VOLUME 27 · NUMBER 33 · NOVEMBER 20 2009

JOURNAL OF CLINICAL ONCOLOGY

SPECIAL ARTICLE

Consensus Report of the National Cancer Institute Clinical Trials Planning Meeting on Pancreas Cancer Treatment

Philip A. Philip, Margaret Mooney, Deborah Jaffe, Gail Eckhardt, Malcolm Moore, Neal Meropol, Leisha Emens, Eileen O'Reilly, Murray Korc, Lee Ellis, Jacqueline Benedetti, Mace Rothenberg, Christopher Willett, Margaret Tempero, Andrew Lowy, James Abbruzzese, Diane Simeone, Sunil Hingorani, Jordan Berlin, and Joel Tepper

See accompanying editorial on page 5487 and articles on pages 5499, 5506, and 5513

From the Karmanos Cancer Institute, Wayne State University, Detroit, MI; National Cancer Institute, National Institutes of Health, Bethesda, MD; University of Colorado, Denver, CO; Princess Margaret Hospital, Toronto, Canada; Fox Chase Cancer Center, Philadelphia, PA; Johns Hopkins Hospital, Baltimore, MD; Memorial Sloan-Kettering Cancer Center, New York, NY; Norris Cotton Cancer Center, Lebanon, NH; M. D. Anderson Cancer Center, Houston, TX; Statistical Center, Southwest Oncology Group, Seattle; Fred Hutchinson Cancer Research Center, Seattle, WA; Pfizer Oncology, New York, NY; University of California San Diego, San Diego; University of California San Francisco Medical Center, San Francisco, CA; Duke University Medical Center, Durham; Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; and University of Michigan, Ann Arbor, MI.

Submitted January 21, 2009; accepted April 15, 2009; published online ahead of print at www.jco.org on October 26, 2009.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Philip A. Philip, MD, PhD, FRCP, Karmanos Cancer Institute, 4 Hudson-Weber Cancer Research Center, 4100 John R St, Detroit, MI 48201; e-mail: philipp@karmanos.org.

The Acknowledgment is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2733-5660/$20.00

DOI: 10.1200/JCO.2009.21.9022

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer mortality, despite significant improvements in diagnostic imaging and operative mortality rates. The 5-year survival rate remains less than 5% because of microscopic or gross metastatic disease at time of diagnosis. The Clinical Trials Planning Meeting in pancreatic cancer was convened by the National Cancer Institute's Gastrointestinal Cancer Steering Committee to discuss the integration of basic and clinical knowledge in the design of clinical trials in PDAC. Major emphasis was placed on the enhancement of research to identify and validate the relevant targets and molecular pathways in PDAC, cancer stem cells, and the microenvironment. Emphasis was also placed on developing rational combinations of targeted agents and the development of predictive biomarkers to assist selection of patient subsets. The development of preclinical tumor models that are better predictive of human PDAC must be supported with wider availability to the research community. Phase III clinical trials should be implemented only if there is a meaningful clinical signal of efficacy and safety in the phase II setting. The emphasis must therefore be on performing well-designed phase II studies with uniform sets of basic entry and evaluation criteria with survival as a primary endpoint. Patients with either metastatic or locally advanced PDAC must be studied separately.

J Clin Oncol 27:5660-5669. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death among both men and women in the United States.[1] Despite significant improvements in diagnostic imaging and operative mortality rates during the past two decades, the 5-year survival rate remains lower than 5%. Most patients present with either locally advanced or clinically evident metastatic disease due in part to a lack of screening tests to detect early stage PDAC. The median survival for optimally staged patients who undergo a pathologically margin negative (R0) resection is approximately 2 years with a 5-year survival of approximately 15% to 20%, and for those with metastatic disease is shorter than 6 months. The median survival of patients with localized but unresectable disease is 8 to 10 months.

The poor survival of patients with resected PDAC is due to the fact that nearly all patients have metastatic disease at the time of initial diagnosis. Gemcitabine has been the most commonly used drug therapy over the past decade. Its very modest benefit over fluorouracil (FU) was first demonstrated in advanced disease.[2,3] Unfortunately, numerous phase III trials testing gemcitabine combined with other cytotoxic drugs have failed to reveal any additional benefit compared with gemcitabine alone.[4] Several targeted agents have been tested in combination with gemcitabine and have similarly failed to confer any added benefit, with the notable exception of erlotinib, a small molecule inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, which conferred a very modest improvement in survival over gemcitabine alone.[5]

While our knowledge of the genetic events that underpin multistep carcinogenesis in PDAC has increased dramatically, and despite a steady identification of new targets and new drugs for clinical testing, researchers still continue to work with an incomplete understanding of how the complex molecular biology contributes to the aggressive behavior of this disease. For example, our understanding of how key signaling pathways interact and the role of the microenvironment (stroma) in initiating and maintaining PDAC remains severely limited. The

complex molecular biology of pancreatic cancer makes it unlikely that we will define new drugs with substantial single-agent activity. However, research aimed at developing therapies that benefit subsets of patients and use of multitargeted approaches should be encouraged. Selected targeted agents now available provide an opportunity to test new strategies that could ultimately improve the treatment of this disease.

The Clinical Trials Planning Meeting in pancreatic cancer was convened by the National Cancer Institute (NCI) Gastrointestinal Cancer Steering Committee (GISC) to discuss the integration of basic and clinical knowledge in the design of clinical trials in PDAC. Participants of this 2-day meeting included clinical, translational, and basic science investigators in pancreas cancer, and representatives from the patient advocacy community, pharmaceutical industry, and government agencies. This meeting was the first substantial follow-up gathering of major stakeholders in pancreas cancer treatment since the Pancreatic Cancer Progress Review Group was held in 2000. The following report summarizes major topics discussed and key recommendations for future research in PDAC.

OBJECTIVES

Major objectives of the meeting were to address critical questions and unmet needs in treatment and translational research in PDAC; to facilitate innovation and collaboration among clinical and basic investigators; to develop key strategic priorities for future clinical trials; and to address how to disseminate these priorities to the relevant oncology communities. The major focus of the meeting was to define the direction for clinical trial investigation for treatment of this disease over the next 3 to 5 years.

PRECLINICAL TARGET IDENTIFICATION AND VALIDATION

Potential Targets

A major challenge to the development of targeted therapies is the molecular heterogeneity of pancreatic cancer, both genetic alterations and epigenetic changes. Frequently occurring mutations, such as those of the *K-ras* oncogene are of high interest. Additional targets of interest include EGFR, PI3 kinase, insulin-like growth factor-1 receptor, vascular endothelial growth factor (VEGF)/ VEGF receptor (VEGFR), c-Met, and the Hedgehog pathways (Table 1). Special emphasis was placed on studying the following targets.

K-ras. Activating K-ras mutations occur in more than 90% of PDAC.[6,7] These mutations are among the earliest detectable genetic changes in PDAC.[8,9] Chemically induced[10] and genetically engineered[11,12] animal models suggest that oncogenic K-ras mutations initiate preinvasive disease. Although preclinical work supports K-ras as a valid target for drug development, its relevance as a therapeutic strategy is not fully established. Specifically, whether pancreas cancers continue to depend on mutant ras activity to maintain their malignant phenotype remains to be determined.

No direct ras inhibitors currently exist. Early anti-ras strategies that were focused on a post-translational modification (farnesylation) necessary for localization of the ras protein to the cell membrane were unsuccessful.[13-16] However, this was likely because these agents did not inhibit alternate prenylation pathways (geranylgeranylation) that preserved ras-mediated signal transduction. A major challenge to targeting ras itself is the intracellular location of the GTPase, which poses difficulties for the development of new drugs against this target.

Table 1. Molecular Targets of Interest in Preclinical and Clinical Target Validation in Pancreas Cancer

Parameter	Target
Tumor cell signaling	K-Ras
	Raf
	MEK
	PI3-K
	EGFR
	IGF-1R
	VEGF/VEFGR
	HIF-1alpha
	TGF-beta
	c-Met
Stem cell signaling	Hedgehog
	CXCR4
	Bmi-1
	Notch
Microenvironment	Stellate cells signaling pathways
	VEGF/VEGFR and other vascular targets
	CTLA-4
	OX-40
	PD-1 and VEGF
	B7-H1/B7-H4
	Tregs
	MDSC
	COX-2
	STATs

Abbreviations: Raf, root abundant factor; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; PI3-K, phosphoinositide 3-kinase; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; HIF-1 alpha, hypoxia-inducible factor-1 alpha; TGF-beta, transforming growth factor-beta; c-MET; mesenchymal-epithelial transition factor; CXCR4, C-X-C chemokine receptor type 4; Bmi-1, BMI1 polycomb ring finger oncogene; CTLA-4, cytotoxic T lymphocyte–associated protein 4; OX-40, a tumor necrosis factor receptor superfamily member; PD-1, prephenate dehydratase; B7-H1, Cd274; B7-H4, V-set domain containing T cell activation inhibitor 1; Tregs, regulatory T cells; MDSC, myeloid-derived suppressor cell; COX-2, cyclooxygenase-2; STATs, signal transducers and activators of transcription.

Another approach to target K-ras–mediated signaling is to inhibit downstream effector molecule(s). These may include Raf and MEK, for which drugs are available (eg, sorafenib and PD0325901, respectively). Other molecules that may be targeted in relation to the K-ras pathway include proteins such as Rac,[17] Aurora,[18] and GGTase I.[19,20] However, the complexity of signaling pathways mediated by K-ras present substantial challenges to effective blockade of ras signaling and therefore the therapeutic index of anti-ras agents as single-agent interventions remains uncertain. Despite these challenges, there was general agreement that developing methods to target K-ras was a high priority that should be pursued at both preclinical and clinical levels. Targeting ras as part of an approach to inhibit multiple signaling pathways to assess whether this improves the efficacy of other signaling inhibitors is also an important strategy to be tested. More preclinical work is also needed to demonstrate reversal of the malignant phenotype by switching off ras-signaling in preclinical models.

Cancer stem cell signaling. Emerging data suggest that malignant tumors are heterogeneous, and that tumors are composed of a small set of cells, termed cancer stem cells (CSCs) that are responsible for tumor initiation and propagation, and a much larger set of more differentiated cells that have limited proliferative potential.[21] These

CSCs are like their normal stem cell counterparts because they possess the ability to self-renew and produce differentiated progeny. Identification of human PDAC stem cells was recently reported[22] and was defined by expression of the cell surface markers CD44+CD24+ESA+ (0.2% to 0.8% of all pancreatic cancer cells). These cells were highly tumorigenic and possessed the ability to both self-renew and produce differentiated progeny, which reflected the heterogeneity of the patient's primary tumor. Upregulation of developmental signaling pathways, including Hedgehog and Bmi-1 signaling was observed in the CSC population.

From a clinical standpoint, the identification of CSCs within human PDAC may have important implications for treatment. In several types of cancer, CSCs have been shown to be resistant to conventional chemotherapy and radiation therapy and these cells are thought to produce metastases and recurrence after clinical remission. Recently published data suggests that pancreatic CSC may also be resistant to chemotherapy and radiation.[23] Hermann and colleagues[24] found that CD133+ populations in pancreatic cancer cells were enriched after exposure to gemcitabine. More detailed studies are needed to understand the biologic properties of CSCs in PDAC. Targeting aberrant signaling pathways regulating self-renewal in pancreatic CSCs may offer improved novel therapies for this disease.

Stromal signaling and angiogenesis. Despite the long-standing recognition of a characteristic fibroinflammatory reaction in primary PDAC,[25-28] the pathophysiologic mechanisms of tumor-stromal signaling and contribution to disease pathogenesis and disease progression are not well characterized. Moreover, the contribution of each component of the host microenvironment (stromal cells, vasculature, and immune cells) to the biology of PDAC is not well understood.[29] It has been suggested that stromal cells, including pancreatic stellate cells might be critical in the activation of pancreas CSCs.[30] There is evidence for a role for hepatocyte growth factor secreted by stromal cells in the activation of CSCs through its receptor C-Met.[31,32] However, the details of the interactions between pancreatic cancer cells and pancreatic stellate cells are just beginning to be investigated.

Even the role of vascular endothelial cells in pancreatic cancer primary tumors and metastases remains uncertain. In light of limited understanding of tumor-endothelial interactions in human pancreatic cancer, the potential benefit of blocking the VEGF-VEGFR pathway was debated with mixed opinion on its importance as a therapeutic target especially with the failure of a recent phase III trial using bevacizumab to demonstrate a prolongation of survival in patients with advanced PDAC.[33] Preclinical studies and ongoing clinical trials of VEGF and VEGFR inhibitors should clarify this issue in the near future.

Also discussed was the probability that the tumor-stromal interactions in metastatic deposits are different from those in the primary tumor.[34] This highlights the ability of metastatic tumor cells to grow independently of the original pancreatic stroma. Understanding these interactions may identify new targets for antimetastatic therapies. The consensus was that tumor-stromal interactions have been underappreciated in pancreatic cancer and greater emphasis needs to be placed on this area of research. New transgenic models of pancreatic cancer appear to reproduce the desmoplastic reaction of human pancreatic cancer with greater fidelity than orthotopic models and may allow for a better understanding of the biology of PDAC.[34,35]

Preclinical Models and Validation Systems

The transition to clinical testing may require multiple models to improve the predictive value of preclinical target identification. Models should be used both for the validation of sensitivity to targeted agents and to identify drug-resistance markers. Use of appropriate preclinical models may shorten the pathway to clinical trials and effective therapies by avoiding testing of ineffective strategies in patients. However, there has been no consistency in the use of preclinical models for drug development in PDAC. Although standardization would benefit the collective effort, no clearly superior model system to predict clinical behavior of human PDAC has yet emerged.

In vitro cell-based assays. Preclinical testing in cell systems, though helpful in determining the mechanism of action of drugs, ignores or minimizes the stromal and CSC contributions to tumor biology and drug response. However, response profiles in molecularly characterized cells from different cell lines may help to identify beneficial new therapies based on molecular markers. Three-dimensional in vitro models may further improve the predictive role of these systems.[36] Another strategy is to more fully characterize available cell lines and to compare these to primary tumors and xenografts using gene expression, comparative genomic hybridization, and methylation arrays. Major challenges include that no single cell line can mimic the heterogeneity of PDAC and it is not clear whether cell lines can mimic differences between primary and metastatic tumors. Taking data directly from in vitro cell lines to clinical testing without additional preclinical validation in vivo is considered inappropriate.

Tumor models. PDAC tumor models are valuable to screen for new drugs and drug combinations, characterize mechanism(s) of action of drugs, and validate biomarkers.[37] Models of preinvasive disease enable exploration of risk factors for disease progression, markers for early detection and strategies for chemoprevention. The major challenge heretofore with many preclinical models has been that they do not faithfully recapitulate human PDAC. Subcutaneous xenograft models using cloned cell lines are not sufficient to study the complex biology of pancreas cancers and metastases. Local factors within the pancreas (eg, very high levels of insulin, interactions with other cellular compartments and elements, including adaptive immunity, neovasculature) may not be replicated in subcutaneous tumor xenograft systems. Although orthotopic xenograft tumor models are considered more useful than subcutaneous xenograft models, both are limited by the lack of heterogeneity in cloned cell lines and the artificial microenvironment in an immune incompetent cohost.

Genetically engineered mouse models. One of the most significant advances in PDAC research has been the development of genetically engineered mouse models.[38] The presence of genetic heterogeneity in human cancers can be simulated by the use of multiple genetically engineered tumor models for drug testing. The defined genetic alterations in these animals allow investigators to study the biology of selected pathways of therapeutic interest and to search for biomarkers of clinical value. Many of these models appear to recapitulate the clinical, histopathologic, and molecular features of the human disease, and have the advantage of generating the disease in the native organ and in the setting of an intact immune system. The role of the host microenvironment in the biology of pancreas cancer can be explored in a number of genetically engineered models of human pancreas cancer.[38]

Primary tumor xenografts. Emerging data supports the potential of primary patient-derived tumor xenografts as a platform for drug screening, biomarker development, and to expand knowledge of the

biology of pancreas cancer.[39,40] Such a system offers advantages over xenografts from cell lines and may recapitulate the heterogeneity seen in patients. The use of pancreas cancer explants may offer a better chance to predict the clinical activity of new drugs, particularly if the mechanism of drug action is confined to the tumor cell and does not involve the stroma. However, the development of primary human tumors for preclinical modeling is limited by the poor availability of fresh tumor tissues. Early evidence suggests that gene expression profiles and drug sensitivity patterns are retained through several generations of primary tumor xenografts.

Available data on primary tumor xenografts, though encouraging, needs to be validated with respect to long-term stability and the accuracy of assessment of antitumor effect. At this time, there is no knowledge of the biologic differences that might exist between xenografts obtained from primary and metastatic tumor sites. The failure of some tumors to engraft may introduce a selection bias in that the tumors that engraft may not identify agents that are active in those patients. Efforts are underway to improve the engraftment rates from the current rate of 50%. An increased effort to develop primary pancreatic cancer xenografts programs would increase access to this preclinical model system to test promising therapeutic agents.

TRANSITION FROM PRECLINICAL TO CLINICAL TESTING

Rational Selection of Target(s)

Empiricism has dominated the identification of new agents for clinical trials. The customary approach to test novel agents in PDAC has been to combine them with gemcitabine. However, these combinations have rarely added benefit over gemcitabine alone and the recent failure of major phase III trials in advanced disease[33,41] challenges the wisdom of this approach. There was strong consensus for the need to develop new strategies based on a rational choice of agents based on better understanding of signaling pathways in PDAC and using agents or combinations that have been previously tested in preclinical models as described above. Other cytotoxic drugs should continue to be evaluated preclinically and clinically especially those that may offer a better platform to combine with targeted agents. Ixabepilone, oxaliplatin/FU, nab-paclitaxel, and S-1 are examples of cytotoxic drugs currently being tested in patients with advanced PDAC. There was no consensus on the importance of assessing the activity of a single agent before inclusion in a combination strategy, but novel approaches to avoid single-agent testing should receive high priority. Clinical testing of combination of agents also presents significant logistic challenges. In particular, cooperation among sponsors in a single study is a major issue that is particularly problematic when testing non-US Food and Drug Administration–approved drugs that are in early development.

Target Validation and Monitoring in Patients

There was a strong consensus that early clinical trials of targeted drugs can be significantly more informative if molecular correlates using validated assays are included. Unless the drug has a major clinical anticancer effect, lack of an assay for assessing drug-target interaction in vivo can make it difficult to devise an efficient strategy for drug development. Phase I trials rarely establish an optimal biologic dose of a drug. Therefore, biospecimens (serum, blood, or tumor tissue) should be obtained from patients treated in phase II trials and tested for pharmacodynamic effects.

Measuring target modulation in patients can be facilitated by an understanding of biomarker(s) that may be affected by intended target modulation. Where the target, the relationship between target and biomarker, and the relationship between biomarker and anticancer effects are known, if a surrogate biomarker cannot be inhibited then the tumor would be unlikely to be affected by that agent. There was a consensus that researchers need to achieve a more comprehensive understanding of the biologic basis why certain drugs do not work in PDAC. With well-validated assays in hand, serial biopsies to evaluate the modulation of specific targets by novel agents could be very informative. Biopsy of liver metastases may be preferred over biopsy of the primary site because of demonstrated safety and acquisition of sufficient cancer cells for histologic examination and molecular studies. However, practical and ethical considerations limit multiple biopsies in a clinical trial. In addition, we need to develop reproducible assays and improve tissue sampling technologies to improve the yield of useful tissue. Surrogates for tumor tissue (normal tissue, blood, and serum) should be explored but have their own limitations. Since there is no universal consensus on what constitutes an effective in vivo inhibition of a target, each target will require separate validation.

Target identification and validation in patients is only one consideration in successful drug development. Effective inhibition of the target by a drug may not be clinically effective because of poor drug delivery to tumor cells (eg, dense stroma in PDAC) or reduced active metabolite concentration. This could also explain differential drug effects between primary and secondary deposits whose stromal content varies.

Role of Functional Imaging

For PDAC, functional imaging will require novel approaches and extensive validation. Current techniques, such as imaging with either positron emission tomography or dynamic contrast-enhanced magnetic resonance imaging, cannot be recommended as surrogates for treatment benefit, but deserve continued investigation. As imaging develops to demonstrate target modulation or predict response to therapy, it will be a critical part of evaluating novel rationally designed therapies.

DESIGN OF PILOT TRIALS

Developing phase II trials that have a high chance of success in subsequent phase III testing is a major priority. If suspected predictive biomarkers are available to select patients, clinical trial designs employing enrichment must be considered whenever possible. Given gemcitabine's limited ability to impact the natural history of PDAC, patient advocates and many investigators indicated the feasibility of performing studies that do not include gemcitabine as initial therapy. Some of the successes of therapy for pediatric cancer were based on the introduction of new therapies before exposure to agents with known clinical activity. Some agents, such as vaccines, may show differential efficacy based on the stage of disease and tumor burden. Therefore clinical trials of these agents should be focused on patients with minimal tumor burden. Assessing the clinical and biologic effects of novel interventions in the preoperative setting (neoadjuvant treatment) carries the advantage of allowing direct examination of the changes in the tumor compartment and microenvironment.

Selection of Patients and Clinicopathologic Parameters

Heterogeneity between studies with respect to patient populations makes it difficult to translate results from one study to another. Reducing this variability will improve the ability to interpret results of clinical trials in PDAC. Patients with unfavorable Eastern Cooperative Oncology Group performance status (≥ 2) should be studied in separate clinical trials from patients with good performance status (zero or one). More emphasis should be placed on describing patient characteristics using additional parameters such as extent of weight loss and nutritional status. In addition early withdrawals from study (eg, within 4 weeks) because of worsening disease and/or overall health and without receiving meaningful treatment should be considered in the analysis of study outcome. Patients with locally advanced and metastatic disease should similarly be separately studied in clinical trials. Eligibility criteria should be uniform across phase II clinical trials and should be similar between phase II and phase III trials of the same agent(s).

The ability to select patients based on predictive biomarkers may reduce the number of patients required in subsequent phase III trials.

Study End Points

Because of the relatively short survival time for patients with pancreatic cancer, overall survival must remain the primary end point for phase II and phase III clinical trials. In addition, tumor shrinkage by either Response Evaluation Criteria in Solid Tumors (RECIST) or WHO criteria is a poor surrogate for survival in this disease and its use as a primary end point is discouraged. Progression-free survival suffers from similar limitations as objective response and can introduce additional biases in the phase III setting, where timing of disease assessment may vary by type of treatment. At this time, changes in serum CA 19-9 levels on therapy have not been established as surrogate for survival.

Statistical Designs

The failure of recent phase III trials to demonstrate clinically meaningful treatment benefit for patients with advanced pancreatic cancer despite the suggestion of benefit in single-arm phase II studies (eg, cetuximab, bevacizumab) has led to much discussion of the reasons for the failure. Possible reasons include phase II population selection bias, inadequate interpretation of historical data, compromises in error rates, overly optimistic interpretation of phase II results inadequate phase II designs, wrong end points, lack of understanding of the target, inadequate agents, and bad luck.

There has been much recent discussion over the relative merits of single arm phase II trials versus randomized phase II trials. In a disease such as pancreatic cancer, where there has been a large body of historical data, it has traditionally been felt that a single-arm trial can be conducted with little concern that the comparison with historic information is biased.[42] Where this is the case, single-arm trials require a smaller sample size, can achieve better error rates, and are easier to conduct, whereas a randomized phase II trial typically requires three to four times as many patients in order to achieve the same error rates (due to the need to account for increased statistical variability in the comparisons between the two treatment groups). However, where there are no reliable historical data, or the study population differs dramatically from patients used in previous studies, randomized trials typically offer the better option.[43]

Current work is ongoing to establish a database of prior phase II and phase III trial results in PDAC that will be analyzed to provide a prognostic model based on baseline covariates. This work is analogous to that performed by Korn et al[44] for melanoma, and is expected to provide a means to assess treatment effect in a single-arm trial, by adjusting patient outcome by what would have historically been expected in patients with a similar baseline profile. Other phase II designs may be appropriate in selected situations and should be explored for feasibility (eg, single-arm estimation with larger sample sizes, selection designs, targeted subgroup trials, and Bayesian designs).

SPECIFIC CONSIDERATIONS FOR TRIALS IN LOCALIZED PANCREAS CANCER

Resectable Pancreas Cancer

The interpretation of results of adjuvant trials in resectable PDAC would be improved by a greater attention to quality control and generally agreed on standards for reporting. Historically, there has been inconsistent reporting of surgical margins that can unintentionally bias treatment arms. Patients with either R0 or R1 resections should be included in clinical trials of adjuvant therapies; however, those with gross residual disease (R2 resections) must be excluded. The use of laparoscopic staging was considered unnecessary for the selection of patients for clinical trials. However, postoperative staging with CT scanning and measurement of CA 19-9 should be done before study entry to exclude patients with known residual or metastatic disease. The primary end point of adjuvant trials should be overall survival.

There was consensus that improvement of overall cure rates and long-term survival after surgical resection are most likely to be achieved by the introduction of novel systemic agents. The testing of new agents should be moved to the resectable population in an expedited fashion after demonstration of activity in advanced disease although certain therapeutics may be selectively cytotoxic to micrometastatic disease (eg, vaccines). Well-conceived correlative studies must be included in adjuvant trials, including obtaining samples from patients at the time of recurrence when possible.

The study of neoadjuvant therapies in pancreas cancer should be encouraged. The safety and feasibility of this approach has been demonstrated in several tertiary referral centers.[45] Borderline resectable patients[46] should ideally be studied in separate clinical trials rather than mixed with the resectable (or the locally advanced) population. The role of radiation in the adjuvant setting remains controversial and warrants further evaluation.

Clinical Trials in Localized Unresectable Disease

Localized unresectable PDAC must be studied in trials that do not include patients with metastatic disease because of the differences in natural history and the potential impact of radiation therapy on survival in patients with localized disease. The treatment schema could consider initial systemic therapy of 3 to 4 months followed by chemoradiation in nonprogressing patients with survival as the primary end point.

VACCINES AND IMMUNE THERAPIES

Molecular Targets

Relevant immune responses can be generated even in the context of advanced PDAC, which has traditionally been considered poorly immunogenic.[47] However, the challenges remain very high for a vaccination strategy to be successful in PDAC. Strategies are needed to manipulate immune check points at both the systemic and tumor microenvironment levels. At the systemic level, regulatory T cells are thought to represent the primary barrier to effective antitumor immunity, and distinct strategies for abrogating their negative influence are under active clinical investigation. Additional targets for immune checkpoint modulation under investigation include CTLA-4,[48] OX-40,[49] and PD-1.[50] Importantly, more focus on regulation within the local tumor microenvironment is needed because multiple immunosuppressive networks map to the local tumor environment. These include intratumoral regulatory T cells and myeloid derived suppressor cells, immunosuppression due to local production of VEGF, and negative signaling through B7-H1/B7-H4 pathways. Tumor biology itself, including signaling through the epidermal growth factor receptor, HER2, cyclooxygenase 2, transforming growth factor β, and STAT pathways, can also antagonize effective antitumor immunity. Advances in molecular and cellular immunology support the use of multitargeted vaccination strategies in the treatment of pancreas cancer.[47]

Clinical Vaccine Trial Designs

Vaccine therapies as a sole intervention are unlikely to have a significant impact on this disease and will need to be integrated with standard therapies. All standard therapies have potential to be combined with vaccines. Emerging data supports the inherent immunogenicity of some chemotherapeutic and targeted agents, suggesting that integrating tumor vaccines with standard cancer therapeutics may be possible. There is also a concern that cytotoxic drugs may depress immune function, thus having a negative impact on vaccine therapy.[51] The infrastructure for vaccine trials should be established to include a select group of specialized centers. Early immunotherapy trials should focus on the optimum biologic dose. Although there are preliminary data in this regard, it is unclear what measures of the immune response would be most likely to correlate with clinical activity. A consensus on appropriate measurements to determine the most effective dose and schedule of vaccines is needed using standardized methods and recording of immune responses.

The tumor cell itself is probably the best source of antigen. This approach has been most fully developed in the context of the GVAX allogeneic whole cell vaccine that has completed phase II assessments and is poised for further development.[52,53] While most of the current work focuses on advanced disease, earlier stage disease or tumors in the locally advanced stage III setting after initial cytoreduction may provide the best opportunity for meaningful intervention with immune-based therapy. Some published data suggest that the extent of tumor burden relative to the level of vaccine-induced T cells may be predictive for the success of cancer vaccines.[54]

BIOSPECIMEN REPOSITORIES

Sample Sets

One of the biggest barriers to conducting translational research in PDAC is the lack of appropriately collected, clinically and molecularly annotated, and properly stored biologic material. Tumor biopsies are the most common source of specimens, but biologic material is limited and often difficult to reserve for research purposes because of diagnostic needs. Unfortunately, a proportion of tissue samples obtained for diagnostic purposes in patients with advanced PDAC are unsuitable for research assays because of poor quality or small sample size or samples that are composed predominantly of stroma. Many available samples are biased toward earlier stage disease.

Ideal sample sets should include tumor tissue plus normal tissue, blood, serum, and serial samples when possible. Surgical resections provide a useful source of biospecimen material, but logistics are crucial. Good quality depends on speed, and immediate processing requires a responsive infrastructure. Autopsy material could be a source of abundant material, but preservation of high-quality tissue is difficult, given the rapid degradation of pancreatic tissue. Rapid autopsy protocols (eg, University of Nebraska, Johns Hopkins) are useful, but expensive. Exfoliated secreted biospecimens have the advantage of easy access, but this material is extremely limited, and distinguishing tumor cells in this type of specimen is sometimes difficult. Circulating tumor cells can be captured with some tumor types, such as breast and colorectal cancer, but so far only in advanced and metastatic pancreas cancer, and even then only in a small percentage of patients.[55] Initiatives to address standards in biospecimen quality should involve exploring surrogates for frozen tissue, such as formalin-fixed, paraffin-embedded tissue, and circulating DNA.

Committed Infrastructure

A committed infrastructure and leadership is critical to establish and maintain a system of specimen collection with the tasks of managing the consent process and following standard operating procedures to acquire and preserve high quality tissue. Experienced pathologists are especially important to the analysis of pancreatic tissue, as it may be difficult to distinguish cancer from pancreatitis, subtleties that are only exaggerated in the context of frozen sections. Complexities also extend to discerning islet cell neoplasms and other nonpancreas cancers that have metastasized to the pancreas.

Strategies to Improve Biorepositories

The adherence to good standards of practice (eg, NCI Harmonization Guidelines and Best Practices) must be in place at all institutions with biorepositories. Storage and tracking mechanisms are key elements of biorepositories and clinical annotation is essential in developing a reference set of biospecimens that will improve and expand the utilization of specimens for research purposes. Work should continue on developing common interinstitution consent forms and intellectual property agreements that facilitate sharing of biospecimens, and move toward the goal of an ideal pancreatic cancer reference set that contains high-quality tissue which is clinically and molecularly annotated and paired with genotype data and serum. The ideal number of high-quality and diverse samples that would be needed for a biorepository could be as high as 300, but 100 may be adequate for

most research purposes. A biorepository could potentially be developed using specimens from patients participating in phase III adjuvant trials. Cooperative groups may play an important role in establishing biorepositories because specimens are best collected under the aegis of a focused scientific question and where clinical annotation is carefully performed.

DEVELOPMENT OF BIOMARKERS

Discovery and Validation of Biomarkers

Biomarkers are powerful tools that can improve PDAC diagnosis and its accuracy, improve clinical trial design, and aid in the identification of patient subsets for clinical management. There is, at this time, no robust surrogate biomarker for an antitumor effect in pancreas cancer. Accordingly, biomarker discovery in PDAC must be accelerated. Molecular profiles that predict response or resistance to therapy with targeted agents may be derived from preclinical models and applied prospectively in clinical trials for the selection of subjects. Appropriate methodology in clinical trial design must be implemented in biomarker validation to distinguish a predictive from a prognostic marker. Uniformity of clinical trial design and adherence to standard operating procedures (eg, standardized acquisition and storage procedures) are essential in studies including biomarkers. It is hoped that ongoing work using proteomics and new platforms such as antibody arrays will provide novel biomarkers that can be validated in therapeutic clinical trials. With incorporation of appropriate biomarker studies larger phase II trials may identify subsets of patients more likely to respond to a targeted agent or regimen and may provide a better estimate of clinical activity. However, the statistical power to identify such a subset of patients is extremely low. Therefore, any observed associations can only be considered as hypothesis generating, requiring validation in a phase III trial. Investing in studies designed to identify specific biomarkers associated with favorable outcomes provides justification to further study a given pathway(s) and to consider subsequent studies in enriched populations. Challenges to such an approach include the requirement for a large number of patients and possibly longer time to develop an agent.

Rather than imposing a strict hierarchy of processes for preclinical biomarker validation, the series of opportunities/methodologies that can be used preclinically (eg, xenograft, genetic models) should be employed in a flexible manner using various models as appropriate for the agent under study. There is no consensus on the value of high throughput systems in the development of biomarkers. One approach suggested for the development of biomarkers and their validation is based on the preclinical platform. The first step would involve a search in human pancreas cancer cell lines (cell-based targets) using expression profiling. Response in these cells to various drugs would be determined and correlated with gene expression profiles. Positive findings would then be validated in a primary cohort maintained as xenografts or genetically engineered mouse models. This approach is based on extensive experience in breast cancer (eg, in the model that developed lapatinib). Another approach is to determine the molecular profiles of patients at the extreme ends of the selected reported outcome to discern differences in selected biomarkers.

Two potential markers were discussed in some detail; serum CA19-9 and tissue markers that characterize the cellular phenomenon of epithelial mesenchymal transition (EMT). There is a suggestion that baseline level of CA 19-9 is a predictor for survival in patients with resected pancreas cancer.[56] Preliminary cell line-based studies are underway to determine whether EMT markers such as vimentin, E-cadherin, nuclear β-catenin, and upregulation of specific nuclear transcription factors such as Zeb-1[57] and Twist[58] may identify cells that are likely to be resistant to certain drugs (eg, anti-EGFR agents).[59-61]

Communication Within the Pancreas Cancer Research Community to Enhance Biomarker Development

Cell lines, pancreatic tissue, and other biospecimens that are important resources for research in pancreatic cancer exist in many locations but are often restricted by cumbersome intellectual property restrictions and are of varying quality. Communication among researchers and clinicians who would benefit from sharing of existing biospecimens is fractured and incomplete. A database of all biospecimens that could be made available to investigators should be generated to maximize use of these materials. Many laboratories have their own libraries of cell lines. However, lab-to-lab differences exist, and some of the biologic characteristics of original cell lines have changed with time. A recommendation was made that notice of grant awards should include a requirement for sharing tissue/mouse models and other biospecimens with other qualified investigators. It was suggested that investigators submit tissue to ATCC via the NCI as a liaison or that the NCI provide money to Specialized Programs of Research Excellence to establish and maintain pancreas cell lines and tissue cores. The Pancreas Cancer Map supported by the NCI (www.cancermap.org) is a resource that is underutilized by the pancreas cancer research community. The majority of pancreas cancer grants are listed on the map. Available pancreatic cancer cell lines could also be posted on this site for easier access.

EXECUTIVE SUMMARY

The NCI and other public and private agencies and organizations must increase funding for basic, clinical and translational research in pancreatic cancer relative to priorities as defined by the community and include methods to evaluate and refine the process in a dynamic manner. Communication between the academic community and the pharmaceutical industry must be improved to benefit patients with this deadly disease. Developing research partnerships that involve academic investigators, pharmaceutical industry, and patient advocacy will best accomplish the goals of decreasing the morbidity and mortality from this disease. There is a need for coordinated and collective effort to implement and ensure progress in the recommendations that were made in this meeting. The GI Steering Committee under the auspices of the NCI is structured to try to maximize collaborations between laboratory investigators, SPORE investigators, early phase clinical trialists as well as the cooperative groups in therapy and imaging. Active involvement by patient advocates and community physicians as well as the ongoing efforts within the NCI to simplify the protocol development process, will hopefully facilitate the necessary advances in this deadly disease. Specific recommendations were as follows:

New Targets for Drug Development (Table 1)

- Enhance research in the identification and validation of relevant targets and molecular pathways in pancreatic cancer, CSCs, and the microenvironment, including the role of angiogenesis in earlier stage disease and as part of multitargeted therapy.
- Establish high-throughput assays systems to accelerate target identification and validation.

Utility of Preclinical Models

- Preclinical tumor models of pancreas cancer may improve the ability to rationally design therapies.
- The development, availability, standardization, and utility of preclinical models for rational drug therapy design and the establishment of predictive biomarkers should be expanded and supported. An infrastructure for developing, validating, and using genetically engineered mice or primary tumor explants should be established and supported.
- A better understanding of the relative strengths of primary tumor xenografts and genetically engineered mouse models is required before recommending widespread adoption by the scientific community. Nevertheless, it is recognized that both model systems will be complementary.
- The model systems should be made freely available to investigators. Some of these models are provided by the NCI via the Mouse Models of Human Cancer Consortium (http://mouse.ncifcrf.gov/).

Future Clinical Trials (Table 2)

- Phase III clinical trials in advanced disease should be implemented only if there is a meaningful clinical signal of efficacy and safety in the phase II setting. The emphasis therefore must be on performing well-designed phase II studies to help define strategies likely to succeed in a phase III setting with survival as the primary end point.
- All high-priority phase III trials must be conducted as intergroup trials without competition and should be designed to include a scientifically appropriate biorepository. The clinical research community in collaboration with the US Food and Drug Administration, industry, and cooperative groups should adopt a consistent set of basic entry and evaluation criteria for phase II trials.
- Emphasis in designing clinical trials should be on rational combinations of targeted agents and the development of predictive biomarkers to assist selection of patient subsets. Government agencies such as the NCI and US Food and Drug Administration should review policies to facilitate, or at least allow the practice of interrogating combinations of unapproved agents that show significant promise in preclinical models. This requires a clinical trial mechanism with intimate planning and coordination between pharmaceutical industry, Cancer Therapy Evaluation Program, US Food and Drug Administration, and the investigators.
- Recent advances in cancer immunology and the development of newer approaches in immune therapy justify the testing of such therapies in patients with pancreatic adenocarcinoma, especially those with earlier stage disease.
- Government agencies, pharmaceutical industry, and clinical investigators should readily provide information on clinical trials outcome, including negative trials to all investigators.

Table 2. Summary of Recommendations for the Conduct of Pilot Trials in Advanced Pancreas Cancer

Parameter	Recommendation
Patient selection	Restrict studies to patients with Eastern Cooperative Oncology Group performance status 0-1
	Study patients with localized unresectable disease separately from those with metastatic disease
	Establish uniform eligibility criteria
	Use of predictive biomarkers for enrichment
Agents to be tested	Maximize input from preclinical data
	Adopt multitargeted approaches based on scientific rationale
	Consider non–gemcitabine-based combinations
Statistical designs	Survival preferred primary end point
	Single arm and multiarm randomized studies each have their place
Correlative science	Incorporate hypothesis-driven correlative research in pilot trials
	Report negative as well as positive study outcomes
	Consider prospective patient selection in pilot study setting based on data relating outcome with a specific molecular profile
Transition to phase III	Consider input from multiple studies
	Implement phase III testing only after a robust signal from pilot study(s)

Establishing Biorepositories

- Clinically and molecularly annotated biorepositories of high quality material will provide a rich source of information and clinical samples that should be utilized by pancreas cancer researchers.
- All randomized and selected single-arm trials should consider inclusion of a related biorepository (eg, serum, blood, tumor tissue) and the infrastructure to allow easy and shared use of this material must be established. Alternate sources for tumor genomic material (eg, blood) should also be developed.
- Access to and sharing of existing biorepositories must be mandated and supported by public-private partnership.

Development of Biomarkers

- The biomarker should be tested preclinically in animal models as part of the process of moving a drug to the clinic.
- Whenever possible, prospective biomarker evaluation should be an integral part of clinical trials in pancreas cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure

Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Philip A. Philip, sanofi-aventis (C), Bristol-Myers Squibb (C), OSI Pharmaceuticals (C); Neal Meropol, sanofi-aventis (C), Biogen Idec (C), Genomic Health (C), Genentech (C), Pfizer (C); Leisha Emens, Genentech (C); Lee Ellis, Schering-Plough (C); Margaret Tempero, Abraxas Bioscience (C), Celgene (C), Elsevier (C), F. Hoffmann-La Roche (C), Merck (C), Myriad Genetics (C), Centocor Ortho Biotech (C), Raven Biotechnologies (C), sanofi-aventis (C); Jordan Berlin, Genentech (C), Bayer Pharmaceuticals (C), AstraZeneca (C), ImClone Systems (C), Bristol-Myers Squibb (C), Abbott Laboratories (C), sanofi-aventis (C), Roche (C), Pfizer (C), Amgen (U), Cephalon (U) **Stock Ownership:** None **Honoraria:** Philip A. Philip, sanofi-aventis, OSI Pharmaceuticals; Malcolm Moore, OSI Pharmaceuticals; Leisha Emens, American Society of Clinical Oncology; Lee Ellis, Genentech **Research Funding:** Philip A. Philip, sanofi-aventis, Bristol-Myers Squibb, Pfizer; Malcolm Moore, OSI Pharmaceuticals; Leisha Emens, Genentech; Lee Ellis, sanofi-aventis; Margaret Tempero, Response Genetics; Diane Simeone, OncoMed Pharmaceuticals **Expert Testimony:** None **Other Remuneration:** Jordan Berlin, Pfizer

AUTHOR CONTRIBUTIONS

Conception and design: Philip A. Philip, Margaret Mooney, Deborah Jaffe, Gail Eckhardt, Malcolm Moore, Neal Meropol, Leisha Emens, Eileen O'Reilly, Lee Ellis, Margaret Tempero, Sunil Hingorani, Jordan Berlin, Joel Tepper

Administrative support: Philip A. Philip, Deborah Jaffe

Collection and assembly of data: Philip A. Philip, Jordan Berlin

Data analysis and interpretation: Philip A. Philip, Margaret Mooney, Gail Eckhardt, Neal Meropol, Eileen O'Reilly, Mace Rothenberg, Diane Simeone

Manuscript writing: Philip A. Philip, Deborah Jaffe, Malcolm Moore, Neal Meropol, Leisha Emens, Eileen O'Reilly, Murray Korc, Lee Ellis, Jacqueline Benedetti, Mace Rothenberg, Christopher Willett, Margaret Tempero, Andrew Lowy, James Abbruzzese, Diane Simeone, Sunil Hingorani, Jordan Berlin, Joel Tepper

Final approval of manuscript: Philip A. Philip, Margaret Mooney, Neal Meropol, Leisha Emens, Eileen O'Reilly, Jacqueline Benedetti, Mace Rothenberg, Margaret Tempero, Andrew Lowy, James Abbruzzese, Jordan Berlin

REFERENCES

1. Jemal A, Siegel R, Ward E, et al: Cancer Statistics, 2008. CA Cancer J Clin 58:71-96, 2008

2. Burris HA III, Moore MJ, Andersen J, et al: Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: A randomized trial. J Clin Oncol 15:2403-2413, 1997

3. Rothenberg M, Moore MJ, Cripps MC, et al: A phase II trial of gemcitabine in patients with 5-FU-refractory pancreas cancer. Ann Oncol 7:347-353, 1996

4. Desai SP, Zalupski MM: Optimum cytotoxic therapy for advanced pancreatic cancer, in Lowy AM, Leach SD, Philip PA (eds): Pancreatic Cancer. New York, NY, Springer, 2008 pp 511-534

5. Moore MJ, Goldstein D, Hamm J, et al: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: A phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25: 1960-1966, 2007

6. Smit VT, Boot AJ, Smits AM, et al: KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. Nucleic Acids Res 25:7773-7782, 1998

7. Almoguera C, Shibata D, Forrester K, et al: Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell 53:549-554, 1988

8. Caldas C, Hahn SA, Hruban RH, et al: Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. Cancer Res 54:3568-3573, 1994

9. Yanagisawa A, Ohtake K, Ohashi K, et al: Frequent c-Ki-ras oncogene activation in mucous cell hyperplasias of pancreas suffering from chronic inflammation. Cancer Res 53:953-956, 1993

10. Cerny WL, Mangold KA, Scarpelli DG: K-ras mutation is an early event in pancreatic duct carcinogenesis in the Syrian golden hamster. Cancer Res 52:4507-4513, 1992

11. Hingorani SR, Petricoin EF, Maitra A, et al: Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 4:437-450, 2003

12. Aguirre AJ, Bardeesy N, Sinha M, et al: Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 17:3112-3126, 2003

13. Zhu K, Hamilton AD, Sebti SM: Farnesyltransferase inhibitors as anticancer agents: Current status. Curr Opin Investig Drugs 4:1428-1435, 2003

14. Cox AD, Der CJ: Ras family signaling: Therapeutic targeting. Cancer Biol Ther 1:599-606, 2002

15. Van Cutsem E, van de Velde H, Karasek P, et al: Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol 22:1430-1438, 2004

16. Rowinsky EK: Lately, it occurs to me what a long, strange trip it's been for the farnesyltransferase inhibitors. J Clin Oncol 24:2981-2984, 2006

17. Tang Y, Yu J, Field J: Signals from the Ras, Rac, and Rho GTPases converge on the Pak protein kinase in Rat-1 fibroblasts. Mol Cell Biol 19:1881-1891, 1999

18. Kanda A, Kawai H, Suto S, et al: Aurora-B/AIM-1 kinase activity is involved in Ras-mediated cell transformation. Oncogene 24:7266-7272, 2005

19. Lerner EC, Zhang TT, Knowles DB, et al: Inhibition of the prenylation of K-Ras, but not H- or N-Ras, is highly resistant to CAAX peptidomimetics and requires both a farnesyltransferase and a geranylgeranyltransferase I inhibitor in human tumor cell lines. Oncogene 15:1283-1288, 1997

20. Sun J, Qian Y, Hamilton AD, et al: Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. Oncogene 16:1467-1473, 1998

21. Visvader JE, Lindeman GJ: Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. Nat Rev Cancer 8:755-768, 2008

22. Li C, Heidt DG, Dalerba P, et al: Identification of pancreatic cancer stem cells. Cancer Res 67: 1030-1037, 2007

23. Simeone DM: Pancreatic cancer stem cells: Implications for the treatment of pancreatic cancer. Clin Cancer Res 14:5646-5648, 2008

24. Hermann PC, Huber SL, Herrler T, et al: Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1:313-323, 2007

25. Cruickshank AH: Solid carcinomas of the exocrine pancreas. In: Pathology of the pancreas. London, United Kingdom, Springer-Verlag, 1986, pp 155-177

26. Jackson L, Evers BM: Chronic inflammation and pathogenesis of GI and pancreatic cancers. Cancer Treat Res 130:39-65, 2006

27. Korc M: Pancreatic cancer-associated stroma production. Am J Surg 194:S84-S86, 2007 (suppl)

28. Farrow B, Sugiyama Y, Chen A, et al: Inflammatory mechanisms contributing to pancreatic cancer development. Ann Surg 239:763-769, 2004

29. Hwang RF, Moore T, Arumugam T, et al: Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res 68:918-926, 2008

30. Vonlaufen A, Joshi S, Qu C, et al: Pancreatic stellate cells: Partners in crime with pancreatic cancer cells. Cancer Res 68:2085-2093, 2008

31. Ide T, Kitajima Y, Miyoshi A, et al: Tumor-stromal cell interaction under hypoxia increases the invasiveness of pancreatic cancer cells through the hepatocyte growth factor/c-Met pathway. Int J Cancer 119:2750-2759, 2006

32. Lee CJ, Li C, Simeone DM: Human pancreatic cancer stem cells: Implications for how we treat pancreatic cancer. Transl Oncol 1:14-18, 2008

33. Kindler HL, Niedzwiecki D, Hollis D, et al: A double-blind, placebo-controlled, randomized phase III trial of gemcitabine plus bevacizumab versus gemcitabine plus placebo in patients with advanced pancreatic cancer: A preliminary analysis of Cancer and Leukemia Group B (CALGB). J Clin Oncol 25: 199s, 2007 (abstr 4508)

34. Mahadevan D, Von Hoff DD: Tumor-stroma interactions in pancreatic ductal adenocarcinoma. Mol Cancer Ther 6:1186-1197, 2007

35. Hezel AF, Kimmelman AC, Stanger BZ, et al: Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 20:1218-1249, 2006

36. Gutierrez-Barrera AM, Menter DG, Abbruzzese JL, et al: Establishment of three-dimensional cultures of human pancreatic duct epithelial cells. Biochem Biophys Res Commun 358:698-703, 2007

37. Azmi AS, Mohammad M, Kaseb AO, et al: Utility of animal models in pancreatic cancer research, in Lowy AM, Leach SD, Philip PA (eds): Pancreatic Cancer, New York, NY. Springer, 2008, pp 577-599

38. Hruban RH, Adsay NV, Albores-Saavedra J, et al: Pathology of genetically engineered mouse models of pancreatic exocrine cancer: Consensus report and recommendations. Cancer Res 66:95-106, 2006

39. Rubio-Viqueira B, Jimeno A, Cusatis G, et al: An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res 12:4652-4661, 2006

40. Rijken AM, Hu J, Perlman EJ, et al: Genomic alterations in distal bile duct carcinoma by comparative genomic hybridization and karyotype analysis. Genes Chromosomes Cancer 26:185-191, 1999

41. Philip PA, Benedetti J, Fenoglio-Preiser C, et al: Phase III study of gemcitabine plus cetuximab versus gemcitabine in patients with locally advanced or metastatic adenocarcinoma: SWOG S0205 study. J Clin Oncol 25:199s, 2007 (abstr LBA4509)

42. Taylor JMG, Braun TM, Li Z: Comparing an experimental agent to a standard agent; relative merits of a one-arm or randomized two-arm phase II design. Clin Trials 3:335-348, 2006

43. Rubinstein LV, Korn EL, Freidlin B, et al: Design issues of randomized phase II trials and a proposal for phase II screening trials. J Clin Oncol 23:7199-7206, 2005

44. Korn EL, Liu P-Y, Lee SJ, et al: Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. J Clin Oncol 26:527-534, 2008

45. Lowy AM: Neoadjuvant therapy for pancreas cancer. J Gastrointest Surg 12:1600-1608, 2008

46. Katz MH, Evans DB, Sun CC, et al: Borderline resectable pancreatic cancer: The importance of the emerging stage of disease. J Am Coll Surg 206:833-846, 2008

47. Rosenberg L, Lipsett M: Biotherapeutic approaches to pancreatic cancer. Expert Opin Biol Ther 3:319-337, 2003

48. Korman A, Yellin M, Keler T, et al: Tumor immunotherapy: Preclinical and clinical activity of anti-CTLA4 antibodies. Curr Opin Investig Drugs 6:582-591, 2005

49. Kuriyama H, Watanabe S, Kjaergaard J, et al: Mechanism of third signals provided by IL-12 and OX-40R ligation in eliciting therapeutic immunity following dendritic-tumor fusion vaccination. Cell Immunol 243:30-40, 2006

50. Fong L, Small EJ: Anti-cytotoxic T-lymphocyte antigen-4 antibody: The first in an emerging class of immunomodulatory antibodies for cancer treatment. J Clin Oncol 26:5275-5283, 2008

51. Plate JM, Plate AE, Shott S, et al: Effect of gemcitabine on immune cells in subjects with adenocarcinoma of the pancreas. Cancer Immunol Immunother 54:915-925, 2005

52. Jaffee EM, Hruban RH, Biedrzycki B, et al: Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. J Clin Oncol 19:145-156, 2001

53. Laheru D, Biedrzycki B, Thomas AM, et al: Development of a cytokine-modified allogeneic whole cell pancreatic cancer vaccine. Methods Mol Med 103:299-327, 2005

54. Emens LA: Chemotherapy and tumor immunity: An unexpected collaboration. Front Biosci 13: 249-257, 2008

55. Kurihara T, Itoi T, Sofuni A, et al: Detection of circulating tumor cells in patients with pancreatic cancer: A preliminary result. J Hepatobiliary Pancreat Surg 15:189-195, 2008

56. Berger AC, Garcia M, Hoffman J, et al: Postresection CA 19-9 predicts overall survival in patients with pancreatic cancer treated with adjuvant chemoradiation: A prospective validation by RTOG 9704. J Clin Oncol 26:5918-5922, 2008

57. Burk U, Schubert J, Wellner U, et al: A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep 9:582-589, 2008

58. Yang J, Mani S, Donaher J, et al: Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927-939, 2004

59. Buck E, Eyzaguirre A, Barr S, et al: Loss of homotypic cell adhesion by epithelial-mesenchymal transition or mutation limits sensitivity to epidermal growth factor receptor inhibition. Mol Cancer Ther 6:532-541, 2007

60. Thomson S, Buck E, Petti F, et al: Epithelial to mesenchymal transition is a determinant of sensitivity of non-small cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. Cancer Res 65:9455-9462, 2005

61. Shah AN, Summy JM, Zhang J, et al: Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 14:3629-3637, 2007

