Abbreviated Title: Subtyping & Mol. Targets in PDAC

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Title: Identification of Molecular Subtypes and Therapeutic Targets in Pancreatic Cancer

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Précis

Background:

- Pancreatic cancer is the deadliest of all cancers worldwide, with a median survival of less than 6 months and 5-year survival of 6%. The dismal prognosis is due to the lack of an effective therapy and reliable biomarkers for early diagnosis.
- Patients who are diagnosed at an early stage undergo surgical resection. However, the median survival even for patients who undergo surgical resection is less than 2 years. Therefore, identification of therapeutic targets is of utmost importance to develop effective treatments.
- Molecular heterogeneity in pancreatic cancer indicates the need for individualized treatment, by identifying targets, based on the subtypes or molecular distinctions among tumors. Understanding tumor biology, a priority recommendation of the Pancreatic Cancer Progress Review Group (convened by NCI), is key to the identification of effective therapeutic targets.

Objectives:

- Role of inflammatory and immune mediators and inflammation signaling pathways in pancreatic cancer progression and disease aggressiveness.
- Identify molecular subtypes of pancreatic ductal adenocarcinoma (PDAC), and metabolic reprogramming by analyzing frozen tumor and surrounding nontumor tissue, and blood samples.
- Investigate the role of inflammatory genes and inflammation-associated miRNAs in regulation of metabolic alterations in tumor aggressiveness in PDAC.

Eligibility:

- Residents from the city of Baltimore and surrounding areas, age ≥18 to less than 90 years, with a diagnosis of PDAC.
- Willingness and ability to give informed consent and be interviewed using a short questionnaire.

Design:

- This is a large-scale, exploratory study of PDAC to identify molecular subtypes, candidate therapeutic targets, and biomarkers for therapeutic outcome. The study will be part of the existing epidemiological infrastructure developed through an NCI-Maryland Resource Contract at the University of Maryland at Baltimore.
- The study will include 500 primary PDAC cases, including 150 cases with surgical resection from which fresh-frozen tumor and surrounding non-cancerous tissue specimens will be obtained for transcriptomic (mRNA and miRNA) and metabolomic analysis.

- Blood samples will be collected from all 500 primary PDAC cases for analysis of candidate biomarkers in serum and plasma. If any immune-related biomarkers are discovered, these will be further validated using peripheral blood mononuclear cells.
- The study will involve administration of a short questionnaire.
- The first 12 months will constitute a pilot phase, during which the recruitment procedure will be refined.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Role of inflammatory and immune mediators and inflammation signaling pathways in pancreatic cancer progression and disease aggressiveness.
- Investigate the role of inflammatory genes and inflammation-associated miRNAs in regulation of metabolic alterations in tumor aggressiveness in PDAC.

1.1.2 Secondary Objective

- Identify molecular subtypes of pancreatic ductal adenocarcinoma (PDAC), and metabolic reprogramming by analyzing frozen tumor and surrounding nontumor tissue, and blood samples.

1.2 BACKGROUND AND RATIONALE

Pancreatic cancer is the fourth leading cause of cancer deaths in the United States and is among the most lethal human malignancies worldwide with a median survival of less than 6 months and 5-year survival of 6%. An estimated 44,920 new cases and 37,390 deaths are expected to occur in the United States in 2012 (1). Among different forms of pancreatic cancer, the most common is PDAC. There has been no significant change in the overall incidence and mortality for last several decades, with an apparent disparity between African-Americans and Caucasians. The etiology of pancreatic cancer is not well defined. Several risk factors, including smoking, alcohol use, inflammation, family history, diabetes, obesity and race have been associated with pancreatic cancer (2-10). However, the evidence of an association between smoking and pancreatic cancer is the strongest and most convincing (11, 12).

The dismal outcome for patients with pancreatic cancer is attributed to the lack of any effective therapeutic drug and the diagnosis of the disease at an advanced stage in majority of patients. In less than 20% of the patients, who are diagnosed at an early stage, surgical resection is an option with some curable potential. However, the median survival even for patients who undergo resection is less than 2 years with more than 80% recurrence within this time period. Therefore, the identification of effective therapeutic targets is of utmost importance to improve patient outcomes in PDAC. The unique tumor biology and underlying mechanisms that confer the highly aggressive nature and resistance to chemotherapy in pancreatic cancer are poorly understood (13). Highly heterogeneous characteristics have been revealed in the genetic analysis of pancreatic cancer xenografts and cell lines (14). In this analysis, an average of 63 genetic abnormalities per tumor were described that were associated with 12 cancer-relevant pathways. However, the altered genes in a specific pathway differed widely among individual tumors, suggesting tremendous tumor heterogeneity. Furthermore, profiling of 95 miRNAs that are functionally related to cancer biology, cell development and apoptosis in pancreatic cancer tissue and cell lines showed different profiling patterns among individual cases and cell lines, suggesting individual molecular makeup in pancreatic cancer (15). Based on gene-expression profiling of 27 primary tumors, a recent report provides the first evidence of the existence of

molecular subtypes in PDAC, with different clinical outcome and therapeutic response (16). However, the sample size was too small in each of the three subtypes for a strong evidence of difference in outcome, measured as overall survival. Further validation of these subtypes and the identification of additional molecularly distinct subgroups with different clinical outcomes and therapeutic response in larger cohorts are needed. Most importantly, the delineation of subtype-specific biology and critical pathways associated with patient outcome is required to identify subtype-specific targets.

In addition to the global strategy using molecular profiling to identify molecular subtypes and subtype-specific candidate therapeutic targets, it is also important to focus on the genes and pathways that are associated with the key features of a specific cancer type. One of the important characteristic features of PDAC is the presence of a large stroma, known as desmoplasia, with evidence of active inflammation. This massive stroma consists of fibroblasts, vascular, and inflammatory cells that are embedded into a distinctly large volume of extracellular matrix [reviewed in (17)]. Evidence from epidemiological and molecular studies have indicated a role of inflammation in the development and progression of pancreatic cancer (8). Increased expression of a number of downstream mediators of inflammation and activation of signaling pathways that link inflammation and cancer are reported in both the precursor lesions and PDAC. Levels of several cytokines are altered in pancreatic cancer and chronic pancreatitis. Cytokines are critical mediators of inflammation and immune regulation, and an imbalance in their optimal level can cause or promote several pathologies, including cancer. *In vitro* studies using pancreatic cancer cell lines have provided evidence that cytokines may play a role in proliferation, cell survival, apoptosis and metastasis (18). Increased levels of MIF, IL-6, IL-8, IL-10 and IL-1RA were found in the serum of patients with pancreatic cancer (19). Furthermore, several of these cytokines are associated with prognosis in patients with pancreatic cancer (18). Polymorphism of IL-1B, resulting in its increased expression, is associated with poor survival in patients with pancreatic cancer (20). Thus, the inflammatory microenvironment of pancreatic cancer may have a critical role in the development and progression of pancreatic cancer. A focused investigation on inflammation-associated genes and pathways in PDAC may provide clues to identify potential targets for therapy.

1.2.1 Rationale for Transcriptomic Profiling of Pancreatic Cancer

Transcriptomics is an important and widely used strategy for an unbiased, global screening of molecular characteristics and/or differences in biological samples, based on the expression level of coding and non-coding genes, including miRNA. However, further validation using a quantitative assay is commonly used to confirm the findings of array-based transcriptomics analysis. Transcriptomic profiling of tumor and nontumor tissue may provide clues to the molecular pathogenesis of pancreatic cancer and identify potential targets for therapy, molecular subtypes with distinct outcomes and therapeutic response, and biomarkers for therapeutic outcome. One of the important goals in expression profiling of pancreatic cancer is to determine an expression signature that is likely to be relevant to tumor biology, and one way to achieve this goal is to first focus on those genes or pathways that are associated with patient outcome. Gene-expression studies have identified differentially expressed genes in pancreatic cancer as compared with nontumorous pancreatic tissue (21). There are also few reports that describe the association of differentially expressed genes with patient outcome. The tight junction protein family, including claudin 4 and 18, and genes related to calcium homeostasis, including annexin

8, have been found to be highly over-expressed in PDAC and its precursor lesions, pancreatic intraepithelial neoplasia (22, 23). Increased claudin 18 expression is associated with significantly better survival in patients with PDAC. Another example is the glycoprotein, mesothelin, which is over-expressed in the majority of pancreatic cancer and is being investigated as a therapeutic target in several clinical trials (23, 24). A six-gene signature has been reported to be associated with survival of patients with localized pancreatic cancer (25).

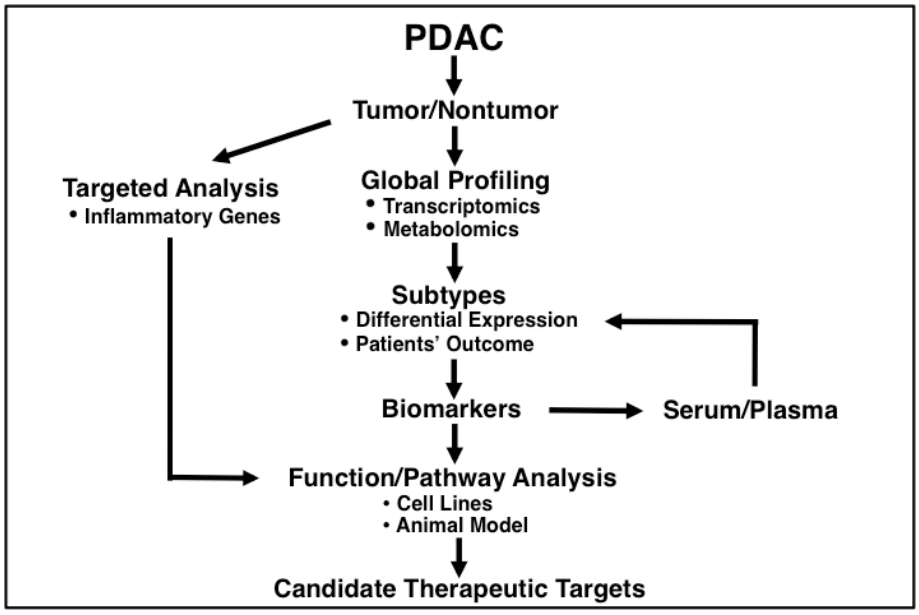
An altered expression of miRNAs is implicated in the development of human cancer (26). The aberrant expression of specific miRNAs can both induce or suppress tumor development. Whereas an overexpression of miR-155 or miR-21 could induce tumors, the overexpression of *let-7a* reduced lung cancer in mouse models (27-29). Expression of a number of miRNAs is found to be either elevated (for example, mir-196, mir-221, mir-186, mir-21 or mir-155) or reduced (for example, mir-let7, mir-34a, mir-96 or miR-216) in pancreatic cancer as compared with non-tumor pancreatic tissue (30). Some of these miRNA expressions are correlated with survival and metastasis in pancreatic cancer. A group of 6 microRNAs, miRs-452, -105, -127, -518a-2, -187 and -30a-3p distinguished long-term survivors with node-positive disease with those who died within 24 months (30). An increased expression of mir-196a-2 and mir-21 predicted poor survival in patients with pancreatic cancer, with a median 14.3 months vs. 26.5 months and 15.2 months vs. 27.7 months, respectively (30, 31). These discoveries indicate that gene expression profiling can identify candidate genes that can be further investigated for their potential utility as therapeutic targets. As stated earlier, our principal goal is to identify molecular subtypes, subtype-specific therapeutic targets and biomarkers for therapeutic response, by first distinguishing coding genes and miRNAs, through gene expression profiling in both the tumor and surrounding non-tumor tissue. Our approach is unique, involving the integration of coding genes and miRNA expression, and metabolite profile to identify distinct subtypes and critical pathways that can be targeted to achieve the most effective treatment outcome.

1.2.2 Rationale for Assessing the Metabolites in Pancreatic Cancer

Metabolomics, a global analysis of metabolites in a biological system, is a promising approach towards early diagnosis, prognosis, therapy guidance and outcome in patients with cancer (32). Metabolites are intermediate and end products of metabolism that represent the physiological state, taking into account the genetic regulation, kinetic activity of enzymes and alterations in metabolic reactions, and yield a functional portrait of the end products of gene expression. Metabolomics allows for sensitive detection of metabolites that are present at low concentrations in tissues or body fluids by utilizing nuclear magnetic resonance spectroscopy and mass spectrometry, the two most commonly accepted methodologies. An increased level of phospholipids, in particular choline-containing compounds, increased glycolytic capacity, citrate and over-expression of pyruvate kinase M2 are some of the markers of an altered metabolic profile in several tumor types [reviewed in (32)] (33, 34). A distinct metabolic profile has been reported in breast, prostate, brain and ovarian tumors that can distinguish cancer patients from normal controls and predict the histological grades in breast cancer (32, 35). A distinct serum metabolic profile is reported in pancreatic ductal adenocarcinoma as compared with benign hepatobiliary disease, including pancreatitis (36). However, a detailed metabolic profile of PDAC is lacking. Integration of metabolomics with transcriptomics is a unique approach of identifying critical genes and pathways associated with tumor progression and patient outcome. Our lab has utilized the strategy of integrating transcriptomics with metabolomics to identify

therapeutic targets in liver cancer (Budhu et al., unpublished data). Paired tumor and surrounding non-tumor samples from PDAC cases will be used for metabolomic analysis at Metabolon, Inc., using ultra-performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry (UPLC-QTOFMS). The tumor-specific metabolites will be analyzed for association with patient outcome (survival) and identification of subtypes of PDAC. Lastly, these metabolomics data will be integrated with transcriptomics data to correlate selected metabolites with genes and their interconnected pathways to identify potential therapeutic targets.

Figure 1: Strategy to Identify Therapeutic Targets in Pancreatic Cancer



1.2.3 Rationale for Conducting the Study in the Greater Baltimore Area

This study will be supported by an existing infrastructure with our Resource Contract at the University of Maryland Medical System (UMMS), Baltimore, MD. The Laboratory of Human Carcinogenesis (LHC) is currently conducting lung, prostate and liver cancer case-case, and case-control studies, which are supported by this resource contract at the same hospital. Based on a recent 5-year record, an average of 150 pancreatic cancer cases each year have been treated at UMMS. This will help us succeed in our goal for collecting fresh-frozen tumor, surrounding non-tumor tissue and blood samples from 150 surgical cases and only blood from an additional 350 cases of primary PDAC.

Table 1: Patients With Pancreatic Cancer Treated at UMMS Over a 5-Year Period

Age	2006	2007	2008	2009	2010
20-29		1			3
30-39	1	1	5	4	1
40-49	14	16	14	15	10
50-59	24	38	34	38	35
60-69	32	52	48	49	51
70-79	35	50	47	49	44
80-89	24	13	20	15	16
Total	**130**	**171**	**168**	**170**	**160**

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have been dignosed with pancreatic cancer including those on treatment with metastatic disease.

2.1.1.2 Age ≥18 years and less than 90 years.

2.1.1.3 Permission from the physician at UMMS, listed as the treating physician to contact the patient.

2.1.1.4 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.5 Physically and mentally capable of participating in the study interview.

2.1.1.6 Must understand English well enough to be interviewed.

2.1.2 Exclusion Criteria

2.1.2.1 Patients, who are 90 years or older are excluded because of co-morbidity considerations.

2.1.2.2 Children are excluded from the proposed study because very few patients with PDAC are younger than 18 years of age.

2.1.2.3 Patients currently residing in an institution, such as a prison, nursing home, or shelter.

2.1.2.4 Severely ill patients in the intensive care unit.

2.1.3 Recruitment Strategies

Please refer to Section 3.1.

2.2 SCREENING EVALUATION

The interviewer will assess the eligibility of patients with pancreatic cancer based on the inclusion and exclusion criteria. The verification of eligibility and enrollment of subjects will follow the procedures outlined in Section 3.1. The Eligibility Checklist (Appendix A) will be completed for each potential subject.

2.3 REGISTRATION PROCEDURES

Accrual to study will not begin until the patient has been deemed eligible. When consent is granted, the Research Study Coordinator at UMMS will register the patient in the UMMS database, where a unique study ID number will be assigned. Basic demographics are entered into the system for tracking purposes.

3 STUDY DESIGN

This study is a case-only study of patients with PDAC. The study is not a clinical investigation but an observational study. Subjects will not receive any type of therapy under the protocol. The collection of blood is the only medical procedure that is performed for this protocol. Patient care is not modified by the protocol. Patients will receive their usual care and are asked to donate tumor specimens that are surgically removed as part of their routine treatment. The surgery will be the same, as if patients were not participating in the study.

The following will be collected as part of this study: 1) tumor and adjacent nontumorous specimens from 150 cases undergoing surgical resection, 2) blood samples from 500 cases, which includes the 150 cases undergoing surgical resection, and 3) questionnaire information from all 500 cases. The duration of the recruitment phase is 5 years.

This study is conducted through an NCI-Maryland Resource Contract at the University of Maryland at Baltimore. All subjects will be enrolled at the UMMS contract site, and all study procedures and data collection will be performed there.

3.1 ENROLLMENT OF PDAC CASES

Cases will be identified through resources including, but not limited to, daily afternoon visits, or phone calls, to the Departments of Pathology, UMMS, to identify all cases diagnosed that day with pancreatic cancer. We will also review the weekly lists of scheduled surgeries. We have established active collaborations with the Departments of Pathology and Department of Surgery, UMMS, to maximize our ability for recruitment of new PDAC cases.

Case recruitment will follow the new HIPAA regulations ("HIPAA Privacy Rule"). However, to better assess eligibility, we intend to review medical and pathology records, cancer center registries and hospital databases at UMMS. Our contractor has been granted a HIPAA waiver to perform the aforementioned study activities for our protocol titled "Resource for the collection and evaluation of human tissues and cells from donors with an epidemiology profile," University of Maryland at Baltimore IRB #0298229. It is our plan to seek the same waiver for this study.

The Department of Pathology and the treating physicians will put a note into the medical record of severely ill pancreatic cancer patients to indicate that we should not contact this patient because of health concerns. If no such note is found in the medical record, we will proceed and contact eligible patients. If a note is found, the interviewer will record that this note was the reason for ineligibility on the eligibility record, in addition to above-mentioned criteria.

After the eligibility has been confirmed, an interviewer will contact patients to get both informed consent and authorization to obtain, use and disclose protected health information for research. The interviewer will administer the questionnaire to those who consented. After the interview, blood will be collected (all recruiters are trained in phlebotomy). The investigators will obtain

informed consent and administer the questionnaire well in advance of either a scheduled surgery or an alternative treatment such as chemotherapy and radiation. It is always at the discretion of the patient to give informed consent on one day but to have the interview or the blood collection on another day that is convenient for him/her. In the situation where we cannot get access to an eligible patient well in advance of surgery, we will approach the patient up to one day before surgery or the day of surgery to seek consent. We will offer to administer the questionnaires and obtain blood after the surgery. However, if the patient wishes to be interviewed and have the blood drawn at the same day when he gives informed consent, we will do so.

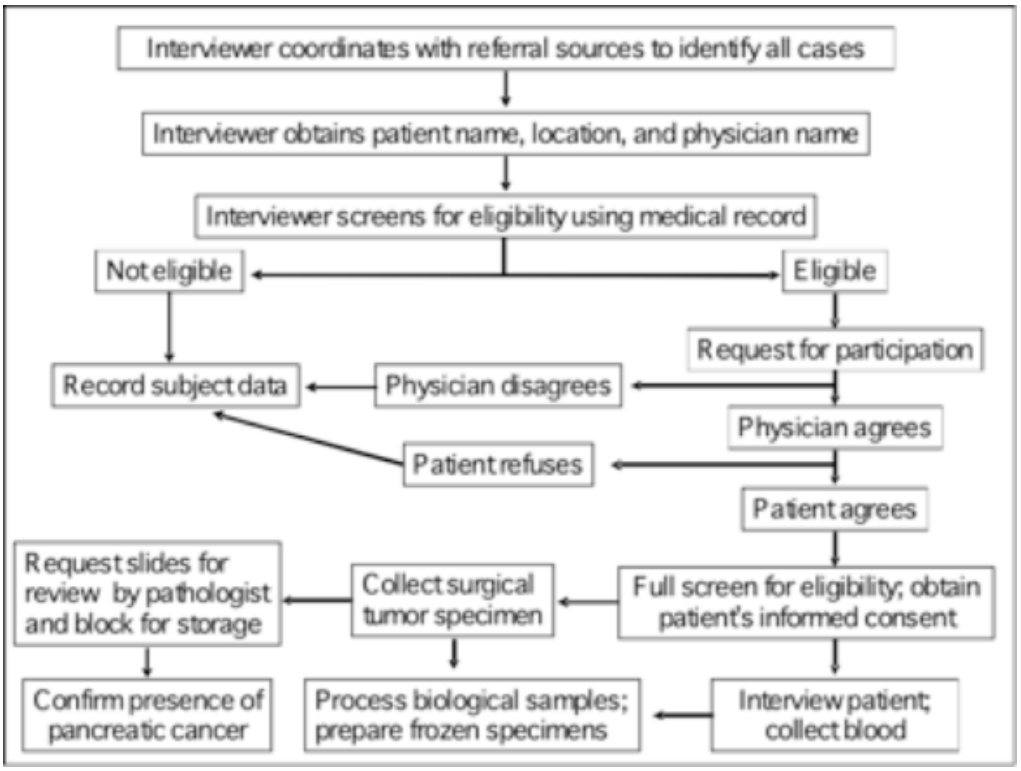
3.2 PROCEDURES

1) Interviewer obtains patient's name, location and physician's name.
2) Interviewer screens medical record for eligibility using eligibility checklist (Appendix A). If eligible, contact the treating physician for permission to contact the patient. If ineligible, record age, race and gender. Record reasons for ineligibility.
3) If physician permits, the interviewer contacts the patient and obtains informed consent and authorization to obtain, use and disclose protected health information for research.
4) If patient refuses (either right away or repeatedly delaying consent [failure to make commitment in response to 3 requests]), we will not contact that patient again. Refusal will be documented on the eligibility record.
5) Perform interview and complete questionnaire for those consented. The interviewer will give a copy of the questionnaire to the patient before the interview begins. The patient will have the opportunity to read a question while being interviewed.
6) Collect 20-40 cc of blood from patient. Collect 10 cc of blood in one red-top tube (serum) and another 10 cc in one green-top tube (plasma). With patient consent, two additional green-top tubes (10 cc each) will be collected for peripheral blood mononuclear cells (PBMC) extraction by gradient centrifugation and will be cryopreserved in the vapor phase of liquid nitrogen. Place tubes with collected blood in a thermos for short-term storage and transport to the University of Maryland Department of Pathology. (Blood should be processed [separation of serum and blood clot; buffy coat (PBMC), plasma and red blood cells] within 8-24 hours at the University of Maryland Department of Pathology).
7) Review pathology report at University of Maryland Department of Pathology to confirm diagnosis and abstract pathological data. Request confirmation of the pathology for the specimens that are collected (performed at University of Maryland Department of Pathology). Request part of tumor and nontumorous block and frozen tumor and nontumorous tissue, if available, for storage and analysis.

3.3 QUESTIONNAIRE

Trained interviewers will administer a short questionnaire (Appendix B) to enrolled patients. The interview will last less than an hour. The questionnaire will be administered before research blood collection and will assess prior medical and cancer history, family medical history, and exposure to known risk factors for pancreatic cancer, for example, smoking and alcohol use. The questionnaire data will allow us to examine, if any biomarkers are related to known risk factors of pancreatic cancer.

Figure 2: Diagram of Study Procedures



3.4 STUDY CALENDAR

Procedure	Screening	After Enrollment
Review medical records	X	
Eligibility checklist[a]	X	
Informed consent	X	
Conduct interview/complete questionnaire[b]		X
Blood samples for research[c]		X
Request tumor and non-tumor tissue (frozen and tissue block)[d]		X
Review pathology report and abstract data		X
Request survival information yearly[e]		X

[a] Appendix A. If ineligible, record age, race, gender and reason for ineligibility.
[b] Appendix B. To be conducted before research blood collection (and preferably before surgery, but may be done afterwards for patient convenience).
[c] After interview, collect 10 cc of blood in one red-top tube (serum) and another 10 cc in one green-top tube (plasma). With patient consent, two additional green-top tubes each with 10 cc of blood will be collected for PBMC extraction. Follow processing and shipping instructions in Section 4.1.2.

[d] Request part of tumor and nontumorous block and frozen tumor and nontumorous tissue, if available, for storage and analysis (see Section 4.1.1).
[e] Survival data, to be obtained from the National Death Index, will be requested annually from the contract site.

3.5 COMPENSATION

Eligible candidates will be offered an incentive with a monetary value of up to $25 to participate in the study.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL AND OFF-STUDY CRITERIA

3.6.1 Off-Study Criteria

Study procedures include completion of the questionnaire, blood collection, and if the participant undergoes surgery, tissue collection. If the patient refuses part of the procedures once enrolled, he/she can still participate. If the patient refuses all parts of the enrollment after signing the consent form, he/she will be withdrawn from the study. The date and reason of patient withdrawal will be documented. Subjects will remain on-study in case they have to be re-contacted for questions regarding their questionnaire data by the contractor. Once a subject is taken off study, no further data can be collected.

4 BIOSPECIMEN COLLECTION

4.1 CORRELATIVE STUDIES FOR RESEARCH

All specimens will be given a unique study identification number at the contract site in the University of Maryland Department of Pathology in Baltimore, MD and stored temporarily at -80°C. No personal identifiable information will be used on the storage vials. On a monthly basis specimens will be sent to LHC, NCI. Each specimen will be entered in the LHC frozen tissue database with double entry system and be given a unique computer generated LHC number before storage at -80°C at Central Tissue Repository at Frederick National Laboratory for Cancer Research, NCI, Frederick, till further analysis. The handling procedures and use of the specimens is outlined below.

4.1.1 Tissue Analysis

4.1.1.1 Gene Expression Analysis at the NCI

- RNA will be isolated from fresh-frozen tissue using a standard TRIZOL protocol (Invitrogen).
- mRNA expression profiling will be done using Affymetrix GeneChip Human 1.0 ST arrays at the LMT core facility at NCI-Frederick.
- Array data will be RMA (Robust Multichip Average)-normalized, and a gene expression summary will be created for each gene using Partek Genomics Suite 6.5 at LHC.

- The microRNA profiling will be done using a nanoString nCounter miRNA assay at the LMT core facility at NCI-Frederick. miRNA expression data will be normalized using Partek Genomic Suite 6.5. Good quality normalized array data will be imported to BRB ArrayTools for further analysis.
- Gene expression analysis is described in detail in Section 7.3.1.

4.1.1.2 Metabolomics Analysis

- Fresh-frozen, paired tumor and adjacent nontumor samples will be used for metabolomic analysis at Metabolon, Inc., using ultra-performance liquid chromatography coupled with quadruple time-of-flight mass spectrometry (UPLC-QTOFMS).
- Quality will be assessed using control samples including blanks, duplicates and endogenous "spike-ins."
- Data analysis will be performed at LHC using Partek and BRB ArrayTools.

4.1.2 Blood Product Analysis

- RNA will be isolated from serum and plasma using an RNA purification kit (Norgen Biotek Corp., Ontario, Canada) as described in manufacturer's protocol.
- MicroRNA expression analysis will be performed by qRT/PCR using pre-made Taqman probes for specific microRNAs purchased from Applied Biosystem as previously described by our lab (37).
- Data analysis will be performed at LHC using BRB ArrayTools and GraphPad Prism.
- If any immune-related biomarkers are discovered by tumor gene expression analysis, those genes will be further validated using peripheral blood mononuclear cells (PBMC). RNA will be isolated from frozen PBMC as described above using an RNA purification kit (Norgen Biotek Corp., Ontario, Canada) according to manufacturer's protocol. Expression of selected immune related genes, discovered in the tumor tissue, will be analyzed in the RNA samples from PBMC using qRT-PCR. Data analyses will be performed as mentioned above.

4.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

- All samples will be labeled with a unique LHC number and will not have any personal identifiers. Samples will be entered using LHC numbers in a secured, password-protected frozen tissue database that was developed at LHC and has been used for more than 20 years. Samples will then be stored at -80°C in the Central Tissue Repository at Frederick National Laboratory for Cancer Research, NCI, Frederick, until further analysis.
- Samples will be ordered and tracked through the password-protected LHC database and will not be sent outside NIH without IRB notification and an executed MTA.

- All specimens obtained in the protocol will be used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss. The PI will also report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI. If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

4.3 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

4.3.1 Description of the Scope of Genetic/Genomic Analysis

Frozen tumor and surrounding non-cancerous tissue specimens obtained from pancreatic cancer patients, undergoing surgical resection, will be analyzed for transcriptomic (mRNA and microRNA) and metabolomic analyses. Serum and plasma samples will be used to analyze selected microRNAs and metabolites that are identified by analysis of tissue specimens. If any immune-related biomarkers are discovered by tumor gene expression analysis, those genes will be further validated using peripheral blood mononuclear cells (PBMC). GWAS, family linkage or germline analysis will NOT be performed.

4.3.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

The protection of study subjects from research risk will be achieved by several mechanisms. Prior to enrolling subjects, we will obtain approval of the study from the IRB at the NCI, followed by the IRB at the University of Maryland School of Medicine. Written informed consent will be required from the study subjects for participation. The form will state that individual results will not be provided to the participants. According to new HIPAA regulations ("HIPAA Privacy Rule"), a separate written authorization to obtain, use and disclose protected health information for research purpose only will be required from the study subjects for participation in the study. Study subjects' confidentiality will be maintained at all times. Subjects will be assigned unique study ID numbers. These unique study numbers will be linked to the subject's identifier information in a database, and to the hard copy of the identifier sheet. This information will be secured at the University of Maryland School of Medicine at Baltimore. The database has two levels of password security, which will only allow authorized individuals to access the information. A log will automatically record who accessed the information, and what information was obtained. Biological samples will be labeled with the unique study number but no other identifier information. Thus, biological samples and results from the analyses of

biological samples can neither be linked back to the participant nor be used in any way to identify the participants.

Identifier information for non-participants will also be recorded to avoid a re-contact. This information will be stored in a database with two levels of password security, which will allow only authorized individuals to access the information. A log will automatically record who accessed the information and what was assessed. Non-participants will be assigned a unique study number. This number will be used for tracking of reasons for non-participation, and for available demographic information. The PI will ensure appropriate IRB review and approval.

4.3.3 Management of Results

The results from this study, including any incidental findings from genetic studies, will not be given to participants, their family members, their health care provider, or the UMMS contractor. We will not analyze any germline mutations in genes. The investigation involves only the relative abundance of gene expression and metabolites in pancreatic tumors, adjacent nontumor tissue and blood. Any research findings will be of an exploratory/investigational nature and will not be of clinical use. The NCI will receive only de-identified samples and will not have a relationship with the patients. Participants will be informed during the consent process that no research results will be returned.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The University of Maryland School of Medicine contractor is responsible for the collection and evaluation of the study data. All data will be kept secured. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained with the Maryland Contractor, which is the only location of personal identifiers with unique subject identification number.

All patient data are transferred electronically to a secured, password-protected centralized study database at the University of Maryland School of Medicine. The centralized database consists of three databases, a tissue, a survey, and a tracking database, that are linked through unique identifiers. The tracking database contains the personal information of each study participants. Access to this database is strictly controlled, and the database is not accessible to NCI researchers. The tissue database is an inventory database for fixed and fresh-frozen tissues, and for blood samples. Updated de-identified files of the tumor database are available to the NCI researcher. The survey database contains the demographic and epidemiological information of the study participants. Access to information in this database has to be requested. Information from the tissue and survey databases cannot be linked to any personal identifiers.

5.1.1 Questionnaire Data

The questionnaire data will be collected electronically using encrypted tablet computer and then downloaded into a secured centralized study database and linked with the unique identifier of the participants. The questionnaires are then verified by security-trained and authorized personnel, approved by University of Maryland IRB and under supervision of the study coordinator. If

errors are detected, the study coordinator will assist with the correction. Monthly reports on coding and editing, data entry and the verification status are prepared and presented at monthly staff meetings and site visits. During the study, quality control will consist of data comparisons among interviewers to determine the quantity and quality of information that they have gathered, by evaluating characteristics such as interview duration, number of interview problems reported, number of refusals, distribution of subject answers, and number of missing and incomplete answers.

5.1.2 Outcome Data

The PI will request the survival information of participants from the contractor on annual basis. The contractor will obtain the survival information using patients file and the National Death Index, which is updated yearly. In addition PI will also request the contractor to provide de-identified clinical information, such as tumor stage, grade, resection margin status, treatments received and clinical outcomes.

5.2 TOXICITY CRITERIA

Subjects will not receive any type of therapy as part of this protocol. The only medical procedure that will be performed as part of this protocol is blood collection; therefore, adverse events are not expected. In the unlikely event that an adverse event occurs during sample collection, the following criteria will be used for grading such events. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate patient areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

6 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

6.1 DEFINITIONS

6.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial, whether or not the event is considered related to the research or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last intervention. AEs that are considered research related, expected, continuing, but not resolvable by 30 days after intervention completion (e.g., alopecia) will not be followed after the 30-day period.

6.1.2 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are expected with similar research, but not specifically mentioned as occurring with this particular research.

6.1.3 Serious

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

6.1.4 Disability

A substantial disruption of a person's ability to conduct normal life functions.

6.1.5 Protocol Deviation (NIH Definition)

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the IRB.

6.1.6 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the study procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

6.1.7 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

6.2 NCI-IRB REPORTING

6.2.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

6.2.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing Review

The protocol PI will report to the NCI-IRB:

1. All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
2. All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
3. All Grade 5 events regardless of attribution;
4. All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

6.3 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS FOR MULTI-CENTER TRIALS

In this study, patients will be enrolled only at UMMS (the contract site). The following will apply for adverse event reporting: The contract site PI must immediately report to the Coordinating Center PI any serious adverse event, whether or not considered research related, including those listed in the protocol and must include an assessment of whether there is a reasonable possibility that the research caused the event within 48 hours of PI awareness of the event. The contract site PI must also report any protocol deviations or violations to the Coordinating Center PI within 7 days of PI awareness. The contract site must also submit the report to their IRB in accordance with their institutional policies.

6.4 DATA AND SAFETY MONITORING PLAN

The resource contractor at University of Maryland will conduct the study and is responsible for data collection and storage and day-to-day oversight of the study. The collected data will be monitored as follows. The contractor is required to perform quality assessment of the

questionnaire data, as described in Section 5.1. The Coordinating Center PI and the NCI Project Officer, together with the consulting epidemiologists under the contract, will monitor the study through weekly conference calls, regular meetings, and an annual site visit. The contractor will be required to provide a written monthly update, a 6-month report, and a year-end report. The data that are particularly monitored are the accrual rates, participation rates, age, race and gender distribution, and the number and stage distribution of fresh-frozen tumor specimens. The PI, the associate investigators, and the consulting epidemiologists, will thoroughly review the study once per year at the annual site visit for the contract.

The resource contractor will collect all data that pertain to the proposed study. The data will be stored in a central database at the University of Maryland, as described in Section 5.1. The contractor will provide data summaries in the semi-annual and annual reports. The database can be queried upon request from NCI investigators. The contractor will provide query results and statistical analysis. The contractor may also provide data in a file format to the NCI researcher for review and analysis at NCI; no participant identifiers will be included.

7 STATISTICAL SECTION

This is a PDAC case-only study that will include a total of 500 cases, of which 150 will be patients undergoing surgical resection. The primary objective of the study is to identify molecular subtypes of PDAC, and biomarkers as subtype-specific candidate therapeutic targets. The secondary objective is to investigate the role of inflammatory genes and inflammation-associated miRNAs in tumor aggressiveness in PDAC.

The objectives of the study will be accomplished with 1) microarray analysis of gene (mRNA and miRNA) expression and metabolite profiling of tumor and nontumor tissues from 150 surgically resected cases; and 2) blood product (serum/plasma) analysis to assess miRNA, metabolites and cytokines, that are associated with subtypes. The study will also involve collection of data from questionnaire, medical and pathology records, and the National Death Index. All statistical analysis will be performed at the LHC.

7.1 AVAILABILITY OF CASES AND JUSTIFICATION OF SAMPLE SIZE

A review of cancer registry data shows that UMMS treated 800 cases of pancreatic cancer from 2006-2010 (Table 1). Most of the patients resided in the greater Baltimore area. We expect to accrue a total of 500 cases, which includes 150 surgical cases, over a 5-year period, based on an average annual accrual of 100 cases.

7.2 POWER CALCULATIONS

We will conduct genome-wide gene expression analysis, and metabolic profiling of tumor and matching nontumorous pancreas tissues to compare expression profiles among PDAC cases. The minimum number of tumors needed to generate a molecular profile that differentiates subtypes cannot be accurately determined for the proposed experiments. We performed power calculations by using Power and Sample Size Calculation Software (version 3.0.43) to first determine if 150 tumors and 150 nontumor samples will have sufficient power to detect a 1.5- to 2-fold difference in gene expression with an alpha (Type I error rate) value of less than 0.001 (**Table 2**). We next determined if 150 tumor samples are sufficient to identify a molecularly different subtype with

significant difference in survival (alpha=0.05). Assuming a molecular subtype as 25% of all the cases (3:1) and a hazard ratio of 2, this sample size (150 cases) will be sufficient to reject the null hypothesis 85% of the time (Table 3). However, recent gene expression studies used fewer tissues than we are proposing for this study and yielded expression signatures that predicted survival (16). Thus, the proposed sample size for this study appears to be adequate.

We then determined if 500 plasma and serum samples would have sufficient power to identify selected biomarkers (miRNA, metabolites and cytokines) representing a subtype of PDAC as determined by analysis of tumors. Our laboratory has earlier reported that 21 out of 29 miRNAs that were selected based on their differential expression in lung cancer could be detected in serum and plasma in the majority of the cases (37). Assuming a molecular subtype as 25% of all the cases (3:1) and a hazard ratio of 1.5, this sample size (500) will be sufficient to reject the null hypothesis 92% of the time (Table 4). The Type I error (alpha) probability associated with this test of this null hypothesis is 0.05.

Table 2: Power calculation for proposed gene expression analysis in tumor (N=150) and non-tumor (N=150) samples with alpha=0.001

FOLD CHANGE	POWER (%)
1.25	16
1.5	91
1.75	99.9
2	99.9

Table 3: Power calculation for proposed survival analysis in PDAC cases (N=150) with alpha=0.05

	POWER		
Hazard Ratio	Subgroup Size (Ratio)		
	3:1	4:1	5:1
1.5	45	33.9	33
1.75	70	63	53
2	85	80	70
2.25	93	89	91
2.5	97	94	88
2.75	98	97	92
3	99	98	95

Table 4: Power calculation for proposed analysis of subtype specific biomarkers in serum/plasma of PDAC cases (N=500) with alpha=0.05

	POWER		
Hazard Ratio	Subgroup Size (Ratio)		
	3:1	4:1	5:1
1.5	92	87	79
1.75	99	98	96
2	1	1	99
2.25	1	1	99
2.5	1	1	1
2.75	1	1	1
3	1	1	1

7.3 DATA ANALYSIS PLAN

Data analysis will be performed by LHC, NCI.

7.3.1 Analysis of Gene Expression Profiles

Our lab routinely uses Partek Genomic Suite and BRB ArrayTool for gene expression microarray analysis (38-40). mRNA expression profile will be determined using the Affymetrix Genechip Human 1.0 ST arrays and microRNA profile using nanostring nCounter platform at the LMT core facility, NCI-Frederick. All gene expression array data will be normalized and gene expression summary will be created using Partek Genomic Suite 6.5. Good quality normalized array data will be imported to BRB ArrayTools for further analysis. All comparisons will be performed using the statistical packages provided in BRB ArrayTools such as class comparison, class prediction and survival analysis. To address multiple comparisons problem, we will use a Benjamini and Hochberg procedure (41). Differentially expressed genes in tumor as compared to nontumor tissues will be used for hierarchical clustering. Genes will be chosen based on statistical p value (p001 or p01) and FDR cutoff (<5%). Kaplan-Meier survival analysis will be used to assess the difference in survival between the clusters employing a log-rank p value. The two clusters will be further analyzed for the difference in gene expression among tumors in these clusters to identify genes that are driving the aggressiveness. The gene set and its association with survival will be further tested using a Cox regression model. The gene signature and each clinical covariate will be first tested by univariate Cox regression analysis. Covariates that are found to be significant ($p<0.05$) will be further tested in a multivariate Cox regression analysis. Pathway analysis on subtype-specific genes will be performed using Ingenuity Pathway Analysis Tool to identify critical pathways associated with disease aggressiveness. Furthermore, we will examine if gene signatures are linked to the exposure to any known risk factors for example smoking, alcohol use, obesity, diabetes, etc. These information will be extracted from the questionnaire data (Appendix B). This novel approach provides a unique opportunity to evaluate

the relative contribution of environmental exposure, medical history and intrinsic tumor biology in the development of gene signatures associated with tumor aggressiveness.

7.3.2 Analysis of Metabolomic Profiles

The data from tumor and nontumor tissue metabolomics will be analyzed using "R" and BRB ArrayTools as described for gene expression analysis. All the comparisons between tumor and nontumor metabolites or metabolites among tumor samples will be performed using the statistical packages provided in BRB ArrayTools such as class comparison, class prediction and survival analysis. Metabolites differentially expressed between tumors vs. non-tumor will be plotted using S-plots. Hierarchical clustering will be performed using the list of metabolites that are differentially expressed in tumors as compared to nontumor tissues. Kaplan-Meier analysis will be used to assess the survival differences between major clusters employing a log rank p-value. The clusters with significant difference in survival ($p<0.05$) will be then analyzed to determine the difference in metabolites in tumors among these clusters. The association of the metabolite signature with survival will be further tested using a Cox regression model. The metabolite signature and each clinical covariate will be first tested by univariate Cox regression. Covariables that are significant ($p<0.05$) will be further tested in a multivariate Cox regression. All clinical variables will be tested for collinearity to determine if the final model meets the proportional hazards assumption. Pathway analysis on subtype-specific metabolites will be performed using Ingenuity Pathway Analysis Tool to identify critical pathways and surrogate genes associated with disease aggressiveness.

8 COLLABORATIVE AGREEMENTS

8.1 AGREEMENT TYPE

This study is conducted under a Resource Contract established with the University of Maryland Medical System (UMMS), Baltimore, MD.

8.2 MULTI-INSTITUTIONAL GUIDELINES

In this study, patients will be enrolled only at UMMS (the contract site). The CCR will serve as the Coordinating Center, and the following guidelines will apply. IRB approval will be obtained at the NCI first, followed by the University of Maryland IRB. The NCI IRB will be provided a copy of the contract site's IRB approval before subjects are enrolled.

8.2.1 IRB Approvals

The CCR PI will provide the NCI IRB with a copy of the contract site's approved yearly continuing review. Registration will be halted at the contract site if a current continuing approval is not on file at the NCI IRB.

8.2.2 Amendments and Consents

The CCR PI will provide the NCI IRB with copies of all amendments, consents and approvals from the contract site.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Men and women age 18 to 89 years of age, of all races and ethnic groups, are eligible for this trial if they meet the criteria outlined in Section 2.1. Patients who are 90 years or older are excluded because of co-morbidity considerations. Efforts will be made to extend the accrual to a representative population. Vulnerable populations, including cognitively impaired individuals, institutionalized individuals, and the seriously ill, are not eligible for the study.

9.2 PARTICIPATION OF CHILDREN

Individuals younger than 18 years old are excluded from this study because very few patients with PDAC are younger than 18 years of age. Inclusion of a rare younger patient will not provide adequate generalizable information to justify their inclusion in this study.

9.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients will not receive any treatment as part of this study. Patient care is not modified by the protocol. Patients will receive their usual care and are asked to donate tumor specimens that are surgically removed as part of their routine treatment. The collection of blood is the only medical procedure that is performed for this protocol. Potential risks include bruising, infection, and minor pain or discomfort associated with vein puncture.

9.4 RISKS/BENEFITS ANALYSIS

This study involves minimal risk to subjects. Although there is no prospect of direct benefit from participation, the knowledge gained from this study may benefit future patients with pancreatic cancer.

9.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The consent process will be done by a nurse at UMMS. After a non-objection statement by the treating physician has been obtained, an interviewer will contact the patient to get both informed consent and authorization to obtain, use and disclose protected health information for research. Severely ill patients in the intensive care unit will not be approached. If a patient is found to be unable to give informed consent, the consent procedure and the enrollment into the study will be stopped. The nurse will explain the purpose of the research study. The patient will be given the option that either he/she reads the form by himself/herself or that the nurse will read the form to him/her. The nurse will answer any questions. The consent form describes the purpose of the study, procedures, risks and potential discomforts, benefits, and the independence of the quality of medical care from the decision to participate in the study. The consent form also explains the confidentiality of the study, the right to withdraw from the study at anytime, and the protection of privacy as it relates to genetic testing. Consent for participation in the study has been obtained when the patient and the research study coordinator have both signed 2 identical consent forms. One form will be given to the patient, and the original signed consent goes to Medical Records; a copy is placed in research record.

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11 APPENDICES

11.1 APPENDIX A: ELIGIBILITY CHECKLIST

NAME_____

DATE_____

DATE OF BIRTH_____

ID# _____

Yes	No	Eligibility Criteria (All responses must be yes for patient to be eligible.)
		Has been diagnosed with pancreatic cancer
		Physician diagnosis based on imaging
		Age ≥18 years and less than 90 years
		A non-objection statement by the physician from the hospital where the patient was identified, or listed as the treating physician by the tumor registry or surgical pathology report, to contact the patient
		Is able to give informed consent
		Is physically and mentally capable of participating in the study interview
		Must understand English well enough to be interviewed
Yes	**No**	**Exclusion Criteria (All responses must be no for patient to be eligible.)**
		Is currently residing in an institution, such as a prison, nursing home, or shelter
		Is a severely ill patient in the intensive care unit

Is subject eligible? _____Yes _____No

For eligible subjects, did subject provide informed consent and sign form?

_____Yes _____No: Unwilling _____No: Unavailable

11.2 APPENDIX B: QUESTIONNAIRE

--Questionnaire Page 1-- **I.D. # __ - __ - __ __ __ __ __ __**

S. Perwez Hussain
National Cancer Institute
Building 37, Room 3044B
Bethesda, Maryland 20892
Phone (301) 496-2048, Fax (301) 496-0497

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--Questionnaire Page 2-- **I.D. # __ - __ - __ __ __ __ __ __**

IDENTIFIER SHEET

1. Interviewer's name: _____ 2. Interviewer's ID __ __

3. Hospital: _____

4. Date of interview: __ __ / __ __ / __ __ __ __

5. Start time: __ __:__ __ am/pm

6. Name _____ / _____ / _____
First Middle Last

7. Date of birth __ __ / __ __ / __ __ __ __

8. Gender: ( ) Male ( ) Female

9. Address

_____
Street Apt. No.

_____ ___ ___ ___ ___ ___ ___ ___ - ___ ___ ___ ___
City State Zip Code

10. Telephone number Home :(__ __ __) __ __ __ - __ __ __ __

Work: (__ __ __) __ __ __ - __ __ __ __ Ext. __ __ __ __

--Questionnaire Page 3-- **I.D. # __ - __ - __ __ __ __ __ __**

DEMOGRAPHIC INFORMATION

Now I would like to ask you some general information about you.

1. Do you consider yourself to be:
   - ( )$_1$ White/Caucasian
   - ( )$_2$ Black/African American
   - ( )$_3$ Asian
   - ( )$_4$ Native Hawaiian/Other Pacific Islander
   - ( )$_5$ American Indian/Alaska Native

2. Do you consider yourself Hispanic/Latino or Non Hispanic/Latino?
   ( )$_1$ Hispanic/Latino ( )$_2$ Non Hispanic/Latino

3. Most people in the United States have ancestors who came from other parts of the world. Please tell me what country or countries your ancestors came from.

   _____

4. What is your age? ____ ____

5. How many cigarettes have you smoked in the last 48 hours? ___ ___ ___

--Questionnaire Page 4-- **I.D. # __ - __ - __ __ __ __ __ __**

MEDICAL HISTORY

Now I would like to ask you some questions about your medical history and your health.

1. Have you ever been diagnosed with cancer (prior to your current diagnosis)?
( )$_0$ No **(Skip to 3)** ( )$_1$ Yes

2. What type of cancer(s)? _____ (cancer organ dictionary, add rows as needed)

3. What is your current weight? ___ ___ ___ lbs

4. What was your weight 10 years ago? ___ ___ ___ lbs

5. What was your weight 2 years ago? ___ ___ ___ lbs

6. How tall are you? _____feet ___ ___ inches

7. Did any doctor ever tell you that you have diabetes (too high or too low sugar level)?
( )$_0$ No **(Skip to 13)** ( )$_1$ Yes ( )$_8$ Don't know **(Skip to 13)**

8. What age was your diabetes diagnosed? ___ ___

9. Do you need any insulin for diabetes?
( )$_0$ No **(Skip to 11)** ( )$_1$ Yes ( )$_8$ Don't know **(Skip to 11)**

10. At what age did you start taking insulin? ___ ___

11. Are you now taking pills to lower your blood sugar? These are sometimes called oral agents or oral hypoglycemic agents.
( )$_0$ No **(Skip to 13)** ( )$_1$ Yes ( )$_8$ Don't know **(Skip to 13)**

12. At what age did you begin to take oral hypoglycemic agents? __ __

13. Do you take Vitamin D?
( )$_0$ No **(Skip to 18)** ( )$_1$ Yes ( )$_8$ Don't know **(Skip to 18)**

14. Which Brand? _____ ( )$_8$ Don't know

15. How many tablets per day? ___ ___ ( )$_8$ Don't know

16. How many milligrams per tablet? ___ ___ ___ ( )$_8$ Don't know

MEDICAL HISTORY: ( )$_1$ Very good ( )$_2$ Good ( )$_3$ Fair ( )$_4$ Poor

--Questionnaire Page 5-- **I.D. # __ - __ - __ __ __ __ __ __**

17. How long you have been taking it? ___ ___ ___ ( )$_1$ weeks
( )$_2$ months
( )$_3$ years
( )$_8$ Don't know

18. Do you take any Multi-vitamin?
( )$_0$ No **(Skip to 23)** ( )$_1$ Yes ( )$_8$ Don't know **(Skip to 23)**

19. Which Brand? _____ ( )$_8$ Don't know

20. How many tablets per day? ___ ___ ( )$_8$ Don't know

21. How many milligrams per tablet? ___ ___ ___ ( )$_8$ Don't know

22. How long you have been taking it? ___ ___ ___ ( )$_1$ weeks
( )$_2$ months
( )$_3$ years
( )$_8$ Don't know

23. Did your doctor ever tell you that you have pancreatitis?
( )$_0$ No **(Skip to next section)**
( )$_1$ Yes
( )$_8$ Don't know **(Skip to next section)**

24. Did the doctor tell you it was chronic or acute?
( )$_1$ Chronic ( )$_2$ Acute ( )$_8$ Don't know

25. What age were you first diagnosed with pancreatitis? ___ ___

26. Do you take any medication for pancreatitis?
( )$_0$ No **(Skip to next section)**
( )$_1$ Yes
( )$_8$ Don't know **(Skip to next section)**

27. What age did you start taking the medication for pancreatitis? ___ ___

28. Has anyone in your family, related to you by blood, ever been diagnosed with pancreatitis?
( )$_0$ No **(Skip to next section)**
( )$_1$ Yes
( )$_8$ Don't know **(Skip to next section)**

FAMILY HISTORY: GENERAL ( )1 Very good ( )2 Good ( )3 Fair ( )4 Poor

--Questionnaire Page 6-- **I.D. # __ - __ - __ __ __ __ __ __**

FAMILY HISTORY: GENERAL

Now, I would like to learn about the members of your family.

1. Has anyone in your family that is related to you by blood, ever been told they have cancer, include children, parents, grandparents, brothers, sisters?

( )0 No **(Skip to next section)** ( )1 Yes

2. Which relative?

3. Where did the cancer start?

TOBACCO HISTORY: GENERAL

Next, I would like to ask you some questions about any smoking history you may have.

1. Have you ever smoked more than 100 cigarettes, which is equivalent to five packs, in your life? ( )0 No **(Skip to next section)** ( )1 Yes

2. *Please tell me about your smoking history. I will be asking you questions about any times you may have stopped or changed your patterns.*

Period	1	2
a. In what year did you start smoking cigarettes or change your patterns?	__ __ __ __	__ __ __ __
b. What was the average number of cigarettes or packs per day you smoked during this time?	__ __ __ __ ( )1cigarettes ( )2 packs	__ __ __ __ ( )1cigarettes ( )2 packs
c. After starting, did you change your patterns or stop smoking for more than 6 months?	( )0 No **(Skip to 3)** ( )1 Stopped smoking ( )2 changed pattern	( )0 No **(Skip to 3)** ( )1 Stopped smoking ( )2 changed pattern

TOBACCO HISTORY: GENERAL ( )$_1$ Very good ( )$_2$ Good ( )$_3$ Fair ( )$_4$ Poor

d. In what year did you stop smoking or change your patterns for more than six months?	— — — — If this is a change of pattern, skip to 2a	— — — — If this is a change of pattern, skip to 2a
e. Did you start smoking again?	( )$_0$ No **(Skip to 3)** ( )$_1$ Yes **(Skip to 2a)**	( )$_0$ No **(Skip to 3)** ( )$_1$ Yes **(Skip to 2a)**

If R stopped smoking more than 6 months ago, Skip to next section

3. Have you increased or decreased your amount of cigarette smoking in the last 6 months?

( )$_0$ No **(Skip to next section)** ( )$_1$ Yes

	Period	1	2	3
4.	How long ago did you change your level of smoking?	__ __ ( )$_1$ weeks ( )$_2$ months	__ __ ( )$_1$ weeks ( )$_2$ months	__ __ ( )$_1$ weeks ( )$_2$ months
5a.	Since then, what is the average amount of cigarettes you smoked per day?	__ __ ( )$_1$ cigarettes ( )$_2$ packs	__ __ ( )$_1$ cigarettes ( )$_2$ packs	__ __ ( )$_1$ cigarettes ( )$_2$ packs
5b.	Did you change your level of smoking again?	( )$_0$ No ( )$_1$ Yes **(Skip to 4)**	( )$_0$ No ( )$_1$ Yes **(Skip to 4)**	( )$_0$ No ( )$_1$ Yes **(Skip to 4)**

--Questionnaire Page 8-- **I.D. # __ - __ - __ __ __ __ __ __**

ALCOHOL HISTORY

Now, I would like to ask you some questions about any alcoholic beverages you may drink on a regular basis.

1. In your entire life, have you ever consumed more than 12 alcoholic beverages per year, such as beer, wine, wine coolers or liquor? ( )$_0$ No **(Skip to 3)**
   ( )$_1$ Yes

2. Tell me about the types of alcohol and when you were drinking them.

Period	1	2	3
a. At what age did you first start to drink/when you next began to drink?	___ ___	___ ___	___ ___
b. How many cans, bottles or 12 oz of beer did/do you drink?	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.
c. How many 4 oz glasses of wine did/do you drink?	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.
d. How many 1 ½ oz. shots of liquor, by itself or in a drink did/do you drink?	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.	___ ___ ( )$_1$ Per day ( )$_2$ Per wk. ( )$_3$ Per mo. ( )$_4$ Per yr.
e. Have you ever stopped drinking or changed your patterns for more than 12 months?	( )$_0$ No **(Skip to 3)** ( )$_1$ Stopped ( )$_2$ Changed pattern	( )$_0$ No **(Skip to 3)** ( )$_1$ Stopped ( )$_2$ Changed pattern	( )$_0$ No **(Skip to 3)** ( )$_1$ Stopped ( )$_2$ Changed pattern
f. What age did you stop drinking or change your patterns for more than 12 months?	___ ___	___ ___	___ ___

ALCOHOL HISTORY ( )$_1$ Very good ( )$_2$ Good ( )$_3$ Fair ( )$_4$ Poor

--Questionnaire Page 9-- **I.D. # __ - __ - __ __ __ __ __ __**

3. Have you had any alcoholic beverages such as beer, wine or liquor in the last 7 days?

( )$_0$ No **(Skip to next section)** ( )$_1$ Yes

4. In the last seven days, how much did you drink of the following?:	Number:
Cans, bottles or 12 oz. glass of beer	__ __ __
4 oz. glasses of wine	__ __ __
1 ½ oz. shots of hard liquor or drinks containing a shot of hard liquor	__ __ __

--Questionnaire Page 10-- **I.D. # __ - __ - __ __ __ __ __ __**

GENERAL INFORMATION

1. Are you having any surgery in the near future?
( )$_0$ No (**Skip to 4**) ( )$_1$ Yes

2. What kind of surgery are you having? _____

3. When are you having this surgery? ___ ___ / ___ ___ /___ ___ ___ ___

4. May we contact you again later if we need to clarify any of the information you have provided? ( )$_0$ No ( )$_1$ Yes

5. Time ended: __ __ : __ ___ ( )$_1$ AM ( )$_2$ PM

6. Interviewer's Signature: _____

INTERVIEWER REMARKS

1. Interview was conducted:
( )$_1$ Home
( )$_2$ Hospital - inpatient
( )$_3$ Hospital - outpatient
( )$_4$ One of the Study Offices
( )$_5$ Other

2. Respondent's cooperation was:
( )$_1$ Very good ( )$_2$ Good ( )$_3$ Fair ( )$_4$ Poor

3. The overall quality of the interview was:
( )$_1$ Very good ( )$_2$ Good ( )$_3$ Fair ( )$_4$ Poor

4. Did any of the following occur during the interview?

a. R did not know enough information regarding the topics	( )$_0$ No ( )$_1$ Yes
b. R did not want to be more specific	( )$_0$ No ( )$_1$ Yes
c. R did not understand or speak English well	( )$_0$ No ( )$_1$ Yes
d. R was upset or depressed	( )$_0$ No ( )$_1$ Yes
e. R had poor hearing or speech	( )$_0$ No ( )$_1$ Yes
f. R was confused by frequent interruptions	( )$_0$ No ( )$_1$ Yes
g. R was emotionally unstable	( )$_0$ No ( )$_1$ Yes
h. Others helped with the answers	( )$_0$ No ( )$_1$ Yes
i. R required a lot of probing	( )$_0$ No ( )$_1$ Yes
j. Patient was reserved	( )$_0$ No ( )$_1$ Yes
k. R was physically ill	( )$_0$ No ( )$_1$ Yes
l. Other, specify _____	( )$_0$ No ( )$_1$ Yes

--Questionnaire Page 11-- **I.D. # __ - __ - __ __ __ __ __ __**

5. Comments/Remarks:
