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Title: A Prospective Study of Outcome in Patients with Pancreatic Cancer with BRCA Mutations.

A. Study Purpose and Rationale

In the United States, pancreatic cancer is the fourth leading cause of death from malignancy in both men and women[1]. Approximately 33,730 patients will be diagnosed with pancreatic adenocarcinoma and 32,300 will die from the disease in 2006. Nationwide, two year survival for pancreatic cancer ranges from 25-30%. This nearly equal incidence and mortality highlights the almost uniform mortality from the disease. Surgical intervention is the only curative treatment for the disease at this time, and many patients present when they are no longer operative candidates. National two year survival for pancreatic cancer ranges from 25-30% and five year survival is 4-5%. While several chemotherapeutic approaches demonstrate increased survival, cure at this point in time can only be demonstrated with lesions <1 cm at the time of resection. Given its rapid progression and late diagnosis, early detection and the development of more effective treatment modalities is essential to affect the impact of this disease on society.

It is estimated that between 4-16% of pancreatic cancers have a hereditary component [2]. In high-risk families with more than two 1st degree relatives with pancreatic cancer, a 2.8 to 18-fold increase in the risk of pancreatic cancer has been previously demonstrated. Several genes and cancer syndromes have been identified which are found in greater frequency in pancreatic cancer. Amongst these are hereditary non-polyposis colorectal cancer (HNPCC), familial atypical multiple mole melanoma (FAMMM) syndrome[3], Peutz-Jeghers syndrome [4], hereditary pancreatitis[5] and BRCA2 mutations. In addition, pancreatic cancer occurs at an increased rate in some families without one of the above cancer syndromes, suggesting that other genetic abnormalities may also be involved in the inherited predisposition to pancreas cancer.

Several studies have shown that germ line BRCA mutations are associated with an increased risk of malignancies, including breast, ovarian and more recently pancreatic cancer. Van Asperen et al examined 139 families with known BRCA mutations and found a relative risk increase for pancreatic cancer of 5.9 (CI 3.2-10)[6]. Other studies have demonstrated approximately 10-fold increase lifetime relative risk of pancreatic cancer in individuals with germ line BRCA2 mutations[2]. A Swedish population based study examined 944,723 families and demonstrated that families with two early onset breast cancers had an increased incidence of early onset ovarian, prostate and pancreatic cancer, suggesting a relationship with BRCA[7]. The BRCA mutations have a higher allelic frequency in those of Ashkenazi Jewish descent, with a combined frequency of approximately 2%[8]. To date, there are no large studies are available which specifically look at BRCA mutations these patients with pancreatic cancer.

Once this increased risk of pancreatic cancer was demonstrated in those harboring BRCA2 mutations, several groups studied both pancreatic cancer tissue and cell lines to find BRCA2 mutations. In one study of 41 pancreatic adenocarcinomas (30 pancreatic

adenocarcinoma xenografts and 11 cell lines), 15 (27%) had allelic loss at the BRCA2 locus while 4 (9.8%) had abnormalities at the second allele [9]. Another study of 26 European families with at least two 1st degree relatives with pancreatic ductal adenocarcinoma revealed that five of these high risk families (19%) had mutations in BRCA2 [10]. Interestingly, none of these families had familial ovarian or breast cancer. Other studies showed less frequent BRCA mutations [11]. Another study of families with 3 or more family members with pancreatic cancer (2 or more first-degree relatives), BRCA2 mutations were identified in 5 of 29 families (17.2%) [12]. They also found one additional BRCA2 point mutation not previously reported.

Screening may reduce mortality from malignancy if it results in earlier treatment, resection of malignant lesions, or resection of pre-malignant tissue. To date, no pancreatic cancer screening method is economically feasible with a risk-benefit ratio to justify screening at a large population level. However, if increased risk of pancreatic cancer may be demonstrated in certain groups, such as those harboring the BRCA mutations, perhaps screening will lead to earlier detection and referral to resection at a curable stage of disease. Only 15-20% of patients present with a resectable lesion at the time of diagnosis[13]. Pancreatic cancer staging follows the tumor, node, metastasis staging system, and can only accurately be assessed during surgery. Pancreatic cancer is usually considered resectable if it is stage T1-T3 and has not extended beyond the pancreas. In addition, other models have been proposed to further characterize tumor progression. Pancreatic intraepithelial neoplasia (PanIN) lesions are graded from 1-3[14]. PanIN-1 lesions are hyperplastic but without dysplasia, PanIN-2 lesions involve dysplasia, and PanIN-3 lesions are analogous to carcinoma in situ. Pan-IN stage may further characterize tumor pathology and outcome. Current beliefs have k-ras mutations occurring early in tumorogenesis, while loss p16 occurs at an intermediate stage. DPC and p53 mutations are relatively late occurrences.

Outcomes of patients harboring BRCA mutations may be improved when compared to those with non-hereditary cancers, as seen breast cancer with BRCA[15], ovarian cancer with BRCA[16] and colon cancer with HNPCC[17]. Furthermore, pathology in these hereditary cancer syndromes generally appears to more aggressive despite equal or even improved survival. BRCA associated breast cancers are more likely to present as histologic grade III and be estrogen receptor-negative. These clinical and histopathologic features are more consistent with a more aggressive tumor. However, survival in these inherited syndromes appears no worse than those without BRCA mutations[15, 18].

BRCA genes normally function in the cell to repair double-strand DNA breaks. Women with BRCA-related ovarian cancer have a better response to cisplatin-based chemotherapeutic regimens[16], which cross-link DNA. These women also have a better overall prognosis when compared with those with sporadic disease, independent of stage of diagnosis. Preliminary data suggests that pancreatic cancer cell lines harboring a BRCA2 mutation respond favorably to DNA alkylating agents when compared to non-BRCA cell lines (unpublished data). Thus further suggests that better characterization of patients harboring BRCA mutations is necessary to improve treatment modalities.

The specific aim of this protocol is a prospective analysis of pancreatic cancer patients to evaluate for differences in survival for those with BRCA mutations. Patients at high-risk for pancreatic cancer as well as those with pancreatic cancer who are of Ashkenazi Jewish descent will be tested for BRCA mutations. Two different populations will be recruited: 1) patients with operable disease will be referred from the surgeons and 2) patients who are not operative candidates will be recruited during ongoing chemotherapy treatment. Many of these patients will already be enrolled another high-risk pancreatic cancer registry here at Columbia University Medical Center. We will follow these patients from time of diagnosis. Primary endpoints will include: death from any cause, disease-free survival, and stage of disease at time of presentation (Pan-In Stage 1-3 and tumor stage). Secondary outcomes will include differences in tissue pathology, risk factors, treatment decisions and development of another malignancy. Additional goals include storing tumor and blood/serum specimens for future genetic and molecular testing.

B. Study Design and Statistical Analysis

Following enrollment, patients will be assigned to either BRCA positive or BRCA negative groups based on genetic testing in a research laboratory. They will be followed prospectively for primary and secondary outcomes discussed above. It is estimated that 200 patients may be referred to this study annually from surgeons, and that 150 of those patients will be eligible for enrollment. Similar numbers of patients will be referred through oncologists and other health-care providers. Based on published data, we estimate that 20% or more of these high-risk patients will be BRCA positive. National two-year survival is for all patients with pancreatic cancer is approximately 25%. We expect to see two-year survival improve to 40% with positive BRCA status. Based on standard alpha and beta errors of .05 and .8 respectively, Chi-squared test of proportions with uneven groups the study would need to accrue 476 BRCA negative patients and 95 BRCA positive patients to demonstrate an effect. It will take under two years to enroll these patients and another two years to follow for our effect.

C. Study Procedures

Once recruited, patients will be asked to fill out a questionnaire (see below). This questionnaire will be extensive during initial evaluation, and will include relevant follow-up data as outlined below. Patients will also provide several tubes of peripheral blood specimens at time of study entry, and following intervention (i.e. surgical resection or chemotherapy). In addition, archived fixed tissue samples will be obtained if tissue had been previously stored. If further surgical intervention is deemed clinically necessary, excess tissue not required for clinical diagnosis or treatment will be collected and appropriately stored for future analysis. No additional tissue will be removed if not clinically indicated. Pathology reports will also be collected. For our research purposes, all patients will be tested for BRCA mutations in a research laboratory. Test results from research laboratories which are not CLIA certified may not be disclosed to patients. If patients wish to know their BRCA status, they will meet with a genetic counselor and discuss the potential risks and benefits to testing for BRCA. They will be offered the

opportunity to be tested commercially through Myriad Laboratories. Following collection of these data, all subjects will be followed for overall survival and disease-free survival. Follow-up questionnaire data will be collected every 3 months or earlier if indicated. Study duration is expected to be four years. However, participants will participate from time of consent through the rest of their life through the high-risk pancreatic registry.

D. Study Drugs

No drugs are to be studied.

E. Study Devices

No devices are to be studied.

F. Study Questionnaires

Questionnaires will be distributed to all study subjects. Data collected will include: medical history, family history of pancreatic and other malignancies, clinical data regarding pancreatic cancer, epidemiologic information. In addition, social and demographic data will be obtained, as well as information regarding follow-up (survival, disease free survival, treatment options, etc.), tumor pathology, frozen specimens, and plasma/blood sample data.

G. Study Subjects

Study subjects will be pancreatic cancer patients at Columbia University Medical Center who are either of Ashkenazi Jewish descent or deemed at high-risk for an inherited syndrome of pancreatic cancer. Unfortunately, genetic testing of all individuals with pancreatic cancer is not economically feasible at this time. To further elucidate the role of BRCA mutations in pancreatic cancer, populations need to be enriched for BRCA mutations, as in those of Ashkenazi descent[8] and those at high risk for pancreatic cancer. Many participants in this study will simultaneously be recruited to the Pancreatic Cancer Registry for High Risk Individuals (IRB-AAAA6154). Pancreatic cancer has a slightly higher RR for men (1.3). We do not expect this to prohibit recruitment of an equal number of men and women. The only exclusion criteria will be the inability to provide informed consent.

H. Recruitment of Subjects

Study participants will be recruited primarily through physician practices at Columbia University Medical Center. Specifically, they will be referred from oncologists, surgeons, geneticists, inpatient consultations and primary care physicians. Patients may also self-refer. Their primary care physician must agree that the patient is appropriate for study participation. Those referring patients to this protocol will first ascertain that the patient is willing to discuss the study with the research team prior to being approached by

investigators. Participants in this study will simultaneously be recruited to the Pancreatic Cancer Registry for High Risk Individuals (IRB-AAAA6154).

I. Confidentiality of Study Data

Blood and tissue specimens, study questionnaires, and other data will be coded and assigned a unique identifying number. Some data may overlap with the Pancreatic Cancer Registry for High Risk Individuals, but duplicate data will be assigned a number which is unique to this protocol. Information which would identify the patient, such as name, social security number, medical record number and other personal identifiers, will not be included in stored data. Data will be stored password-locked computers, and paper data will be stored in the Principal Investigators locked office. Blood and tissue specimens will also be de-identified and stored in locked freezers.

J. Potential Conflict of Interest

No conflicts of interest are identified.

K. Location of Study

Study location will be clinical care areas at Columbia University Medical Center.

L. Potential Risks

There are no additional risks to participating in this study. Invasive procedures will only be performed if clinical indicated, and no additional tissue will be removed for study purposes. Psychological stress may be a factor in those who decide to undergo commercial testing for BRCA mutations and a genetic counselor will be available at all stages of diagnosis and treatment to discuss the implications of testing, both to the individual and to their families. Blood samples will be stored for future analysis. Risks involved in blood draws are minimal and blood draws will be performed in conjunction with regularly scheduled blood draws as clinically indicated.

M. Potential Benefits

No additional benefits will be conferred to patients in this study. Testing for BRCA status through a CLIA certified laboratory (Myriad Laboratories) if patients wish. As the full extent of BRCA involvement in pancreatic cancer if unknown, this is unlikely to confer benefit to the patient. Patients will be offered treatment compatible with the standard of care, which includes (but is not limited to): clinical evaluation by experts in this field, access to genetic counseling and testing if indicated (beyond BRCA testing), and timely diagnosis and treatment. Further characterization of the role BRCA mutations play in pancreatic cancer may have societal implications, including earlier diagnosis and modifications of treatment for those with pancreatic cancer.

N. Alternative Therapies

This is not a therapeutic study, and no alternative therapies apply.

O. Compensation of Subjects

No compensation will be provided to study subjects.

P. Cost to Subjects

No cost will be incurred by study participants. BRCA testing will be performed in a non-CLIA certified laboratory at Columbia University Medical Center. If study participants wish to know the results of their BRCA testing, they can apply to Myriad Laboratories to conduct the testing. Myriad then discusses payment for testing with the patient's insurance company.

Q. Minors as Research Subjects

No minors will be recruited for this study. If minors are referred to participate in this study, they will be deferred until they are of age to consent.

R. Radiation or Radioactive Substances

Radiation and radioactive substances will not be used in this study. The only possible use of these materials would be in analyzing laboratory data; patients would not be exposed to radiation.

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