancer tissues in comparison to normal controls. Ihh, Patched and Smoothened were expressed in the islet cells of normal and cancerous tissues and in pancreatic cancer cells. The growth of pancreatic cancer cells was dose-dependently inhibited by cyclopamine through G0/G1 arrest. In contrast, Ihh agonists exhibited no significant effect on pancreatic cancer cell growth. TGF-β1 repressed Ihh transcription in a TGF-β1-responsive pancreatic cancer cell line, but had no effect on the other tested cell lines. Ihh and its receptors are expressed in pancreatic cancer, and blockage of Hh signaling results in inhibition of pancreatic cancer cell growth, suggesting that aberrant activation of the Ihh signaling pathway contributes to tumor development in this malignancy (Kayed H, etal, Int J Cancer. 2004 Jul 10;110(5):668-76).

In addition to inhibition of Shh, investigators at Johns Hopkins and elsewhere are developing modulators of Smoothened. SAG, a chlorobenzothiophene-containing Hh pathway agonist, binds to the Smoothened heptahelical bundle, also the binding site of cyclopamine, but yet antagonizes cyclopamine action. To understand Smoothened regulation through small molecule binding better, investigators also identified additional effectors of the pathway including compounds SANT-1 through SANT-4, that potently inhibit Shh signaling by binding directly to Smoothened Other small molecule Hh pathway inhibitors are also in development that appear to act downstream of Smoothened (Chen JK, etal, PNAS USA, 29 Oct 2002;99(22):14071-6).

Cyclopamine, a plant-derived steroidal alkaloid originally isolated from an extract of a lily, *Veratrum californicum*, found in the western USA, is a specific inhibitor of the Hh pathway. Cyclopamine has both teratogenic and antitumor activities arising from its ability to specifically block cellular responses to vertebrate Hh signaling. This inhibitory effect is mediated by direct binding of cyclopamine to the heptahelical bundle of Smoothened.

Cyclopamine also can reverse retention of partially misfolded Smoothened in the endoplasmic reticulum, presumably through binding-mediated effects on protein conformation. These observations reveal the mechanism of cyclopamine's teratogenic and antitumor activities and further suggest a role for small molecules in the physiological regulation of Smoothened (Chen JK, etal, Genes Dev, 1 Nov 2002;16(21):2743-8).

The agent itself may not become an anticancer drug, in part because it is already in the public domain, but it is serving as a platform for the development of mimics. A patent application, describing the use of cyclopamine to selectively block the Hh pathway for therapeutic purposes, was filed by the Johns Hopkins University School of Medicine, and subsequently licensed to Curis (Cambridge, MA).

HhAntag are small molecule drugs or MAb that inhibit the hedgehog pathway, being developed as cancer therapeutics by Curis, in collaboration with Genentech. Curis has successfully developed several promising Hh inhibitors,

including small molecule Hh antagonists, and an Hh MAb, that are effective in blocking tumor growth in cancer models.

In June 2003, Curis licensed its novel small molecule and antibody inhibitors of the Hedgehog signaling pathway to Genentech for applications in cancer therapy. Included among the small molecules covered by this agreement is CUR-61414, a small molecule that inhibits the hedgehog pathway, being developed as a locally administered therapy and tissue-sparing alternative to surgery for basal cell carcinoma (BCC). Under terms of the agreement, Genentech has agreed to pay Curis a license fee of $8.5 million ($5 million in cash and $3.5 million in equity) and additional fees over two years. The agreement also provides for Genentech to make cash payments to Curis upon the successful achievement of clinical development and drug approval milestones. Genentech will also pay a royalty on net product sales, which increases with increasing sales volume.

Mammalian Target of Rapamycin (mTOR) Signaling Pathway Modulators

The mammalian target of rapamycin (mTOR) is a large serine/threonine kinase involved in the initiation of mRNA translation. mTOR is a member of the phosphoinositide 3-kinase related kinase (PIKK) family. It regulates essential signal-transduction pathways, is involved in the coupling of growth stimuli with cell-cycle progression, and initiates mRNA translation in response to a favorable nutrient environment. It regulates many aspects of cell growth, including membrane traffic, protein degradation, protein kinase C signaling, ribosome biogenesis, and transcription. It acts as a checkpoint for ribosome biogenesis long recognized as a predictor of cancer progression; increase in size and number of nucleoli is known to be associated with the most aggressive tumors and poor prognosis (Kozma SC, etal, AACR02, Abs. 5628).

mTOR plays a critical role in transducing proliferative signals mediated through the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) signaling pathway, principally by activating downstream protein kinases that are required for both ribosomal biosynthesis and translation of key mRNA of proteins required for G1 to S phase transition (Mita MM, etal, Cancer Biol Ther, Jul-Aug 2003;2(4 Suppl 1):S169-77).

mTOR inhibition results in cell-cycle arrest in G1 phase. Inhibitors of mTOR also prevent cyclin-dependent kinase (CDK) activation, inhibit retinoblastoma (Rb) protein phosphorylation, and accelerate the turnover of cyclin D1, leading to a deficiency of active CDK4/cyclin D1 complexes, all of which may help cause G1-phase arrest. Malignancies with phosphatase and tensin homolog (PTEN) tumor suppressor gene mutations associated with constitutive activation of the PI3K/Akt pathway that are relatively resistant to apoptosis, may be particularly sensitive to mTOR (Peralba J-M, etal, AACR03, Abs. R3638, and Panwalkar A, etal, Cancer, 15 Feb 2004;100(4):657-66; comment:1478, author reply:1478).

Rapamycin, now referred to as sirolimus, is the original natural agent, initially isolated from the soil bacteria *Streptomyces hygroscopicus* that is the basis for most mTOR inhibitors currently in development. Sirolimus, a complex macrolide and highly potent fungicide, immunosuppressant, and anticancer agent, is a highly specific inhibitor of mTOR. By targeting mTOR, rapamycin inhibits signals required for cell-cycle progression, cell growth, and proliferation. Rapamycin analogs with relatively favorable pharmaceutical properties are under investigation in patients with solid tumors and hematologic malignancies.

Researchers examined whether inhibition of the mTOR pathway affects mitogen-induced proliferation and cell-cycle progression of human pancreatic cancer cells *in vitro*. Rapamycin inhibited phosphorylation of mTOR in BxPC3 and PANC-1 human pancreatic cancer cells at cell-cycle entry, inducing G1 cell-cycle arrest. In both cell lines, rapamycin inhibited serum-induced proliferation without affecting apoptosis. Akt phosphorylation was not affected, indicating mTOR's specificity to rapamycin. mTOR signaling appears to be necessary for G1-to-S phase progression and proliferation in pancreatic cancer cells (Shah S, etal, J Surg Res 15 May 2001;97(2):123-30).

AP23573, under development by Ariad Pharmaceuticals (Cambridge, MA), is a phosphorus-containing sirolimus analog that inhibits mTOR. Ariad used structure-based drug design to synthesize analogs of rapamycin with optimal pharmacologic properties as antitumor agents. AP23573, one of the most potent compounds, was selected for further study and development. The C43 modification of AP23573 was stable in organic solvents, aqueous solutions at a variety of pH, and in plasma and whole blood, both *in vitro* and *in vivo*. These stability studies along with *in vitro* metabolism studies also indicated that AP23573 is not a prodrug for rapamycin (Metcalf CA III, etal, AACR04, Abs. 2476).

AP23573 shrinks tumors by a novel mode of action that causes cancer-cell starvation (metabolic arrest) through inhibition of nutrient uptake by tumor cells, as well as inhibition of growth factor stimulation. This broadly applicable approach to cancer treatment is anticipated to make AP23573 particularly useful for treatment of advanced solid tumors. Also, studies indicate that mTOR inhibition effectiveness may be even more pronounced in tumor cells with a mutated or inactivated form of PTEN. Tumors without PTEN protein represent many of the major difficult-to-treat malignancies, including prostate, uterine, pancreatic, and ovarian cancer, as well as melanoma, leukemia and glioma. Because cancer patients with PTEN-deficient tumor cells can be identified using readily available tests, it may be possible to select those who may benefit most from AP23573.

In May 2003, Ariad reported results of ongoing comprehensive *in vivo* studies of AP23573, exploring a variety of doses and dosing regimens, alone or in combination with other antitumor agents to further characterize the antitumor activity of the compound (Clackson T, etal, ASCO03, Abs. 882:220). Mouse xenograft studies were performed using six different human tumor cell lines of diverse tissue origin, including U-87 MG, PC-3 prostate, MCF7 breast, PANC-1 pancreas, A549 lung, and HCT-116 colon. Mice were administered AP23573, IP or PO, at doses ranging from 0.1 to 10 mg/kg. The two intermittent dosing schedules used to circumvent potential immunosuppressive effects of daily dosing, were five consecutive daily doses followed by a 9-day rest period, or weekly dosing. When treatment was initiated at an early stage of tumor growth, dosing on either schedule induced regressions of up to 90% that persisted for up to two weeks. Dosing at a later stage also resulted in substantial reductions in the rate of growth of all six tumor types. Parallel studies demonstrated that intermittently administered doses that were efficacious in xenograft studies were non-immunosuppressive in a mouse skin allograft rejection model. Molecular analysis of tumors removed from treated animals confirmed that mTOR signaling was completely abolished one day after a single administration of AP23573, and that this inhibition persisted for 2 to 3 days. These findings form the basis for using biomarkers to determine patterns of responsiveness to AP23573 therapy in clinical trials of patients with cancer. *In vitro* studies also demonstrated that the antiproliferative activity of AP23573 is additive with that of camptothecin, cisplatin and 5-FU, supporting the use of AP23573 in combination regimens tailored to treat specific malignancies.

AP23573 is presently being clinically evaluated in hematologic malignancies and in glioma.

Everolimus (RAD001, Certican), a hydroxyethyl ether derivative of sirolimus, is an orally administered specific inhibitor of mTOR. RAD001 possesses potent immunosuppressive and anticancer activities. The drug, developed by Novartis, was approved in 2003 in Europe as an immunosuppressive agent in solid organ transplant patients.

RAD001 is a cell-cycle inhibitor, arresting cells in the G1 phase. It inhibits oncogenic signaling in tumor cells and angiogenic signaling in vascular endothelial cells. RAD001 inhibits the growth of a wide range of histologically diverse tumor cells. In cancer, RAD001 is being developed as a cytostatic agent to delay the time to tumor recurrence/progression, or to extend survival of patients with various malignancies. RAD001 is particularly applicable to the treatment of leukemia.

In preclinical studies RAD001 had an antiproliferative effect on tumor cells *in vitro* and in tumor-bearing rodents, including CA20948 pancreatic tumor-bearing rats (Boulay A, etal, Cancer Res. 2004 Jan 1;64(1):252-61 and AACR03, Abs. 2984). RAD001 demonstrated dose-dependent antitumor activity with daily and weekly administration schedules. The agent was well tolerated and exhibited antitumor potency similar to that of 5-FU. Moreover, the efficacy of intermittent treatment suggested a therapeutic window allowing differentiation of antitumor activity from the immunosuppressive properties of this agent.

In a phase I clinical trial in patients with advanced cancer, RAD001 was administered to successive cohorts at increasing weekly doses estimated to reach blood levels associated with significant antitumor activity in the CA20948 preclinical *in vivo* study. Assuming that for effective antitumor activity in patients the predicted kinase inhibition must be as great as that observed in the rat study, 20 mg once weekly was identified as the initial dose for subsequent clinical investigation, in which the pharmacodynamic activity of RAD001 is to be determined in tumor tissue itself (Lane H, etal, ASCO03, Abs. 951).

RAD001 is being currently evaluated in phase I/II clinical trials in hematologic malignancies and various solid tumors.

Temsirolimus (CCI-779), under development by Wyeth, is a structural analog of the sirolimus. When investigators at Kyoto University Graduate School of Medicine, in Japan, examined the expression pattern of mTOR signaling pathways in six human PDAC cell lines, and 20 tissue specimens of PDAC, PTEN expression was detected in four cell lines except for KMP-3 and KMP-4 cells. CCI-779 had additive effects with gemcitabine in these two cell lines resistant to treatment with CCI-779. Furthermore, CCI-779 induced antitumor activity in AsPC-1 xenografts. The average volume of AsPC-1 subcutaneous xenografts was 1514 mm^3 in control mice and 1249 mm^3 in gemcitabine alone-treated mice, whereas the average volume was 375 mm^3 in CCI-779-treated mice and 444 mm^3 in CCI-779 with gemcitabine-treated mice. These results suggest that mTOR may be a promising target addressed by CCI-779 for the treatment of PDAC (Ito D, etal, AACR04, Abs. 3886).

A phase II clinical trial (protocol IDs: MDA-2003-0530; NCI-6182) was initiated at the University of Texas M. D. Anderson Cancer Center, in December 2003, to determine the overall survival at 6 months of patients with locally advanced or metastatic pancreatic cancer treated with CCI-779. Principal investigators are Henry Qinghua Xiong MD, PhD, and James L. Abbruzzese, MD. A total of 40 patients are to be administered IV CCI-779 over 30 minutes once weekly, every 4 weeks, in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months.

Other Novel Targeted Therapeutics

Numerous agents targeting various biomarkers are being investigated both clinically and preclinically in the treatment of solid tumors. A good number of these will probably prove applicable in PDAC. The agents described here have shown activity in PDAC but the list is by no

means inclusive. Targeted therapeutics acting as antiangiogenesis or metastatic agents are covered in Part V of this series.

AGRO100, under development by Aptamera (Louisville, KY), is a G-rich oligonucleotide that binds to nucleolin, a protein found on the surface of tumor cells. Nucleolin is an abundant, multifunctional phosphoprotein predominantly located in the nucleolus of proliferating cells. The level of nucleolin is related to the rate of cell proliferation. AGRO100 is an aptamer, i.e. an oligonucleotide that binds to protein targets based on its 3-dimensional shape.

In preclinical studies AGRO100 exhibited potent anticancer activity against multiple malignancies. In September 2003, Aptamera initiated a dose-escalation phase I clinical trial with AGRO100, at the University of Louisville's James Graham Brown Cancer Center, in patients with various advanced solid tumors. The primary objective of the trial is to determine the appropriate dose levels of AGRO100 that can be safely administered to patients, and to establish the pharmacokinetic profile of the drug. Enrollment in this trial, expected to involve up to 20 patients, had been completed as of August 2004.

Although AGRO100 appears to be an effective treatment of many types of malignancies, based on its research efforts, Aptamera concluded that pancreatic cancer would be an appropriate primary disease target for further clinical development. A clinical trial in this indication is to be initiated in 2004. In August 2004, the FDA granted orphan drug designation to AGRO100 in the treatment of PDAC.

In August 2003, Aptamera signed a product development and license agreement with Archemix (Cambridge, MA) for AGRO100. As part of this agreement, Archemix, the exclusive holder of rights to the SELEX portfolio for aptamer therapeutics, will provide Aptamera with a nonexclusive license to aptamer technology for therapeutic uses of AGRO100 and AGRO100 derivatives. In addition, Archemix will provide Aptamera expertise and know-how in support of these programs. Aptamera remains responsible for the clinical development, and ultimately, marketing of AGRO100 and its derivatives.

AP 12009, under development by Antisense Pharma (Regensburg, Germany), a phosphorothioate antisense oligonucleotide specific for the human transforming growth factor-β 2 (TGF-β2) isoform mRNA, is a specific inhibitor of TGF-β2 protein production. Secretion of high levels of TGF-β by tumor cells prevents the host immune system from recognizing and destroying these cells. Overexpression of TGF-β2 in malignancies is associated with advanced tumor stage and correlates with decreased survival.

TGF-β2 levels were significantly elevated in patients with pancreatic cancer (median=1198 pg/ml; range=319-1713 pg/ml) compared to healthy controls (median=378 pg/ml; range=241-575 pg/ml). AP 12009 significantly reduced TGF-β2 secretion in several human pancreatic cancer cell lines Hup-T3, Hup-T4, and PA-TU 8902 by up to 76%. Migration of PA-TU 8902 cells was completely blocked as compared to untreated controls, while a TGF-β2 antibody had no effect. Furthermore, AP 12009 reversed TGF-β2 mediated immunosuppression in an allogeneic PA-TU 8902 system targeted by lymphokine-activated killer (LAK) cells derived from healthy donors. After AP 12009 treatment, LAK cell cytotoxicity was increased in a donor-dependent manner up to 708% of the untreated control (Stauder G, etal, ECCO03, Abs. 229, Eur J Cancer September 2003;Suppl;1(5):S71, and ASCO04, Abs. 4106).

Safety of AP 12009, assessed in cynomolgus monkeys, did not indicate any untoward side effects. Based on the preclinical efficacy in tumor proliferation, migration and inhibition of escape from immunosurveillance, the favorable toxicity profile in animal studies, and the successful application of AP 12009 in clinical studies with glioma patients, a multicenter dose-escalation trial with AP 12009 in pancreatic carcinoma is currently in preparation (Schlingensiepen K-H, etal, AACR04, Abs. 2955). In phase I/II clinical trials in high grade glioma, the efficacy of AP 12009 was manifested as a significant and persistent tumor response, including one CR and prolonged overall MST compared to literature data on temozolomide (Temodar; Schering-Plough).

ARRY-142886 (AZD6244), under development by Array BioPharma (Boulder, CO), is a selective, orally active inhibitor of the protein target MAPK/ERK/kinase (MEK), which represents one step in a critical cellular hyperproliferation pathway in human cancer cells. The drug's mechanism of inhibition is noncompetitive with respect to ATP. ARRY-142886 has demonstrated no inhibitory effects on a wide variety of both tyrosine and serine/threonine kinases, but inhibits the MEK pathway with an IC_{50} of 10 nM (Lyssikatos J, etal, AACR04, Abs. 3888).

In preclinical studies, efficacy of this agent was demonstrated in *in vitro* and *in vivo* tumor models of malignant melanoma and pancreatic, colon, lung, and breast cancer. No significant adverse effects were observed in early toxicity testing with this agent.

In June 2004, Array BioPharma initiated a multicenter phase I clinical trial designed to evaluate tolerability and pharmacokinetics of ARRY-142886 following oral administration in patients with advanced cancer. In addition, the trial is designed to examine patients for indications of biologic activity using tumor biomarkers and pharmacodynamic measurements.

In December 2003, AstraZeneca acquired exclusive worldwide rights to ARRY-142886 and certain second-generation compounds for all oncology indications. Array retains the rights to all therapeutic indications for compounds not selected by AstraZeneca as part of the collaboration. The parties have created a joint steering committee to oversee the drug development process. Array is

responsible for filing the IND, phase I clinical trials, and certain aspects of process research for the development of ARRY-142886. In addition, Array is responsible for creating a select number of second-generation compounds, and cGMP manufacturing of phase I clinical materials. AstraZeneca is responsible for all other aspects of clinical development and commercialization. In addition to the MEK program, the two parties have agreed to a further collaboration aimed at targets selected by AstraZeneca. Under this agreement, Array will receive an upfront payment of $10 million, research funding, potential development milestones of over $85 million (dependent upon the number of successfully commercialized products), and royalties on product sales. In June 2004, Array became entitled to a $4 million milestone payment for initiating a phase I clinical trial with ARRY-142886.

ARQ 501 (β-lapachone), in development by ArQule (Woburn, MA) in collaboration with Roche, is a naturally occurring tricyclic O-naphthoquinone that is a checkpoint regulator of the E2F pathway. E2F selectively activates checkpoint-mediated apoptosis in cancer cells without causing damage to normal cells. The pathway influences the activation of E2F, a key cell-death regulator. Direct checkpoint activation is a strategy against cancer. Activation of E2F plays a critical role in apoptosis of cancer cells, while leaving healthy cells unharmed. Unlike most cytotoxic drugs that kill cancer cells by indirectly activating checkpoint-mediated apoptosis after creating nonselective damage to DNA or microtubules, which accounts for their toxicity toward normal cells, ARQ 501 directly activates checkpoint regulators without creating such damage. ARQ 501 selectively induces apoptosis in cancer cells without causing the death of non-transformed cells in culture. This unusual selectivity against cancer cells is preceded by activation of S-phase checkpoint and selective induction of E2F1, a regulator of checkpoint-mediated apoptosis (Li Y, etal, PNAS USA, 4 Mar 2003;100(5):2674-8).

The E2F transcription factor family of proteins is involved in the complex processes of both cellular proliferation and apoptosis. E2F is needed for cellular proliferation; however high levels of activated E2F or failed down-regulation of E2F can also trigger apoptosis through p53 dependent or independent mechanisms. The activity of E2F is regulated through its interaction with the retinoblastoma (Rb) family of proteins which, when phosphorylated, release E2F, allowing it to transcribe E2F-responsive genes. ARQ 501 seeks to modulate the E2F/Rb pathway to control cellular proliferation and apoptosis. Apoptosis can be selectively triggered in cancer cells without harming normal cells by rapidly raising the concentration of the checkpoint regulatory protein E2F1. ArQule uses its Activated Checkpoint Therapy (ACT) platform to activate multiple checkpoints simultaneously to enable cancer cells to detect and respond to DNA damage, so that they undergo apoptosis, while normal cells are spared. The approach demonstrates greater specificity and lower toxicity than traditional cancer therapies, while maintaining wide spectrum activity to counteract tumor heterogeneity.

ArQule obtained ARQ 501, then CO-501, via its acquisition of Cyclis Pharmaceuticals, now ArQule Biomedical Institute (Norwood, MA), that had licensed full rights to ARQ 501 from the Dana-Farber Cancer Institute where it was discovered by a team of scientists led by Arthur B. Pardee, PhD. In October 2002, Cyclis also licensed exclusive worldwide rights to broad technology from the Beth Israel Deaconess Medical Center and the Dana-Farber Cancer Institute that significantly expands the company's Activated Checkpoint Therapy (ACT) platform, including two patents that broadly protect the concept of checkpoint activation as a strategy for cancer therapy. In addition to these two new patents, ArQule has additional issued and pending international patent applications to protect the technology.

In April 2004, Roche and ArQule established a partnership to discover and develop drug candidates targeting the E2F pathway, including ARQ 501. Under the terms of the agreement, Roche obtained an option to ArQule's E2F program in the oncology field. Roche provided immediate research funding of $15 million, and will assume significant financial support for ongoing R&D. ArQule is responsible for advancing drug candidates from early stage development into phase II trials. Roche may opt to license worldwide rights for the development and commercialization of products resulting from this collaboration, by paying an option fee. Assuming the successful development and commercialization of a compound under the program, ArQule could receive up to $276 million in milestone payments, plus royalties. Additionally, ArQule has the option to copromote E2F-related products in the USA. ArQule retains all rights to programs associated with its Activated Checkpoint Therapy platform that work independently of the E2F pathway.

In preclinical studies as well as in animal models, ARQ 501 demonstrated potency and selectivity in the activation of faulty cellular checkpoint machinery in cancer cells, both as monotherapy and in combination with cytotoxic drugs. ArQule 501 was active as monotherapy and in combination with gemcitabine in PDAC. ArQule 501, in combination with gemcitabine, eradicated tumors in this model; at the end of 41 days there was no tumor tissue visible and no recurrences occurred over that period of time.

In September 2003, ArQule initiated the first human clinical trial with IV ARQ-501, an open label, single arm, dose-escalation, phase I clinical trial (protocol ID: ARQ 501-101), to evaluate the safety, and pharmacokinetic and pharmacodynamic properties of this drug in patients with refractory advanced solid tumors, and to determine MDT and DLT. The trial is being conducted at three Boston hospitals, Dana-Farber Cancer Institute, Massachusetts General Hospital and Beth Israel Deaconess Medical Center.

The trial is expected to enroll between 20 and 40 patients. The drug is administered IV, once a week, for one month. Then, patients are able to continue on therapy at the prescribing physicians discretion.

Interim results from this trial were presented at the 2004 EORTC-NCI-AACR meeting in Geneva in October 2004. The trial has explored 9 dose levels ranging from 10 mg/m^2 to 550 mg/m^2, in 18 patients with a wide range of solid tumors who had failed standard chemotherapy. MTD and DLT have yet to be determined. The clinical trial will continue enrolling patients until MTD. Because at the higher doses of 390 mg/m^2 and 550 mg/m^2 evidence of reversible hemolytic anemia was observed, it was proposed that dosing be changed from once weekly over 4 weeks to 2 out of 3 weeks, to accommodate higher doses. Thus far, it has been possible to continue escalating the dose without any side effects preventing escalation. Also, potential biomarkers have been identified in peripheral blood that may provide clues as to activity levels in these patients. Activity was observed in 14 evaluable patients treated with weekly drug infusions at levels up to 390 mg/m^2. Disease stabilization, ranging from 16 weeks to 51 weeks, was observed in 3 patients. A 20% shrinkage of a pulmonary lesion was observed in a patient with stable disease lasting 51 weeks. Reported adverse events, possibly associated with ARQ-501, include mild injection-site reactions, decreased appetite, fatigue, myalgia, chills and anemia. Reversible hemolytic anemia and hyperbilirubinemia occurred at doses between 390 mg/m^2 and 550 mg/m^2. No signs of myelosuppression were observed. This safety profile is consistent with the preclinical toxicology of ARQ 501.

As of November 2004, two open label, phase Ib clinical trials are being planned with ARQ 501, one in combination with docetaxel (Taxotere; sanofi-aventis) and the other with gemcitabine, for treatment of advanced solid tumors.

BMS-354825, under development by Bristol-Myers Squibb, is a small molecule dual function Src/Abl tyrosine kinase inhibitor designed to overcome mechanisms that give rise to resistance to treatment with imatinib mesylate (Gleevec; Novartis). BMS-354825 binds to the active form of Abl, which closely resembles the active configuration of Src, another well known cancer causing enzyme. In contrast, imatinib does not bind to the active form of the Bcr-Abl fusion, thereby allowing resistance to arise when mutations lock Abl in an active state (Shah NP, etal, Science, 16 July 2004;305 (5682);399-401).

BMS-354825 is considered the next generation of Gleevec. It has been refined and improved by structural biology so that the drug 'fits' its target. Also taken into account is how mutations may alter the shape of this target.

Investigators at M. D. Anderson Cancer Center have shown that BMS-354825 inhibits Src kinase in pancreatic cancer cells. Inhibition of Src kinase then blocks VEGF in these cells.

BMS-354825 is being currently evaluated in phase I clinical trials in chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST).

Bortezomib (Velcade), a boronic acid dipeptide derivative, is a selective and potent proteasome inhibitor commercialized by Millennium Pharmaceuticals (Cambridge, MA), in collaboration with Johnson & Johnson companies Ortho Biotech (Raritan, NJ) and Janssen-Cilag (Berchem, Belgium).

The 26S proteasome is a primary component of the cell's machinery, responsible for the degradation of most cellular proteins. Rapid elimination of targeted proteins is key to the activation or repression of many cellular processes, including cell-cycle progression and apoptosis. The importance of the proteasome in regulating cell growth, survival, and metastasis of cancer cells makes it an attractive therapeutic target.

Investigators at M. D. Anderson Cancer Center examined the antitumor activity of bortezomib in two pancreatic cancer cell lines, MiaPaCa-2 and L3.6pl. Bortezomib proved a potent inhibitor of cell-cycle progression by stabilizing p21 and p27 in both cell lines. Drug treatment also resulted in inhibition of the kinases, Cdk1 and Cdk2. Bortezomib effectively reduced tumor cell viability. Tumor weight and volume were reduced in orthotopic models of both pancreatic cancer cell lines. Tumor burden reduction was associated with decreased proliferating cell nuclear antigen (PCNA) expression. It appears that bortezomib has cytostatic and cytotoxic effects, both of which contribute to the drug's antineoplastic properties (Nawrocki ST and McConkey DJ, AACR04, Abs. 4005).

CC-401, under development by Celgene (Warren, NJ), is the lead of several oral small molecule inhibitors of c-Jun N-terminal kinase (JNK). In 1993, Dr. Michael Karin at the University of California San Diego, and Dr. Roger Davis of the University of Massachusetts (Amherst, MA), discovered two novel kinases JNK1 and JNK2 in the JNK pathway that are pivotal activators of c-Jun, a component of AP-1 and other transcription factors, and genes under the control of c-Jun. Celgene licensed patents from the University of California concerning the JNK signaling pathway.

There are three isoforms of JNK, JNK1, JNK2 and JNK3 that act as an integration point for multiple intracellular biochemical signals involved in a variety of cellular processes such as proliferation, differentiation, apoptosis, migration, transcriptional regulation, and development. JNK2 binds c-Jun approximately 25 times more efficiently than JNK1. Individual members of the JNK family may selectively target specific transcription factors *in vivo*.

JNK inhibitors tested by Celgene in *in vivo* models of various diseases, were well tolerated and demonstrated significant activity.

A phase I/II clinical trial with CC-401 in patients with advanced solid tumors is ongoing. A double blind, placebo-controlled, ascending, single IV dose, phase I clinical

with CC-401 in healthy human volunteers, that began in October 2002, was completed in May 2003.

GTI 2040, under development by Lorus Therapeutics (Toronto, Ontario, Canada), is a phosphorothioate 20-mer antisense oligonucleotide directed against the R2 subunit of ribonucleotide reductase (RNR).

In *in vivo* studies in nude and/or severe combined immunodeficient mice, GTI-2040 significantly inhibited growth of PDAC, among other solid tumors. No such antitumor effects were noted using an oligonucleotide containing four mismatches to the R2 sequence or with a scrambled sequence containing the same base content but not complementary to R2. This suggests that an antisense mechanism is responsible for the *in vivo* observations (Lee Y, etal, Cancer Res, 1 Jun 2003;63(11):2802-11).

An open label, dose-escalation, phase I clinical trial (protocol IDs: CTRC-IDD-0306; NCI-6090) of GTI-2040, in combination with gemcitabine, in patients with metastatic or inoperable solid tumors, was initiated in February 2004 at the Cancer Therapy and Research Center (CTRC; San Antonio, TX), under PI Chris Takimoto, MD, PhD. The trial's primary objective is to determine the toxicity profile and MDT of GTI-2040 in this setting. Secondary objectives are to determine the pharmacokinetics and pharmacodynamics of this regimen in these patients. According to the protocol, patients are treated with GTI-2040 by continuous IV (CIV) on days 2 to 16 of course 1, and on days 1 to 16 of all subsequent courses, and with IV gemcitabine over 30 minutes on days 1, 8, and 15 of course 1, and on days 2, 9, and 16 of all subsequent courses. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3 to 6 patients are treated with escalating doses of GTI-2040 and gemcitabine until MTD is determined. Once MTD is determined, 10 additional patients are treated at that dose. Approximately 18 to 40 patients will be accrued for this trial.

LY900003 (Affinitak), under development by Isis Pharmaceuticals (Carlsbad, CA) in collaboration with Eli Lilly, is a 20-mer phosphorothioate antisense oligonucleotide inhibitor of protein kinase C α (PKC-α) isoform gene expression via an RNase H-mediated mechanism.

Positive results were obtained in several human tumor xenografts treated with LY900003 including MIA PaCa-2 pancreatic carcinoma. Tumor growth was largely abolished or significantly delayed in mice treated with LY900003, but not with saline alone or control oligonucleotide (Yang XH, etal, ASC003, Abs. 861).

Affinitak has been evaluated in phase II and III clinical trials in a variety of solid tumors.

PD0325901, under development by Pfizer, was shown to be exquisitely specific for MAPK/ERK/kinase (MEK) and, particularly, MEK1. The highly specific nature of MEK inhibition in response to treatment with PD0325901, coupled with its improved pharmaceutical properties, may provide ultimate validity (or lack thereof) of the viability of MEK as an anticancer drug target (Sebolt-Leopold JS, etal, AACR-NCI-EORTC03, Plenary Session).

PD0325901 is a significantly more potent analog of CI-1040 with an improved pharmaceutical profile. Development of CI-1040 was discontinued in 2003. Although phase I data with CI-1040 demonstrated clinical activity as reflected by a 30% incidence of stable disease and a PR in a patient with pancreatic cancer (LoRusso PM, ASCO02, Abs. 321), results of phase II clinical trials with this agent were disappointing.

Perifosine, under development by Æterna Zentaris (Quebec City, Canada), is a novel orally available heterocyclic alkylphospholipid (alkylphosphocholine derivative) analog of hexadecylphosphocholine (miltefosine). Perifosine belongs to a new class of alkylphosphocholines that are lipid-related compounds that disrupt lipid-mediated signal transduction pathways necessary for tumor cell growth and survival.

In addition to its potential efficacy as a single agent, perifosine may provide synergistic effects when combined with established cancer treatments such as radiotherapy, chemotherapy, tyrosine kinase inhibition with commercially available EGFr inhibitors, and endocrine therapies.

In September 2002, Æterna Zentaris licensed to Access Oncology, now owned by Keryx Pharmaceuticals (New York, NY), exclusive rights to develop and market perifosine in the USA, Canada and Mexico. Under terms of the agreement Keryx made an upfront payment and will pay milestone payments in excess of $18 million, pay royalties on net sales of perifosine and fund all further development of perifosine in the territory, including the completion of phase II and phase III clinical trials.

A multicenter phase II clinical trial (protocol ID: E-E1202) to determine the activity of perifosine treatment in patients with locally advanced, inoperable, or metastatic (Stage II, III, IVa and IVb) PDAC, sponsored by Eastern Cooperative Oncology Group (ECOG), was initiated in July 2003. A total of 35-84 patients will be accrued for this trial within 21 months. Oral perifosine is administered every 6 hours for a total of 6 doses, and then once daily in the absence of disease progression or unacceptable toxicity. Patients are followed every 3 months for 3 years. Robert de W. Marsh, MD, of Shands Hospital and Clinics, University of Florida (Gainesville, FL) and Caio Max S. Rocha Lima, MD, at H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL), are Study Chairs.

A phase II clinical trial (protocol ID: PMH-PHL-015; NCI-5983) of perifosine as second line therapy in patients with advanced PDAC, was initiated in August 2003 at Princess Margaret Hospital (Toronto, Canada). In this trial the drug is administered daily for 3 weeks. Courses are repeated every 4 weeks in the absence of disease progression or unacceptable toxicity. Patients are followed every

3 months. Malcolm J. Moore is Study Chair. This trial was temporarily closed in April 2004.

RAV12, under development by Raven Biotechnologies (South San Francisco, CA), is a chimeric form of glycotope-specific IgG1 MAb KID3 that exhibits potent antiproliferative activity against colon and gastric tumor cell lines *in vitro*. RAV12 was created using gene-splicing techniques to replace more than 80% of the mouse amino acids in KID3 with human gene sequences.

RAV12 recognizes the KID3 cell-surface antigen, which is highly expressed in more than half of cases of gastric and colon cancer and PDAC, and in smaller proportions in other solid tumors. Normal human tissue expression of KID3 antigen was limited to some types of ductal epithelium (sweat gland, bile, pancreatic), and gastrointestinal epithelium. KID3 exhibits potent growth inhibitory activity against multiple gut epithelium-derived tumor cell lines. Furthermore, *in vivo* analysis confirmed that this activity correlates with antitumor activity in rodent subcapsular and subcutaneous models (Loo D, AACR04, Abs. 5349 and AACR04, Abs. 712).

SDX-102, under development by Salmedix (San Diego, CA), an amino acid analog (L-alanosine), is a small molecule that inhibits a key enzyme in the *de novo* synthesis pathway for adenosine monophosphate (AMP), the precursor for ATP. 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 malignancies. 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. L-alanosine induced apoptosis in two MTAP-deleted pancreatic cancer cell lines, PANC-1 and AsPC-1.

Scientists at the University of California San Diego developed a novel, patented molecular diagnostic test, which Salmedix believes will permit selecting patients in whom SDX-102 will have optimal efficacy. Salmedix has licensed patents covering the tumor-genotyping test, the use of SDX-102 in the genetically-selected patient group, and the use of drugs, which act by a common mechanism to SDX-102.

In April 2003, a multicenter (n=23), open label, phase II clinical trial (protocol ID: MSKCC-03029, SALMEDIX-SDX-102-01) was initiated with SDX-102, in patients with MTAP deficient cancer, including PDAC. Trial endpoints are to determine response rates, time-to-response, duration of response, PFS, safety and tolerability, and pharmacokinetics. The trial is to enroll between 50-145 patients to be treated with SDX-102 as a continuous infusion on days 1-5. Treatment repeats every 21 days for up to 9 courses in the absence of disease progression or unacceptable toxicity and patients are followed at 28 days. Lee Krug, MD, of the Memorial Sloan-Kettering Cancer Center, is Protocol Chair.

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