University of Pennsylvania

Annual Progress Report: 2006 Formula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Formula Grant Overview

The University of Pennsylvania received $8,472,940 in formula funds for the grant award period January 1, 2007 through December 31, 2010. Accomplishments for the reporting period are described below.

Research Infrastructure Project 1: Project Title and Purpose

Health Services Research Data Center (HSRDC) – Research Infrastructure - The purpose of this multi-year infrastructure project is to make administrative data available to researchers seeking to understand and improve the organization, delivery, financing, and management of health care, and the social forces that shape health. The HSRDC coordinates the acquisition, storage, and analysis of data from private and government sources (e.g., Medicare, Medicaid, and others) and facilitates its use by researchers to answer important questions in health policy, delivery of health care, and effects of health care and social forces on the health of individuals and populations.

Anticipated Duration of Project

1/1/2007 - 12/31/2010

Project Overview

The overall objective of this project is to develop and sustain a health services research data center that will support acquisition, secure storage, and analysis of data to address three key goals:

- Expand access to health care and understand and eliminate disparities in health and health care;
- Reduce medical errors, increase patient safety, and improve medical quality;
- Improve the health and health care decisions made by individuals, patients, clinicians, and policy makers.

The goal of the HSRDC is to house public and privately collected data in a secure environment, with appropriate statistical tools and technical support to facilitate use by single and multiple investigators. Data centers of this kind are essential for high quality work in health services and health policy research because equipment needs are large (storage is often required for files that are terabytes in size); storage must be secured and data encrypted; and complex regulatory and oversight protocols are necessary to simultaneously protect data from unauthorized use and yet

facilitate authorized use. Data centers are thus in many ways akin to vivaria or other core infrastructures that require the protection of animals and the facilitation of their use.

The center represents a partnership with existing hardware capacity available from the Biomedical Informatics Program, with occasional upgrades to hardware and software as necessary for specific projects, increased capacity needs, or technologic obsolescence.

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Expected Research Outcomes and Benefits

Outcomes: the success of this infrastructure project will be determined by the number of research projects utilizing the function, and their quantity, quality, and impact.

Benefits: currently, data that appear to be available to researchers are in practice hard to use. They require substantial hardware investments to store them. They require secure computing environments to protect them, particularly if they contain identifiable patient information. They are subject to intense and often highly variable regulatory rules and data use agreements that are complex to negotiate individually. Each of these issues represents a barrier to effective use of these data to answer important social and health policy questions. This project will lower the transaction costs of data use so that research can be accomplished with better facility.

The longer term benefits are that these data will be put to use in analyzing and ultimately improving the health and health care of the public, through the identification and evaluation of specific health policies and clinical programs and approaches. As described, these projects will emphasize Equity, Quality, and Choice.

Summary of Research Completed

<u>Health Services Research Data Center (HSRDC) Overview</u>
The HSRDC successfully coordinates the acquisition, secure storage, and analysis of data and facilitates its use by researchers. As a shared research resource, the HSRDC is currently utilized by numerous independent investigators at the University of Pennsylvania.

Since the last annual Health Research Formula Fund (HRFF) report, in July 2009, the HSRDC has expanded its capability to permit additional Penn faculty, staff, and students to use a unified, synergized analytic platform to perform state-of-the-art statistical analyses on highly sensitive federal, state, and private sector health care data. Grant support from the HRFF provides sustaining funding for the HSRDC, bridging the inevitable variation in grant funding that supports individual research projects. HRFF's support has also permitted mentored trainees to use the system in close collaboration with funded Penn faculty members, and has allowed unfunded Penn faculty members to perform pilot research in preparation for grant applications. The data center's research infrastructure has facilitated rapid responses to numerous requests for applications (including several related to 2009 ARRA stimulus funding opportunities) and it has encouraged numerous collaborations on increasingly interdisciplinary research that includes investigators from Wharton and the Schools of Medicine, Nursing, and Dentistry.

HSRDC Personnel Update

The Data Center Coordinator (Mollie Epstein, MA) ensures that the HSRDC system is accessible to researchers, functional, and compliant with federal regulations governing secure computer systems (i.e., the Federal Information Security Management Act). Ms. Epstein maintains a Microsoft Access database to record HSRDC file access permissions at the user, investigator team, and project level, as well as status and date of each user's cyber security training updates. The mandatory annual cyber security training recertification program has been implemented by the HSRDC for over a year, with all server users completing cyber security training prior to obtaining server access. Training must be updated and certification renewed on an annual basis. A webpage describing policies for server use was created and linked to Penn's Leonard Davis Institute website: http://www.upenn.edu/ldi/faq.html. Achievement of these security-related goals continues to be a high priority.

HSRDC Hardware and Software Update

All data are stored and analyses performed on a secure Dell PowerEdge Rack 900 server running the Red Hat Enterprise Linux 5 operating system. It is a new (2008), high-performance server with four 2.4 GHz E7330 Xeon quad-processors and with a 10 terabyte storage area network. Both SAS and STATA statistical software packages have been installed on the server, and are upgraded regularly as updates are released.

This year, a new Fujitsu Linux server (Eternus DX-80) with an attached 80 terabyte storage area network was purchased and installed to upgrade the data center's analytic capacity, and upgrades to both major statistical software packages (SAS 9.2 and STATA MP4 v.11) were installed on the server. These hardware and software improvements dramatically increased the capability of the shared computational environment.

HSRDC Users Update

A total of 52 researchers are active on the HSRDC server, an increase of 10 users, or 24%, since the last annual report. These include funded University faculty members and members of their respective project teams (primarily biostatisticians and programmer/analysts); unfunded faculty members performing pilot research in preparation for external grant proposals; and students in the Robert Wood Johnson Clinical Scholars and Master of Science in Health Policy Research Programs.

HSRDC Hosted Projects Update

Thirteen externally-funded research projects currently utilize the HSRDC server (seven new this year, six ongoing); four additional studies completed during the past year. Included below are brief descriptions of the seven new projects that have used the HSRDC server for data storage and statistical analysis during FY2010:

New Projects

• *Improving the Value of Pay-for-Performance.* An Evaluation in Hospitals. Led by Co-Investigators Rachel Werner, MD, PhD, and Dan Polsky, PhD, this new study for FY2010 is funded by the Agency for Healthcare Research and Quality (AHRQ). It will focus on the impact of P4P on the value of health care, examine which providers are the most likely to show value improvements under P4P, and test whether P4P reduces costs or improves the value of care for patients with acute myocardial infarction (AMI) within the setting of the Centers for Medicare and Medicaid Services' hospital P4P demonstration project.

• *The Role of Long-Term Acute-Care Hospitals in Chronic Critical Care Illness.* Led by Co-Investigators Jeremy Kahn, MD, MsC and David Asch, MD, MBA, and funded by the University (School of Medicine Pilot Research Funds), this pilot research project will investigate the role of long-term acute-care (LTAC) hospitals for patients with chronic critical illness and prolonged mechanical ventilation (PMV). The investigators aim to describe the incidence and outcomes of patients with PMV and the impact of LTAC utilization following critical illness on clinical and economic outcomes for these patients.

• *Evaluation Plan for the Commonwealth Fund's Demonstrations Project on Reducing Re-Hospitalizations.* This study is funded by a Commonwealth Fund grant to Penn State, from which Kevin Volpp, MD, PhD was awarded a subcontract. Dr. Volpp's research aims to provide technical assistance to hospitals and other stakeholders in order to reduce avoidable re-hospitalizations, and to create a robust learning community of medical providers so as to improve quality of care.

• *Diffusion of Percutaneous Breast Biopsy.* This is an AHRQ-funded observational study of Medicare beneficiaries diagnosed with breast cancer, led by Seema Sonnad, PhD. The research aims are to assess self-reported physician practice and understanding of percutaneous vs. open breast biopsies, and examine diffusion of and disparities in use of percutaneous breast biopsies.

• *Community and Provider Determinants of Breast Cancer Stage at Diagnosis, Treatment, & Mortality.* Katrina Armstrong, M.D., M.S.C.E and Keerthi Gogineni, MD aim to identify community- and provider-level characteristics associated with increased disparities in breast cancer outcomes that would potentially highlight critical shortcomings in the continuum of care. The ultimate goal of this research, funded by the American Society of Clinical Oncology (ASCO) Cancer Foundation, is to identify sources of disparity in breast cancer epidemiology and outcomes so interventions and policy recommendations can be developed to reduce the clinical burden of disease.

• *Do Nursing Home Report Cards Improve the Quality of Care for Persons with Dementia?* Investigator R. Tamara Konetzka is studying the effect of nursing home report cards on the quality of care for residents with AD and other dementia. The project, funded by the Illinois Department of Public Health, is examining the extent to which demented nursing home residents have shared in quality improvements from public reporting and whether quality improvements in reported measures have been made at the expense of improvement in unreported measures thought to be important for persons with dementia.

• *The Comparative Effectiveness of Cardiovascular devices and Medicare Cost Growth.* This project, funded by AHRQ and led by Peter Groeneveld, MD, MS will examine in detail how new technology increases health care costs, and it will investigate how cost growth due to new technology is influenced by conditions in local health care markets as well as Medicare's coverage policies.

All projects—new, ongoing from previous periods, and completed/closed during this reporting period—are listed in Table A.

Publications

Use of the HSRDC server for data storage and analysis resulted in 11 scientific publications during FY2009 (Table B).

Table A. FY 2009 Research Projects Using the HSR Data Center

Projects currently active on HSR Data Center		
Investigator(s)	Project	Funder
Katrina Armstrong, MD, MSCE and Tim Rebbeck, PhD	Segregation and Racial Disparities in Prostate Cancer	National Cancer Institute
Justin Bekelman, MD and Katrina Armstrong, MD, MSCE	Quality of Care in Urologic Cancers	University of Pennsylvania School of Medicine
Virginia Chang, MD, PhD	Influence of Obesity on Quality of Care	Robert Wood Johnson Foundation (RWJF)
Peter Groeneveld, MD, MS	Implications of Cardiovascular Technology Diffusion among Medicare Beneficiaries	National Heart, Lung, and Blood Institute (NIH)
Daniel Polsky, PhD	Market Competition and the Quality of Home Health Services	National Heart, Lung, and Blood Institute (NIH)
Kevin Volpp, MD, PhD	Impact of Treatment Profitability on Hospital Responses to Financial Stress	RWJF/ Health Care Financing and Organization
Kevin Volpp, MD, PhD	Reducing Re-Hospitalizations: A Proposal from the Institute for Healthcare Improvement	Commonwealth Fund (subcontract)
Rachel Werner, MD, PhD	The Impact of Public Reporting on the Quality of Post-Acute Care	Agency for Healthcare Research and Quality (AHRQ)

Projects completed during FY09		
Investigator(s)	Project	Funder
Peter Groeneveld, MD, MS	Impact of Advanced Medical Devices and Procedures on Cardiovascular Diseases	Institute for Health Technologies
Jack Iwashyna, MD, PhD	ICU Networks	American Thoracic Society (ATS)
Rachel Werner, MD, PhD	Evaluating Performance Measurement in Older Clinically Complex Patients	Robert Wood Johnson Foundation

Table B. Peer-Reviewed Publications of Scientific Work Conducted on the HSR Data Center, FY 2009

Hutchinson RN, Putt MA, Dean LT, Long JA, Montagnet CA, Armstrong K. *Neighborhood racial composition, social capital and black all-cause mortality in Philadelphia.* Soc Sci Med. (1982). 68(10): p. 1859-65, May 2009.
Paulson EC, Wirtalla C, Armstrong K, Kelz R, Mahmoud N, *National Cancer Institute designation predicts improved outcomes in colorectal cancer surgery.* Ann Surg. 248(4): p. 675-86, October 2008.
Paulson EC, Ra J, Armstrong K, Wirtalla C, Spitz F, Kelz RR. *Underutilization of esophagectomy as treatment for resectable esophageal cancer.* Arch Surg. 143: p. 1198-1203, 2008.
Pollack CE, Armstrong K. *The geographic accessibility of retail clinics for underserved populations.* Arch Intern Med. 169(10): p. 945-9, May 2009.
Polsky D, Jha AK, Lave J, Pauly MV, Cen L, Klusaritz H, Chen Z, Volpp KG. *Short- and long-term mortality after an acute illness for elderly whites and blacks.* Health Serv Res. 43(4): p. 1388-402, August 2008.
Putt M, Long JA, Montagnet C, Silber JH, Chang VW, Liao K, Schwartz JS, Pollack CE, Wong YN, Armstrong K. *Racial differences in the impact of comorbidities on survival among elderly men with prostate cancer*. Med Care Res Rev. Epub Apr 2009.
Rosen AK, Loveland SA, Romano PS, Itani KM, Silber JH, Even-Shoshan OO, Halenar MJ, Teng Y, Zhu J, Volpp KG. *Effects of resident duty hour reform on surgical and procedural patient safety indicators.* Med Care. 47(7): p. 723-31, July 2009.
Werner RM, Konetzka RT, Kruse GB. *Impact of public reporting on unreported quality of care.* Health Serv Res. 44(2 Pt 1): p. 379-98, April 2009.
Werner RM, Konetzka RT, Stuart EA, Norton EC, Polsky D, Park J. *Impact of public reporting on quality of postacute care.* Health Serv Res. Epub March 2009.

Wong YN, Freedland SJ, Egleston B, Hudes G, Schwartz JS, Armstrong K, *Role of androgen deprivation therapy for node-positive prostate cancer*. J Clin Onc. 27(1): p. 100-5, Jan 2009.

Wong YN, Freedland SJ, Egleston B, Vapiwala N, Uzzo R, Armstrong K. *The role of primary androgen deprivation therapy in localized prostate cancer*. Eur Urol. Epub Apr 2009.

Research Project 2: Project Title and Purpose

Role of Cancer Stem Cells in Tumor Progression and Response to Therapy - Growing evidence indicates that the majority of cells within human cancers have a limited ability to divide and that only a minority of cells – termed cancer stem cells – have the ability to proliferate extensively and form new tumors. If true, these findings would have critical implications for the manner in which we treat cancer, since the properties of cancer stem cells are predicted to differ substantially from the majority of cells present in tumors. The purpose of this project is to understand the biology and response to therapy of cancer stem cells in common epithelial malignancies, such as carcinomas of the breast, colon, lung and prostate. Since eradicating cancer stem cells may be required to cure cancer, such studies are of paramount importance.

Anticipated Duration of Project

7/1/2008 - 12/31/2010

Project Overview

An emerging hypothesis suggests that the frequent inability of traditional antineoplastic therapies to cure human cancers may be due to a failure of such therapies to eradicate the small populations of cancer stem cells present within tumors. Although cancer stem cells have been demonstrated to exist in some human leukemias, proof for their existence in most common solid tumors is currently lacking. The goal of this project is to use genetically engineered mouse models to understand the molecular and cellular biology of cancer stem cells in common solid tumors and to determine their response to traditional as well as targeted antineoplastic therapies. This project is composed of three specific aims:

Aim 1: Identify and characterize cancer stem cells in common epithelial malignancies.

Aim 2: Determine whether cancer stem cells contribute to aggressive tumor behavior.

Aim 3: Compare the impact of traditional therapies and therapies that target cancer stem cells on common epithelial malignancies.

Aim 1 will use transplantation of prospectively sorted cells from tumors arising in genetically engineered mice to determine whether cells enriched for tumorigenic potential – i.e. cancer stem cells – exist in common epithelial malignancies, such as those of the breast, lung, colon, and

prostate. Additional experiments will characterize the functional and molecular properties of these cells. Aim 2 will use mouse models for tumor progression to examine the contribution of cancer stem cells to the aggressive behavior of solid tumors, as manifested by metastasis and recurrence. Aim 3 will use genetically engineered mouse models to compare the antineoplastic activity of traditional therapies to therapies targeted against cancer stem cells. Therapies will be selected that target oncogenic signaling pathways known to play a role in stem cell self-renewal and maintenance, as well as pathways demonstrated by biochemical or genetic approaches to be important for cancer stem cell maintenance. Together, these studies should permit important inroads towards the goal of understanding the biology of cancer stem cells and their response to therapy and, in doing so, should facilitate the development of more effective therapies targeted against this critical subpopulation of tumor cells.

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None

Expected Research Outcomes and Benefits

Accumulating experimental evidence suggests that only a small proportion of cells within solid tumors can form new tumors following transplantation and that it is this small subpopulation of cells that is ultimately responsible for the aggressive behavior of cancers and their ability to kill patients. The remaining cells, which constitute the overwhelming majority of cells within tumors, are postulated in this model to represent non-tumorigenic daughter cells of cancer stem cells that do not lead to metastasis, recurrence, or death. If true, this theory would have major implications for the manner in which we treat human cancers, since the properties of cancer stem cells – including their sensitivity to anti-cancer agents – are predicted to differ substantially from the majority of cells present in breast cancers. Furthermore, since it is only by eradicating cancer stem cells – and not their non-tumorigenic daughter cells – that cancers may be cured, and since many cancer therapies are evaluated in clinical trials according to their ability to cause tumors to shrink in size, it is possible that the ability of antineoplastic therapies to cause tumor shrinkage may be irrelevant to their ability to cure patients. Since it is this small population of cancer stem cells that is believed to be responsible for the failure of traditional therapies to cure common human solid tumors, such as those of the breast, lung, colon, and prostate, understanding how to identify and kill these cells is of critical importance. The studies in this project should advance the goal of understanding the biology of cancer stem cells as well as their susceptibility to therapies used to treat human cancers. As such, the outcome of this research will promote the

development of more potent therapies targeted against this important subpopulation of tumor cells.

Summary of Research Completed

During the current reporting period, to achieve the goals of this grant of identifying and characterizing cancer stem cells in common epithelial malignancies we generated inducible MMTV-rtTA/TetO-H-*Ras* (MTB/TRAS) bitransgenic mice to determine whether cytokeratin-8 (CK8)/cytokeratin-14 (CK14) double-positive cells induced by overexpressing oncogenic H-Ras in the mouse mammary gland are also positive for other luminal and myoepithelial markers, as well as presumably progenitor cell markers.

We have previously observed that c-MYC-induced invasive mammary tumors harboring *Kras2* mutations fail to undergo complete regression when MYC is down-regulated. A plausible hypothesis to explain the recurrence of mammary adenocarcinomas in deinduced MTB/TOM mice would be the outgrowth of a small number of cancer stem cells harboring activating *ras* mutations that were either present in the tumor at the time of deinduction or that subsequently arose. Notably, we have now found that a subset of aberrantly proliferating cells in mammary glands overexpressing *HRas* are positive for both luminal epithelial and myoepithelial cell markers, e.g, CK8, CK14 (Figure 1). There are a number of examples in tissues in which cells expressing lineage markers from different lineages are in fact a type of stem or progenitor cell. Therefore, it is possible that c-MYC-induced mammary tumors contain a tumorigenic stem cell population that drives tumor growth and gives rise to both the tumorigenic stem cells as well as their nontumorigenic progeny. The former population may harbor activated ras mutation and be responsible for the failing of complete tumor regression under transgene deinduction as well as the tumor recurrence. Therefore, we reasoned that it would be important to test the stem-like properties of residiual cells in the mice with fully regressed tumors.

In order to directly test whether the activated Ras initiates cancer stem cells (CSCs) and the CSCs in residual disease of MTB/TOM mice are responsible for the recurrence of c-MYC-induced tumors, we generated MMTV-rtTA/TetO-H-*ras* (MTB/TRAS) inducible bitransgenic mice. We first determined whether CK8/CK14 double-positive cells are positive for other luminal epithelial cell markers (e.g., CK18) or myoepithelial cell markers (e.g., smooth muscle actin (SMA) or p63). Although technically difficult to co-stain for three different markers simultaneously (e.g. CK8 and CK14 with CK18, SMA, or p63) by immunofluorescence, our results clearly demonstrated that a population of cells exists in the mammary gland following Hras activation that are double-positive for CK18/CK14, CK8/SMA, and CK8/p63 (Figure 2).

It has been reported that p63+ and CK6+ cells are putative mammary progenitor markers. Typically, p63 is expressed in myoepithelial and CK6 in luminal epithelial layers in the normal mammary gland. Therefore, some cells expressing both CK6 and p63 may represent bipotent progenitors committed to generating cells of luminal epithelial and myoepithelial cell lineages. Cells expressing both markers most likely represent primitive progenitors. Notably, activated Ras induced dramatic expansion of CK6+ cells as well as some cells in the mammary gland positive for both CK6 and p63 markers (Figure 3). Taken together, these results would be

consistent with a model in which oncogenic Ras activation may promote mammary stem cell differentiation to primitive progenitors of lumnial and myoepithelial cells.

We have also attempted to test whether the above double-positive cells are positive for other putative stem/ progenitor cell markers, such as Abcg2, Sca1, and long-term retention of BrdU metabolic label (LRC). However, we have found that Abcg2 is not a reliable stem/progenitor marker in the mammary gland due to its widespread expression in differentiated mammary epithelial cells during lactation. We also have not found a clean Sca1 antibody to costain with epithelial (e.g., CK8, CK18) or myoepithelial (e.g., CK14, p63) markers. Therefore, we turned to reports that CD24+/CD29^{hi}/Lin- and CD24+/CD49f^{hi}/Lin- cells are enriched for mamamry gland stem cells, suggesting that these markers would represent superior candidates to test the effects of c-MYC activation on mammary gland stem cells.

We next used inducible MTB/TRAS bitransgenic mice to determine the candidate genes and biological pathways that are responsible for the generation of CK8/CK14 double-positive cells, given that these cells may be related to cancer stem cells that are responsible for the recurrence of MYC-induced tumors.

Mammary gland samples from MTB/TRAS mice uninduced or 96hrs following doxycycline-induced Ras activation were generated. Biotinylated cRNA was generated and hybridized to Affymetrix MG-U74Av2 oligonucleotide microarrays according to the manufacturer's instructions. The scanned raw chip data were analyzed using Affymetrix GeneChip5.0 (MAS5) with default normalization to a target signal of 150. The MAS5 signals were used in the downstream data analysis. After applying a computational deconvolution model to adjust the MAS5 signals, genes were sorted based on signal-to-noise ratio between the induced MTB/TRAS and uninduced mice (3 replicates/group). Gene Set Enrichment Analysis (GSEA) was performed on the sorted gene list. GSEA indicated that biological processes and cellular pathways related to inflammation, cell proliferation, angiogenesis, TGF-β, and NF-κB pathways were significantly associated with genes upregulated upon acute oncogenic Ras activation. Further biochemical and histological analysis confirmed our gene expression analysis, demonstrated by the abundant infiltration of macrophages, rapid activation of the TGF-β and NF-κB pathways, and significant increases of pro-inflammatory chemokines in induced mammary glands. Notably, unlike many in vitro studies in tumor cells showing that TGF-β pathway antagonizes Ras-initiated mammary epithelial cell transformation, we obtained evidence that TGF-β signaling was induced by the acute Ras activation to promote transformation of epithelial cells in vivo. Furthermore, the crosstalk of TGF-β and Ras signaling may also be responsible for transformation of the normal progenitor/stem cell to tumor initiation cells.

Finally, during the current study period, we sought to define the stem-like nature of cells in residual tumor cells in fully regressed tumor-bearing glands. In order to define the stem-like nature of cells that survive oncogene down-regulation in tumor-bearing bitransgenic mice, and that ultimately give rise to recurrent mammary tumors, we have attempted to identify reliable markers for cancer stem cells in MMTV-rtTA/TetO-MYC (MTB/TOM) tumors. As noted above, CD24+/CD29^{hi}/Lin- double-positive cells have been demonstrated to be enriched for mammary gland stem cells. We therefore tested if activation of c-MYC would influence this cell population. We found that acute activation c-MYC in mammary glands did not significantly

change the proportions of CD24+/CD29^{hi}/Lin- cell populations after 4 days and 2 weeks of c-MYC activation. In fact, the proportion of this cell population was slightly decreased in primary tumors induced by c-MYC overexpression (Figure 4). Given that oncogenic c-MYC induced mammary epithelial hyperplasia and thus expanded the epithelial cell population, our results would be consistent with a model in which activated c-MYC might maintain stem cells pool by promoting asymmetric proliferation, but would not expand this population of cells. Additional studies are planned using an automated high-content analysis system to more fully characterize the nature of this critical population of cells.

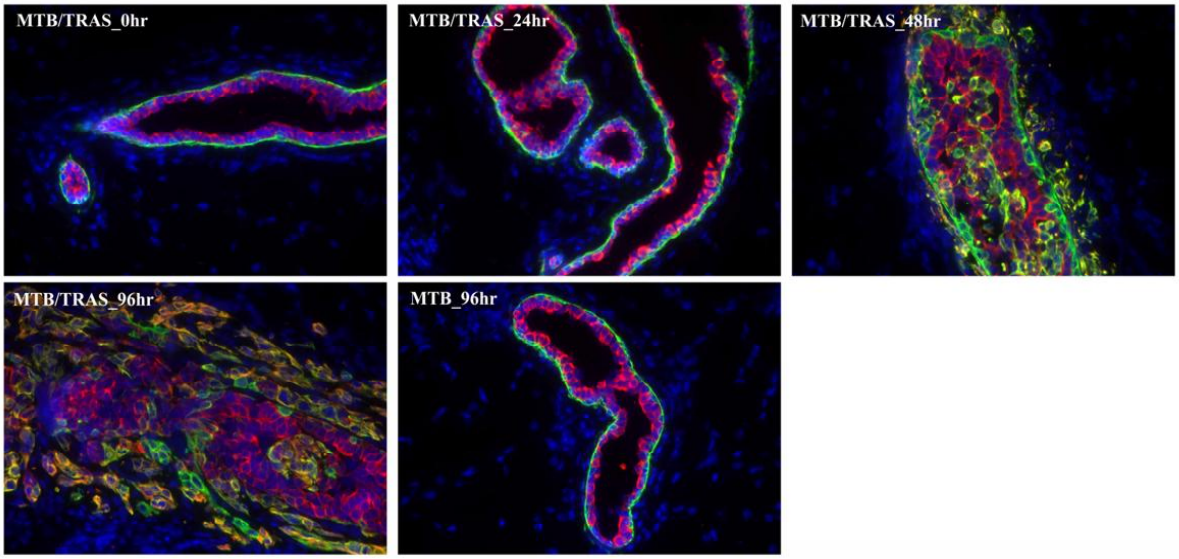

Figure 1: Induction of oncogenic Hras in the mammary gland results in the appearance and proliferation of a subset of epithelial cells that are double-positive for luminal epithelial cell markers (cytokeratin 8, red) and myoepithelial cell markers (cytokeratin 14, green). Nuclei were counterstained with Hoechst (blue). MMTV-rtTA/TetO-Hras (MTB/TRAS) bitransgenic mice were induced at 6 weeks of age with 2 mg/mL doxycycline and the number 4 mammary glands were harvested at the indicated timepoints for immunofluorescence analysis.

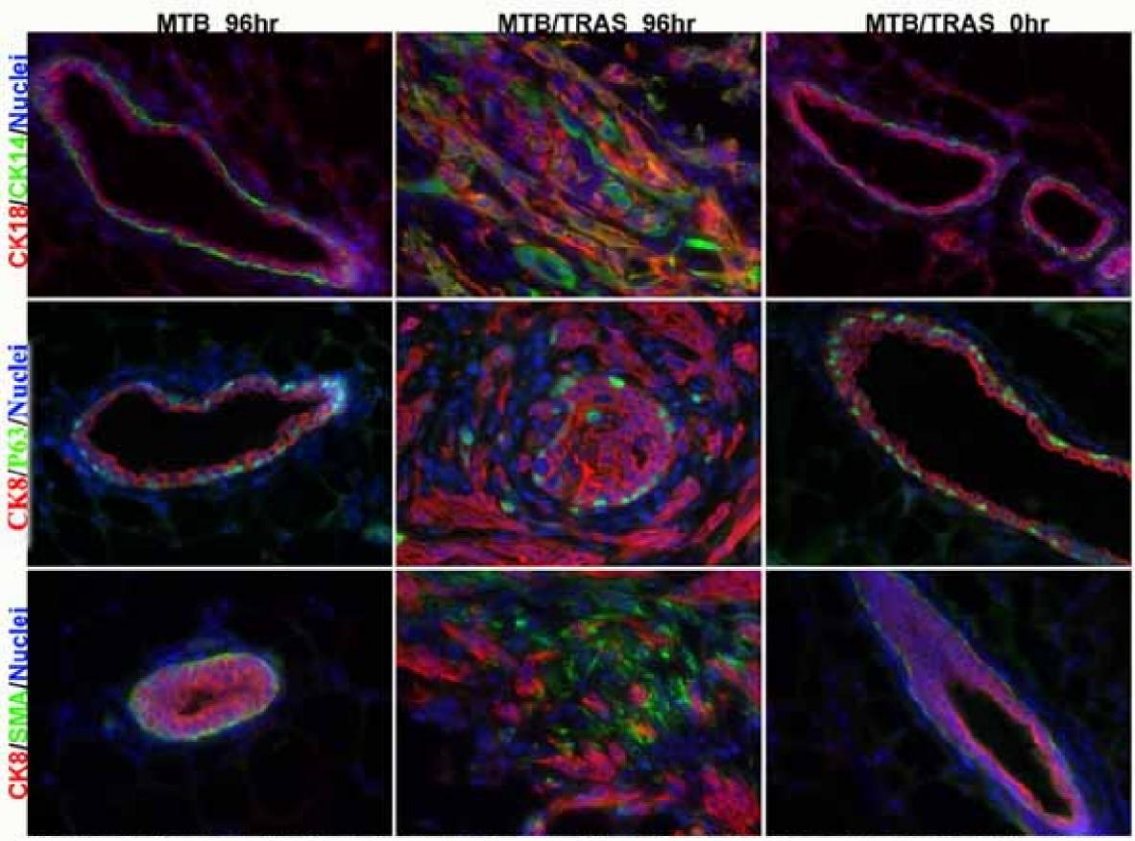

Figure 2: Some cells in the mammary glands of MTB/TRAS mice express both luminal and myeopithelial markers following the induction of oncogenic Hras. Glands were stained for CK18 (red) and CK14 (green), CK8 (red) and p63 (green), or CK8 (red) and smooth muscle actin (green). Nuclei were counterstained with Hoechst (blue). 400x.

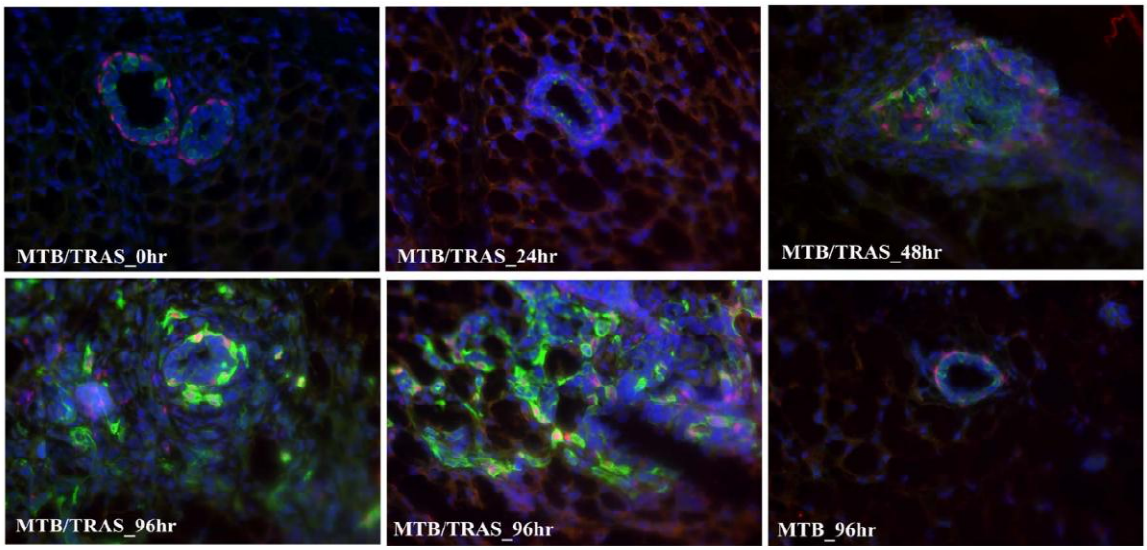

Figure 3: Hras activation expands the number of cytokeratin 6 (CK6)-positive cells and induces the appearance of some cells that co-express CK6 (green) and the myoepithelial marker p63 (red). Nuclei were counterstained with Hoechst (blue). 400x.

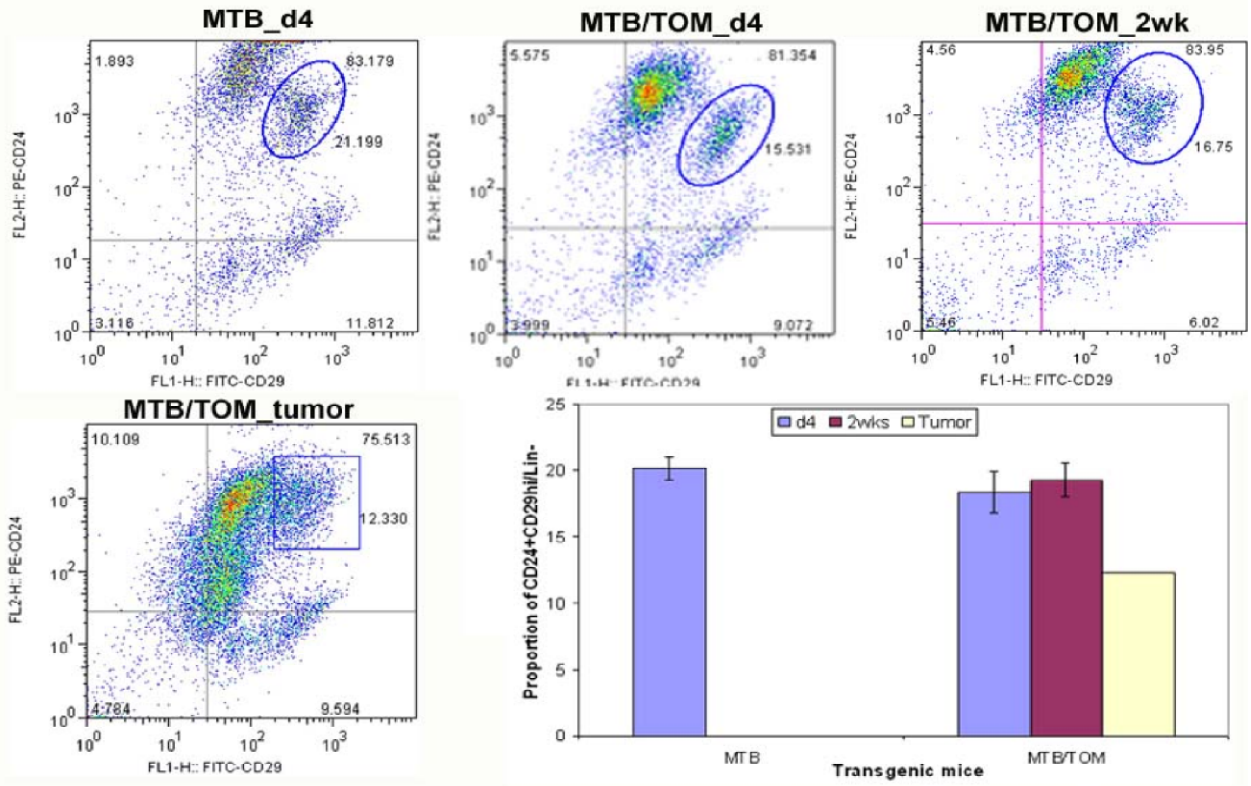

Figure 4: Overexpression of oncogenic c-MYC does not significantly alter the proportion of CD24+/CD29hi/Lin- cells in the mammary gland or in mammary tumors, suggesting that MYC may not induce the appearance of additional mammary stem cells or cancer stem cells.

Research Infrastructure Project 3: Project Title and Purpose

Tumor Tissue Bank for Cancer Research: Phase IV of a Research Infrastructure Project - The purpose of this project is to support the establishment and development of an infrastructure for operations of a Tumor Tissue Bank and Biospecimen Repository. The Tumor Tissue Bank and Biospecimen Repository will foster and stimulate transdisciplinary research into the etiology, diagnosis and treatment of human cancer. This infrastructure project is a continuation of the 2005-06 project.

Duration of Project

7/1/2008 - 12/31/2009

Project Overview

The objective of this research infrastructure project is to develop a state-of-the-art Tumor Tissue Bank and Biospecimen Repository that allows easy access to properly collected and maintained normal and malignant tissue, blood components and other types of biospecimens that are needed to conduct state of the art clinical and translational research now and, as new technologies are developed, well into the future.

More specifically, development will include the following aims:
A. Construction of Tumor Tissue Bank and Biospecimen Repository
B. Development of bioinformatics infrastructure
C. Establishment of a tissue culture room
D. Identification of any other space needs.

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Expected Research Outcomes and Benefits

The development of the Tumor Tissue Bank and Biospecimen Repository is expected to ultimately result in the identification of new diagnostics, prognostics, and treatments for cancer. Immediate benefits include:

- Improved ability for investigators to conduct innovative and competitive research due to the ease of access to normal and malignant tissue, and other biospecimens that are properly prepared and stored.
- Increased reliability of data collected due to adoption and adherence to best practices and guidelines for biospecimen banking standards.
- An increase in the number of samples available for research due to the ability to access results from previously performed tests and experiments so as not to duplicate efforts.
- Identification of factors giving rise to different tumor types involved in health disparities for different groups or populations.

Summary of Research Completed

A. Construction of tumor tissue bank and repository

Funds from the Tobacco grant were used to support the setup of freezers storage systems, computer systems to monitor freezer storage temperatures, computer systems to manage freezer inventory and labeling devices. In addition a histopathology service has been established for investigators who need either frozen tissue slides or paraffin tissue slides prepared from our tissue banked samples. As a result of these efforts, the Tumor Tissue and Biosample Core laboratory currently supports 18 research protocols across multiple areas of cancer. Biosample collected ion 2009 are shown in table 1. In aggregate since its inception, TTAB has collected nearly 20,000 samples (years 2007-current date).

Table 1: Biosamples by study area in 2009

Study group	Number samples
Multiple Myeloma	387
Non-group related (broad range of cancers)	2495
Gyn Oncology group	1204
Breast Cancer group	3
Renal cell carcinoma group	27
Viral tumor group	135
Warfarin Anticoagulation	899
Prostate Cancer group	162
Mesothelioma group	1035
Total biosample collected 2009	6,347

B. Development of bioinformatics infrastructure

The Tumor Tissue and Biosample laboratory has been using software developed by the NCI to manage its biosample inventory. This software, called caTissue is currently at version 1.2. This version of software was lacking in several features, most notably.

1. Ability to use a bulk upload feature – this key piece of functionality will be used import existing inventories data into caTissue for investigators that do not have a robust inventory management system. These other systems include excel spreadsheets, filemaker databases and accesss databases.
2. Ability to manage the movement of samples from inventory to analysis lab, processing of samples and return of samples with new data elements attached back into inventory. Currently, this has been a manual process which is frought with potential for errors. An electronic process to send and receive samples and new data elements needed to be created in caTissue
3. Molecular diagnosis laboratory frequently receives samples that are not managed as part of a TTAB's Biosample collection. The molecular laboratory needed a way to upload these into caTissue so that they could use caTissue to track this internal biosample inventory rather than rely on spreadsheets for their inventory management needs. A bulk upload tool needed to be created for this purpose

Funds from the tobacco project were used to customize caTissue to support the three missing features described above. Efforts included defining use cases, software testing, software training, software documentation and now ongoing software support. These new enhancements are now being incorporated into the next NCI release of caTissue, version 2.0 which is scheduled to be relased in October 2010.

C. Establishment of a tissue culture room and other space needs

A dedicated room was constructed for tissue culture in the TTAB space. This room houses a tissue culture hood and adjacent to this room are two CO2 incubators for use in tissue culture

experiments. Use of the tissue culture facility has been limited, despite Cancer Center surveys that indicated a need from investigators. This is likely due to other tissue culture facilities around campus.

The tissue culture room serves double duty, housing the tissue banks image analysis hardware and software. As part of our needs requirement gathering, end users identified automated image analysis of tumor tissues coming from our banked material as an important and unmet service need. Funds from the tobacco grant were used to purchase two new computer workstations and a 4 Terabyte storage device to store files. These computers complement the multispectral image analysis hardware that is part of the tissue bank's tissue analytic service.

Research Project 4: Project Title and Purpose

Nicotine Effects on Lung Cancer Promotion: From Cells to Patients - The goals of this project are to: (1) examine the role of nicotine on lung cancer in vitro (cell lines) and in vivo (mouse models), and (2) to examine genetic variation in lung cancer development and outcomes. The ultimate clinical goal of this project is to identify novel molecular targets for drug development for lung cancer.

Duration of Project

7/1/2007 - 12/31/2009

Project Overview

Background: Neuronal-type nicotinic receptors (nAChRs), which are ligand-gated ion channels, are expressed in normal human bronchial epithelium and non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) cell lines. In normal human bronchial epithelial cells, nicotine activates the Akt pathway, a pro-growth and anti-apoptotic pathway. In NSCLC cell lines, nicotine upregulates cell survival proteins (survivin, XIAP) in an Akt-dependent manner, and inhibits the apoptotic effects of chemotherapeutic agents. Lung cancer cells lines also express the proteins necessary to synthesize and transport acetylcholine (ACh), the endogenous ligand for nAChRs, providing an autocrine signaling mechanism that may regulate cell growth in the absence of nicotine exposure.

Aims: (1) To examine nicotine effects on lung cancer cell lines as well as lung tumor growth and chemotherapy response in mouse models, and (2) To perform an nAChR SNP pathway analysis of smoking behavior and lung cancer.

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Expected Research Outcomes and Benefits

The results of the proposed research program will address two important questions that could have a significant impact on lung cancer morbidity and mortality. First, experiments conducted in mouse models will address the question of whether nicotine promotes the progression of lung cancer and reduces the efficacy of chemotherapy. Evidence that nicotine promotes the progression of lung cancer would lead to a change in standard of care with respect to the use of nicotine replacement therapies for smoking cessation among lung cancer patients. Second, the genomic analyses on human DNA from lung cancer cases and smoking controls may reveal novel genetic variants associated with smoking behavior and lung cancer. This work may identify novel molecular targets for drug development for this fatal disease.

Summary of Research Completed

The activity on this project was largely completed in the prior reporting cycle, with results detailed in the prior report. Specifically, we completed Aim 1, as well as the nAChR pathway analysis of smoking behavior and cessation in Aim 2.

During the current project period, we completed the manuscript describing the nAChR pathway analysis of smoking behavior. The project and results were described in the previous progress report. The paper was published in 2010 (Convergent evidence that choline acetyltransferase gene variation is associated with prospective smoking cessation and nicotine dependence. Ray R, Mitra N, Baldwin D, Guo M, Patterson F, Heitjan DF, Jepson C, Wileyto EP, Wei J, Payne T, Ma JZ, Li MD, Lerman C. *Neuropsychopharmacology*. 2010 May;35(6):1374-82)

In the current project period, we have also completed the SNP analysis of lung cancer as described below.

Study Population: Our control group was comprised of treatment-seeking smokers responding to advertisements for a free smoking cessation program at the University of Pennsylvania between 2004-2008. Inclusion criteria included ages 18-65 and a smoking rate of $\geq$10 cigarettes per day. The exclusion criteria were: DSM IV Axis I psychiatric or substance abuse disorder (based on the Structured Clinical Interview-Non-Patient (SCID-NP); use of psychotropic medications (e.g., anti-depressants, anxiolytics, anti-psychotics); and pregnancy or lactation. In the full cohort of 571 trial participants, there were 472 smokers of self-reported European ancestry. Forty-two percent of participants were female and 34% were college graduates. The mean age was 45 years (sd = 10.4), with an average length of smoking of 29.5 years (sd=10.9). In addition, controls

were recruited from the general medicine and pulmonary outpatient practices at Penn, and included both current and previous smokers without evidence of lung cancer.

Cases were comprised of subjects with incident non-small cell lung cancer, recruited from the thoracic surgery, medical oncology, and pulmonary practices at Penn. In the full cohort of 443 cases, there were 400 individuals of self-reported European ancestry. The mean age was 67 years (sd= 10.2).

Genotyping: We utilized a custom Illumina GoldenGate® genotyping array, to genotype 169 SNPs in 11 candidate genes in the nAChR system, as well as 359 ancestry informative markers and technical control SNPs found in the pre-designed Illumina DNA Test Panel. The Illumina Assay Design Tool (www.illumina.com) was used to identify all SNPs within, or 10 kb up- or down-stream from, the 11 targeted genes. The resulting SNP list was filtered for Illumina designability rank of 1 (a prediction of assay performance) and minor allele frequency >0.1, and one SNP from any pairs separated by less than 60 bp was chosen by design score prioritization to prevent interference in the multiplex genotyping assay. The remaining SNPs were selected using HapMap linkage disequilibrium (LD) maps to ensure that at least two SNPs were included from each LD block in or around the targeted genes. Non-synonymous coding SNPs and targeted SNPs from previous association studies were included regardless of minor allele frequency or design score.

GoldenGate® 768-plex genotyping assays were performed in the Sentrix Array Matrix format (Illumina, Inc. San Diego CA) using 250 ng of each subject's genomic DNA according to the manufacturer's instructions. The Illumina BeadStudio software was used to assign genotype calls with default parameters and signal thresholds. Failed SNP assays (n = 18) and DNA samples with low call rates (n = 65 from the entire sample of 571 trial participants) were removed from the data set. Replicate concordance was confirmed before removing duplicate samples from the data set. There were no deviations from Hardy-Weinberg equilibrium (HWE) using chi-squared tests; 34 SNPs with minor allele frequencies (MAF) < 0.05 in the discovery cohort were excluded from analysis.

Data Analysis Methods: Cases and controls were matched on age using Rosenbaum's optimal matching algorithm using Euclidean distances. After matching, there were 205 cases and 420 controls. Because self-reported race/ethnicity is a complex composite variable comprised of self-identified concept of ancestry, culture and behavior, we recognize that using self-reported race/ethnicity as a proxy for ancestral background can be problematic. Hence, potential population stratification within our study cohort was adjusted for using principle components estimated from ancestry informative markers. Principle components analysis is a method for minimizing the difference between "mapped" distances on *k* axes and actual distance in a much larger-*n* space. In our analyses, we adjusted for the top 10 principle components to adequately adjust for population stratification. Individual SNP associations with case-control status were assessed using two-sided chi-squared tests or Fisher's exact tests. Both the one degree of freedom genotypic trend test (analogous to the Cochran-Armitage test) and the two degree of freedom tests of independence were performed. Individual SNPs were coded as dummy variables under different genetic models (dominant, recessive, or additive). Conditional logistic regression models provide adjusted odds ratios and 95% confidence intervals adjusting for tobacco use, sex and the

top 10 PCs. A conditional logistic regression model was used to account for the matching on age. Because of the exploratory nature of this study aim, we focused this secondary analysis on SNPs in *CHAT* and *CHRNB2* based on our prior data associating these genes with nicotine dependence and smoking cessation, *CHRNA5/CHRNA3/CHRNB4* based on prior associations with nicotine dependence and lung cancer, and on a novel candidate gene, *CTLA4*, involved in immune and inflammatory responses.

Results: SNPs with nominal associations with case-control status are shown in the table below. Preliminary evidence suggests a signal for association with *CHAT*, *CHRNA5*, *CHRNB2* and *CTLA4*. However, given the relatively small sample size, this preliminary study should be considered hypothesis-generating. Future studies to replicate these associations in larger samples, and to examine predictors of lung cancer survival are planned.

GENE	**RSNUMBER**	**Minor ALLELE Frequency in CASEs (n=205)**	**Minor ALLELE Frequency in CONTROLs (n=420)**	**Odds Ratios**	**95% CI**	**P_value**
CHAT	RS4838392(G/A)	166(40.9%)	303(36.5%)	1.716	0.945-3.11	0.076
	RS7094248(C/G)	198(48.8%)	393(47.3%)	2.312	1.141-4.68	0.020
	RS7903612(G/C)	148(36.1%)	217(26.0%)	2.069	1.148-3.72	0.016
	RS8178990(A/G)	5(1.2%)	27(3.2%)	6.024	0.761-47.6	0.089
	RS868750(A/G)	77(18.9%)	259(30.8%)	1.611	0.923-2.81	0.094
CHRNA5	RS4275821(G/A)	142(35.5%)	410(48.9%)	0.560	0.309-1.01	0.055
CHRNB2	RS1127326(A/G)	126(30.9%)	236(28.2%)	1.588	0.926-2.72	0.093
	RS2072659(C/G)	92(36.2%)	87(14.0%)	3.482	1.542-7.86	0.003
CTLA4	RS231775(G/A)	116(34.1%)	279(39.1%)	2.156	1.055-4.40	0.035
	RS4553808(G/A)	42(19.3%)	218(26.3%)	0.251	0.092-0.68	0.007
*A/G=Minor Allele/Major Allele						

Research Project 5: Project Title and Purpose

Inhibition of the PI3 Kinase Pathway in Leukemic Stem Cells - Acute myeloid leukemia or AML is a malignant disease of the blood that is still fatal in over half of patients who develop the disease. Recent advances in cancer biology have demonstrated that by understanding the molecular control of growth in malignant cells, new therapies can be developed that inhibit these growth regulatory mechanisms. The PI3 kinase pathway is critical for the survival of leukemic cells and leukemic stem cells. In this project, experiments will be performed to better understand the function of this and other pathways in leukemia cells. Tests will be performed of new therapies to inhibit these pathways in mouse models of leukemia. The aim is to develop clinical trials to improve the therapy of AML.

Duration of Project

7/1/2007 - 6/30/2010

Project Overview

Acute myeloid leukemia (AML) is a malignant disease of the blood that occurs with increasing incidence as individuals age. Despite major advances in understanding the molecular biology of AML in the last ten years, there have been few new therapeutic advances. On the other hand, dramatic progress has been made in chronic myeloid leukemia (CML) by focusing on the mechanisms of disordered growth regulation in CML. The proposal was made that an increase in the understanding of growth dysregulation in AML, can lead to development of novel therapeutics for this disease. Consistent with this hypothesis, it was demonstrated that the molecular signaling pathway from the phosphotidylinositol 3 (PI3) kinase to the mammalian target of rapamycin (mTOR) protein is critical for survival of AML stem cells. Inhibition of mTOR in AML cells did not kill cells by itself but made them more sensitive to chemotherapy. Based on this observation, a clinical trial was recently completed of the mTOR inhibitor, rapamycin, with chemotherapy. However, preliminary results from this trial do not demonstrate a dramatic increase in obtaining complete remission in treated individuals suggesting that further understanding of growth regulation in AML and better optimization of drug schedules in vivo is necessary to translate our observations into improved therapy for AML. In order to achieve this Aim, three Specific Aims are proposed: 1) Study the function of the Akt kinase, a clinically targetable mediator of the effects of PI3 kinase, in AML cells. AML cells will be treated with Akt inhibitors and then engrafted samples in NOD/SCID mice to determine if Akt activation is necessary for survival of AML stem cells. 2) Study the clinical effects of different schedules of administration of rapamycin with chemotherapy in a NOD/SCID xenograft model of AML. 3) Study the role of the STAT5 protein in survival of AML cells. Preliminary data suggests that inhibition of STAT5 with siRNA decreases the growth of AML cells. The project will also investigate the role STAT5 plays in the survival of AML cells.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Acute myeloid leukemia is still treated with high dose chemotherapy. This therapy is expensive, toxic and can only be administered safely to younger patients. The therapy is efficacious in achieving clinical response but is curative in less than 40% of patients. The experiments proposed are designed to achieve two goals. First, an improved understanding of the molecular pathways that control cell survival in AML cells will be investigated. Focus will be on molecular pathways that can be modified with either existing drugs or drugs being developed by the

pharmaceutical industry so that we can rapidly translate these scientific studies into new therapies. Thus, the second goal is to develop new therapies for AML that are less toxic and more efficacious. Previous work has focused on the PI3 kinase signaling pathway in AML and the already developed clinical trials based on these observations, however to date, improved treatment for AML has not clearly been shown. Possible explanations are that either using a drug which is not effective in suppressing signaling in patients or that the wrong schedule of drug administration was used. Aims 1 and 2 of this proposal will continue to study this signaling pathway. New drugs that inhibit activation of a protein called the Akt protein will be investigated. Drugs to inhibit Akt are in development and the expectation is that clinical trials of Akt inhibitors in AML can be planned shortly. The goal is to determine how best to do such studies. For the mTOR protein, which is another target of the PI3 kinase pathway, a clinical trial has been performed using rapamycin, a commercially available mTOR inhibitor. Although the drug is safe, it has not yet been demonstrated that it improves treatment. The wrong schedule may have been used for administering the drug. The preliminary data suggests that another biochemical pathway involving the STAT5 protein is involved. In Aim 3, the project will determine what the STAT5 pathway does by itself and how it interacts with the PI3 kinase signaling pathway.

Summary of Research Completed

We have made substantial progress on all 3 Aims with significant translational implications. Results are summarized by Aim below.

Specific Aim 1) Study the function of the Akt kinase, a clinically targetable mediator of the effects of PI3 kinase, in AML cells. AML cells will be treated with Akt inhibitors and then engrafted samples in NOD/SCID mice to determine if Akt activation is necessary for survival of AML stem cells.

This Aim was completed in this grant period. We performed in vitro assays of the effect of the Merck Akt inhibitor on survival of AML colonies using 7 different AML samples. Surprisingly, the drug was largely ineffective at inhibiting colony forming activity in AML cells. AML cells were plated in methylcellulose using complete media. Cells were grown for 14 days and then assayed for colony forming units. Controls were either untreated cells or cells treated with the PI3 kinase inhibitor, LY294002, which we have previously shown to completely inhibit colony forming ability in AML cells. Akt inhibitor A from Merck was used at 500 nM, a relatively high but still specific concentration. Akt inhibitor A is an Akt1-2 inhibitor which spares Akt3 at this concentration. In one of 7 samples, Akt inhibitor A inhibited colony formation over 90%. However, in other samples (n=6) there was either no inhibition of survival or partial inhibition of survival (up to 50%). Importantly, at 500nM normal bone marrow colony forming ability was also inhibited by 50%. These studies suggested to us that there would not be a therapeutic index for the compound. Importantly, from other research projects, we know that many AML samples have activation of Akt3 (which is not normally activated in hematopoietic cells). Overall, we hypothesize that Akt3 must provide substantial survival benefit for AML cells and this Akt 1-2 inhibitor is unlikely to have therapeutic benefit. For this reason, we have not pursued further studies of the compound.

Specific Aim 2) Study the clinical effects of different schedules of administration of rapamycin with chemotherapy in a NOD/SCID xenograft model of AML.

Further studies on this topic are being pursued by the research group, but not funded by this project. Briefly, we have decided that studies of rapamycin in AML have developed to the point where they are best pursued in patients and not in the animal model.

Specific Aim 3) Study the role of the STAT5 protein in survival of AML cells.

As we previously noted, we had developed preliminary data that STAT5 is necessary for AML cells and that STAT5 is activated by src family kinases (SFKs) such as lyn and lck. We had previously submitted a paper on this topic but the reviewers pointed out the wide heterogeneity of the findings from sample to sample. We are currently working to study a larger cohort of patient samples before re-submitting this data. These studies will be funded by a recently awarded Merit Award to Dr. Carroll from the Veterans Administration ("Therapeutic targeting of Src kinase signal transduction pathways in AML", 1 I01 BX000918-01)

Research Project 6: Project Title and Purpose

Clinical Trials of Novel Targeted Therapies in Patients with Advanced Cancers - The treatment of the most common adult cancers, once they have become metastatic, is inadequate. Currently available systemic therapies, such as chemotherapy, rarely induce complete remissions, and partial remissions are followed by the emergence of tumors resistant to further therapy. Progress has been made in understanding the mechanisms by which cancers become established and spread to distant sites. The development of drugs that counteract these processes offers an approach that is unique compared to chemotherapy. This project focuses on two elements of this biology: tumor blood vessel formation and the abnormal function of enzymes within the tumor cell. Clinical trials will be conducted evaluating novel strategies to inhibitor tumor blood vessel formation and, separately, to block the function of abnormal enzymes within tumor cells.

Anticipated Duration of Project

7/1/2007 - 12/31/2010

Project Overview

Specific Aim 1: To evaluate the effect of combining drugs targeting different aspects of tumor blood vessel formation (angiogenesis) in clinical trials.
Specific Aim 1a: To determine the therapeutic effect of novel anti-angiogenesis therapies in patients with advanced renal cell carcinoma.
Specific Aim 1b: To determine the effect of novel anti-angiogenesis therapies on radiographic measures of tumor perfusion.
Specific Aim 2: To evaluate the effect of drugs targeting mutated oncogenes that are prevalent in common adult cancers.
Specific Aim 2a: To determine the therapeutic effect of novel BRAF inhibitors in patients with advanced melanoma, colon cancer and thyroid cancer.

Specific Aim 2b: To determine the effect of novel BRAF inhibitors on intracellular signal transduction pathways in tumor samples obtained from patients with metastatic melanoma.

Renal cell carcinoma is a cancer for which blood vessel formation is particularly critical for its development and progression. The first generation of therapies that target vascular endothelial growth factor (VEGF) have demonstrated single-agent efficacy in patients with metastatic disease, however, tumors ultimately circumvent these therapies and form new blood vessels despite their continued administration. Several factors have been identified which appear to promote angiogenesis in addition to VEGF. Targeting these entities in combination with VEGF is a theoretically promising strategy and will be evaluated in phase I and phase II clinical trials.

Activating mutations in the serine threonine kinase BRAF have been identified in 7% of all solid tumors. These mutations appear to play a significant role in tumor cell growth and resistance to conventional chemotherapies. They are particularly common in melanoma and thyroid cancer, and are also found in a subset of colon and ovarian cancers. Potent and specific inhibitors of BRAF have very recently become available for clinical evaluation. Phase I and phase II trials will be conducted among patients with these cancers to determine their therapeutic impact for patients and effect on tumor cell signaling.

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Expected Research Outcomes and Benefits

The clinical trials proposed in this project will produce preliminary evidence of therapeutic effect among the patients treated. These data will serve as the basis for proposing subsequent phase III clinical trials to demonstrate definitive benefit in comparison to standard therapy. Such trials would be appropriately conducted with National Cancer Institute sponsorship through the cooperative groups.

The development of novel regimens targeting tumor blood vessel formation will be broadly applicable to common adult cancers. The first generation of these therapies have already demonstrated the ability to slow the progression of metastatic colon, lung, breast and kidney cancers. Thus, combination regimens that more effectively inhibit this process will likely translate into further benefit for patients with these and other types of cancers.

Understanding the consequences of BRAF inhibition within melanoma will provide essential insight into the therapeutic value of this molecular target. Given that mutations in BRAF are found in 7% of adult cancers, this information will translate into improved therapy for a significant subset of cancer patients. It is critical to understand which cancers are susceptible to single-agent BRAF targeted therapy and which cancers are responsive to regimens that combine BRAF inhibitors with other therapies. Determining the optimal chemotherapy or targeted therapy to combine with BRAF inhibition will be critical in each of the cancer types where BRAF mutations are found (melanoma, thyroid, colon and ovarian cancers).

Summary of Research Completed

Specific Aim 1 - This project has continued to make progress toward the ultimate goal of understanding the effect of agents that target tumor angiogenesis among patients with metastatic clear cell renal cell carcinoma (ccRCC). We previously reported studies of VHL mutation status and HIF expression in ccRCC which led to the identification of three subgroups of ccRCC: 1) *VHL* wildtype; 2) *VHL* mutant/HIF1α/ HIF2α expressing (H1H2) and 3) *VHL* mutant/ HIF2α expressing only (H2). The three types of tumors exhibit patterns of gene expression that allow them to cluster together and each has distinct molecular characteristics with potential impact on clinical course and prognosis in the absence of treatment and on response to treatment.

Previously, we also reported that study of mouse tumors treated with selected antiangiogenic agents revealed characteristic changes in endothelial cell signaling and the pharmacodynamics of these agents. We began development of a histopathology analysis platform that would allow similar analysis of endothelial cell (as well as tumor cell) signaling in patient tumors. This platform analyzes immunostained histopathology specimens by quantifying antigen staining at the level of individual cells. In this system, slides are stained for multiple antigens per slide: Some antigens are for identifying cell types of interest, other antigens reveal the analyte(s) being studied. The multiplex-stained slides are imaged multispectrally in brightfield and fluorescence mode using a microscope fitted with a multispectral imager (CRi, Woburn MA). Software is then used to "unmix" the multispectral images into the component chromogens and fluorochromes present on the slide and in their images: The individual chromogens and fluorochrome are placed into separate "channels". Analysis proceeds by (step 1) delineating the nuclear and cell boundary of cells in images using the stain channels that define nuclei, cytoplasm and plasma membrane (nuclear/cell "segmentation"); (step 2) classifying the segmented cells into types of interest, e.g. endothelial cells and tumor cells, based on stains for cell-type antigens (e.g. CD34 for endothelial cells and epithelial cytokeratins for carcinoma cells); and (step 3) referencing analyte staining to the nuclei/cells identified in the previous two steps. When analyte expression is determined by individual cells (cytometrically), the event/process represented is examined by the units in which it occurs; when multiple analytes are stained on a slide, the many events/processes represented are associated in the units in which they co-exist and may interact. Our cytometric approach to analyzing antigen expression, termed "histocytometry", presents data in biologically meaningful terms/context and provides biological insights in addition to diagnostic aid to allow the study of biological processes in tissues.

Development of the histocytometry analysis platform
Multiplex immunostaining - Each ccRCC slide has to be multiplex stained for cellular structures

and antigens to allow identification of cell nuclei, endothelial cell (EC) and tumor cell (TC) antigens and analyte. To allow analytes (p-ERK, p-STAT3, p-AKT or Ki67) to be studied in tumor EC and TC, we have developed immunostaining/staining of each ccRCC slide for the following: one analyte, an EC antigen (CD34), a TC antigen (CA IX), a pericyte antigen (SMA) and nuclei (hematoxylin). A different reporter must be used for each antigen to allow their resolution. After testing various combination of reporters for the different antigens in multiplex combination, we have settled on staining for analyte by immunohistochemistry (IHC) using DAB chromogen and staining for the three cell-typing antigens by immunofluorescence (IF) using a set of resolvable fluorochromes (e.g. Alexa Fluor 488, 555 and 594 or 647). Analyte IHC staining is performed first, so that analyte staining (and quantification) is not influenced by the other immunostaining steps.

Multispectral imaging - Multiplex-stained slides are imaged multispectrally in brightfield and fluorescence modes using a multispectral microscope setup (Vectra®, CRi, Woburn, MA). This is followed by use of Nuance® software (CRi, Woburn, MA) to spectrally "unmix" the brightfield and fluorescent spectral image data (image cubes) to resolve the different chromogens and fluorochromes in the images into separate channels.

FARSIGHT image analysis - FARSIGHT is the name of the image analysis software that is used to process the information in the different channels of stain information to accomplish steps 1-3. Following spectral unmixing of staining for the four antigens plus hematoxylin in each image, FARSIGHT uses information in the hematoxylin channel to segment individual nuclei. Nuclei belonging to TC are typed by their association with staining in the CA IX channel, and boundaries of individual TC are segmented using the pattern of staining in this channel. Nuclei belonging to EC are typed by their association with staining in the CD34 channel and their nonassociation with staining in the SMA and CA IX channels (this eliminates nuclei of pericytes and TC situated near vessels). Analyte staining by IHC using DAB reporter provides maximum sensitivity of detection for these rarer antigens, many of which are poorly revealed by less sensitive IF staining.

FARSIGHT analytical software - We have successfully adapted the FARSIGHT system to analyze ccRCC specimens imaged by the Vectra multispectral microscope. A long list of software improvements were made in order to address the special needs of endothelial cell analysis in ccRCC specimens. These include fundamental improvements resulting in core segmentation algorithms that produce more accurate results faster, additional cell features specifically designed to help identify vascular endothelial cells and to separate them from pericytes, a fully automatic cell classification algorithm based on the kernel partial least squares (k-PLS) method that requires no parameter settings aside from a training set, advanced editing functions, and several user interface improvements. Figure 1 shows a screen view of the current system. In the image panel, the blue channel shows the DAPI nuclear signal, the yellow channel shows SMA labeling, the green channel shows CD34 labeling, and the red signal shows renal cell marker C IX labeling. The system displays and stores a color-coded summary of cell classification results that can be used to identify cell subpopulations. In Figure 1, colored dots on each cell indicate the following cell types: Yellow: endothelial cells, Pink: pericytes, Cyan: tumor cells, and Red: all other cell types. The FARSIGHT system is currently undergoing extensive testing and application to newly generated ccRCC datasets in the PI's laboratory.

Clinical trial and specimens - The histocytometry platform described will be used to analyze tumors from ccRCC patients previously entered into a clinical trial of sorafenib conducted at Penn and in an ongoing clinical trial of sunitinib as first line therapy of metastatic ccRCC (UPCC03809). In the latter, which has already accrued 13 patients, patients undergo DCE-MRI to evaluate tumor radiographic and perfusion parameters just before and 2-3 weeks after the initiation of sunitinib therapy. Tumor material from the time of nephrectomy or other tumor resection will be analyzed by histocytometry. The results of histocytometry analysis, the paired DCE-MRI studies (including Ktrans), and clinical parameters of response will be then be correlated.

Specific Aim 2 - The focus of this aim has been the clinical investigation of novel BRAF targeted therapies. *BRAF* is mutated in 7% of all human cancers. The cancer in which these mutations are most commonly found is melanoma, with a prevalence of 60%. We have completed a phase I/II trial of PLX4032, the first potent and selective inhibitor of BRAF, and will be reporting the results in an upcoming issue of the *New England Journal of Medicine*. In the first-in-human trial, 49 of 55 patients enrolled in the dose escalation portion of the study had metastatic melanoma. There was enrichment for patients with V600EBRAF mutations as many patients' tumors were prospectively evaluated prior to study entry, particularly at the higher dose levels. An additional 32 patients with metastatic melanoma harboring BRAF mutations were enrolled as an extension cohort at the maximum tolerated dose. Toxicity was clearly related to dose with increasing frequency and severity of rash, fatigue, and arthralgia at the highest doses. Efficacy was first clearly demonstrated at doses that produced drug exposure that was commensurate with that required to achieve tumor regression of V600EBRAF melanoma xenografts preclinically. Objective responses were observed in the vast majority of patients - 81% of the 32 patients in the extension cohort. FDG-PET scans revealed metabolic changes as early as two weeks into therapy. Selected patients underwent biopsy of superficial tumors for the purposes of confirming downregulation of Erk activation and Ki67. As single-agent trials are underway with the aim of establishing single-agent BRAF inhibition as a new standard of care for the BRAF mutated subpopulation, attention now turns to understanding mechanisms of resistance and rational combination approaches.

These results strongly validate the single-agent activity of a potent and specific BRAF inhibitor in patients whose tumors harbor the common activating BRAF mutation (V600E). This agent is currently also being evaluated in patients with other cancers (e.g. colorectal) with V600E-BRAF mutations. Current work is focused on understanding the factors other than V600E-BRAF that determine response and development of resistance on the setting of ongoing therapy.

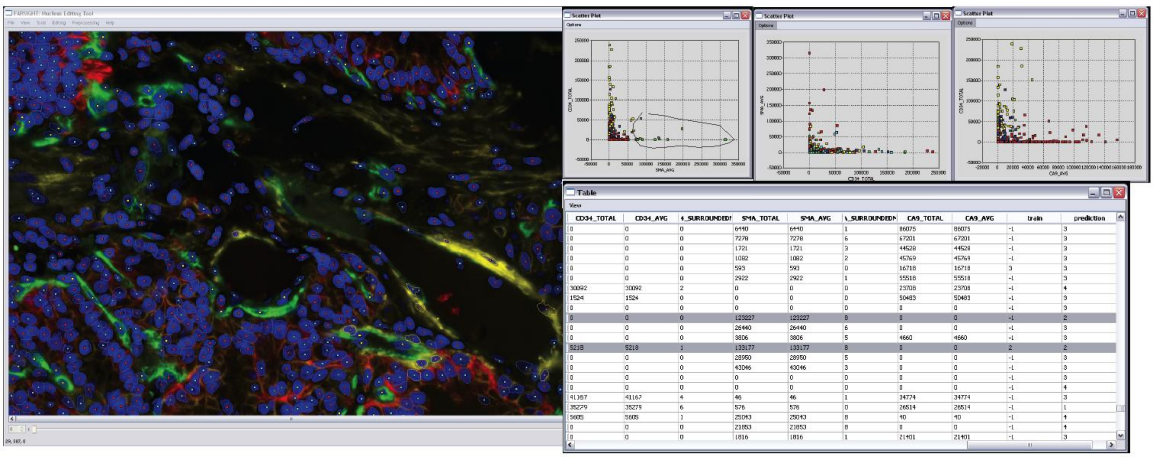

Figure 1. A screen view of the FARSIGHT system demonstrating successful cell segmentation and typing. The panel on the left is the screen view showing the image data and overlay of segmentation and cell classification results. The three panels on the upper right-hand section are scatter plots of quantitative measurements. The table on the lower right displays measurements.

Research Project 7: Project Title and Purpose

Acceleration of Umbilical Cord Blood (UCB) Grafts with Activated T Cells - Hematopoietic Stem Cell Transplantation has emerged as a very promising therapy for a variety of life-threatening diseases. However, patients receiving UCB transplants performed up until now have proven to recover donor blood counts slowly, and in some cases incompletely. This slow engraftment results in a heightened risk of life-threatening infection. But if the problem of slow engraftment could be circumvented, UCB transplantation would become the dominant and, hopefully, decisively curative mode of blood stem cell transplantation. In this new program, we will take advantage of a newly developed approach to activate the T lymphocytes in the donor UCB graft as a technique for stimulating UCB stem cell engraftment. In 2009 this project was expanded in order to allow for the evaluation of cardiac stem cells in addition to cord blood stem cells for the development of novel therapies for human illness.

Anticipated Duration of Project

1/1/2007 - 12/31/2010

Project Overview

Activated adult T cells have long been known to amplify blood stem cell growth in test tubes and in vivo. In addition, UCB T cells have long been known to be immunologically immature, and non-activated. Over the past year we have developed a protocol to remove the T cells from a UCB sample, activate them by exposure to microbeads coated with anti-T cell receptor and costimulatory receptor antibodies, and remix the activated T cells with the UCB sample. In preliminary experiments, investigators have found that UCB activated in this fashion (UCB*) is able to engraft Non-Obese Diabetic/Gamma Chain Deficient (NOG) mice to a higher extent than non-manipulated UCB cells. Penn investigators are now in the position to ask whether these UCB* are able to engraft NOG mice more rapidly than non-manipulated UCB.

For these studies, UCB or UCB* will be transplanted into irradiated NOG mice, and peripheral blood will be sampled every week for eight weeks. The percentage of human CD45+ circulating white blood cells will be measured by flow cytometry, and engraftment of specific lymphoid and myeloid lineages will be compared by using lineage-specific antibody markers. In these studies, cohorts of 6-8 mice per group will be used, so as to obtain reliable statistics. Results from these studies will be reported annually, beginning year 1. (Years 1-3)

NOG Transplantation Facility: To accomplish this study three distinct phases for fit-out and operations at a new NOG Transplantation Facility will be completed:

1) Equipping of the new NOG Transplantation Facility in Hill Pavilion with an Irradiator capable of delivering fixed dose X-rays to microisolated mice, and with a Xenogen in vivo imaging system so that investigators can track fluorescently labeled cells in vivo in live mice. (Year 1)

2) Developing the NOG colony on site in the new facility. This requires extensive inbreeding, with concomitant testing for pathogens. (Years 1-2)

3) Validating the protocols for establishing human-NOG transplantation protocols, including radiation dosing, antibiotic treatment, monitoring by periodic blood sampling and in vivo fluorescence imaging. (Years 1-3)

Expanded Project: Prior to 2009 the work of this project focused on blood cells and was aimed at allowing for the expansion of umbilical cord blood stem cells for therapeutic use. This work is ongoing and highly productive. New discoveries in science in the last few years make this work even more attractive and applicable to a wide range of human illness. The ability to produce pluripotent stem cells (iPS cells) from human fibroblasts means that we may be able to produce mature tissues from these stem cells for the treatment of multiple diseases. Significant work has correlated blood stem cells with cardiac stem cells with regard to shared characteristics of progressive lineage restriction, expansion and differentiation. In the expanded project, we will extend our analysis of blood stem cells to include parallel analysis of cardiac stem cells. We will examine the ability of stem cells expressing a cell surface marker called c-kit to contribute to the muscle of the heart. This extension of the previous work involves the use of additional cell types with altered cell surface markers for use in parallel studies.

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Expected Research Outcomes and Benefits

UCB is a rich source of stem cells. UCB stem cells like those retrieved from bone marrow can be used to treat a wide array of genetic disorders that affect the immune system, leukemia and certain cancers, and inherited disorders of body chemistry. At present, more than 45 disorders are treatable with UCB stem cells.

Since UCB stem cells could potentially be collected and stored from literally every birth, physicians envision the possibility of universally available UCB stem cells as a major medical resource, circumventing the current problem of trying to locate rare tissue type matches for patients in need of a transplant. In addition, in contrast to bone marrow or blood stem cell grafts, umbilical cord blood (UCB) stem cell transplants appear to cause less toxicity to the patient (Graft Versus Host Disease ─ GVHD).

If the studies conducted on NOG mice are successful at demonstrating accelerated engraftment, the principal investigators would plan a clinical trial of UCB* for human patients without other hematopoietic stem cell donors, with the plan to monitor engraftment and toxicity in these patients. Such studies, if successful, would pioneer the expansion of UCB* grafts at a nationwide level.

NOG Transplantation Facility: The new NOG Transplantation Facility is critical to this study and will allow investigators to track the ability of individual stem cell subsets to form specific human (or non-mouse animal species) tissues after transplantation, whether blood, cardiac muscle, breast tissue, neurons, etc., either under basal conditions or under the influence of specific biochemical therapies (e.g. peptides, miRNAs, etc.). Similar approaches will allow investigators to ask whether specific tissue engineering approaches, e.g., cell growth in bioreactors, will allow for the production of whole tissues that can function after transplantation in vivo.

Expected outcomes of the expanded project include the ability to determine if c-kit expressing cells can contribute to developing or regenerating cardiac tissue in mouse models. In the future, we believe that this work will contribute to our ability to promote cardiac regeneration and repair using stem cells and regeneration biology.

Summary of Research Completed

As in previous years, we note that there are both technologic and biologic Aims associated with this project. The description of progress on these Aims is broken out according to these different Aims below. In particular, development of the NSG Transplantation Facility required for this project is again summarized as a separate section:

To accomplish this study three distinct phases for fit-out and operations at a new NSG Transplantation Facility will be completed:

1) Equipping of the new NOG Transplantation Facility in Hill Pavilion with an Irradiator capable of delivering fixed dose X-rays to microisolated mice, and with a Xenogen in vivo imaging system so that investigators can track fluorescently labeled cells in vivo in live mice. (Year 1)

As reported previously, the NSG Transplantation Facility in the Hill pavilion has been established. This is an animal barrier space solely dedicated to our immune-deficient mouse colonies.

Currently, our maximum capacity is 6 x 140 cages = 840 cages (4200 mice). If necessary, our Hill space could accommodate 3 additional racks for a maximum capacity of 1260 cages (6300 mice). This room is also equipped with a Xenogen Spectrum system designed for bioluminescence and fluorescence in vivo imaging. The Hill animal facility is equipped with a dedicated cesium source irradiator (GammaCell 40) specifically designed for small animal irradiation and is housed within the barrier. Thus, the equipment aspects of this part of the proposal are completed.

In the last grant year, we have focused on developing use of the Xenogen Imaging System. Last year we described development of fluorescent dyes introduced via viral vectors to track hematopoietic stem cells. We concluded that the Katuska flourophore provides the best imaging for deep tissues such as the bone marrow where stem cells reside. Unfortunately, this has not proven as useful as we had hoped for quantitation of stem cell engraftment since the sensitivity is not sufficient currently. However, this methodology has been adapted by other projects using the Core facility to study stem cell localization in the bone marrow in order to characterize the hematopoietic stem cell niche. These studies are still in development but promise to interact with the enhancement of stem cell engraftment studies described in more detail below.

2) Developing the NSG colony on site in the new facility. This requires extensive inbreeding, with concomitant testing for pathogens. (Years 1-2)

After a Klebsielle infection resulted in the loss of many breeder pairs and experimental animals (as reported last year), we are pleased to report that the new sterility protocols introduced in 2008-2009 have been highly successful. We have had no significant infections during this grant period. We have been able to successfully expand the NSG breeding and are currently producing an average of 40 pups per week (approximately 2000 animals per year).

3) Validating the protocols for establishing human-NSG transplantation protocols, including radiation dosing, antibiotic treatment, monitoring by periodic blood sampling and in vivo fluorescence imaging. (Years 1-3)

We have continued to work with the Maternal and Fetal Health Group at the Hospital of the University of Pennsylvania and to maintain discussions with the Penn IRB about these studies. Our cord blood collections are now integrated into the Maternal and Fetal Health Group studies

so as to avoid conflict between researchers. The IRB has informed us that current NIH best practices are to provide an information sheet to expectant mothers stating that cord blood will be collected for research studies. Since umbilical cord blood is medical waste, no consent is required. We are working with staff from Maternal and Fetal Health to develop this information sheet and, once developed, will have it approved by the IRB so these studies can continue.

For engraftment studies, we are continuing to optimize the human engraftment studies. Although consistent engraftment can be obtained using $1x10^5$ CD34 purified cord blood stem cells per mouse, we have observed that transplanting increased numbers of CD34 cells leads to higher levels of engraftment. This suggests that, under the experimental conditions used, in which the mouse is prepared with sub-lethal irradiation, we are setting up a competitive repopulation experiment in which human and mouse stem cells are competing for stem cell space and expansion potential. We are currently undertaking a more controlled experiment transplanting animals with a range of CD34 cell numbers from $1x10^5$ to $51x10^5$. At this point, the intent of these experiments is simply to better understand the experimental system but as we develop more quantitative studies of hematopoietic stem cell engraftment as described below, this may be critical for understanding the sensitivity of detection of HSC.

<u>Scientific Studies:</u> *The initial goal of the scientific aspect of this project is to test the hypothesis that ex vivo activation of umbilical cord blood (UCB) derived T cells may enhance engraftment of cord blood stem cells.*

We have initiated a new approach to the ex vivo expansion of umbilical cord stem cells. The concept for this work comes from our collaborator, Dr. Peter Klein, in the Division of Hematology and Oncology at University of Pennsylvania. Dr. Klein has previously published that in a murine system incubation of stem cells with a combination of inhibition of glycogen synthase kinse 3 (GSK3) and an mTOR inhibitor (rapamycin) increases stem cell numbers in vitro as assayed by bone marrow transplantation into a recipient mouse. We are now collaborating with Dr. Klein to determine if the combination of the chemical GSK3 inhibitor, lithium, with rapamycin will lead to expansion of hematopoietic stem cells ex vivo. If this proves to be true, this would generate a potentially robust and simple approach to stem cell expansion that could be used for clinical applications. We will continue to pursue this avenue of investigation in the next grant period.

In the spring of 2009 we received permission to add an additional aim to this grant which involves a genetic approach to determine the extent to which c-kit expressing cells in the adult can contribute to myocardium of the heart. The aim requires generation of a genetically modified mouse in which the gene encoding Cre recombinase (tamoxifen inducible) is knocked into the endogenous c-kit locus. We have successfully generated targeted embryonic stem (ES) cells and we have generated chimeric mice using these cells. However, we experienced an unanticipated delay because the chimeric mouse did not generate germline transmission of the targeted locus, despite analysis of over 6 litters. The type of ES cell used is called “TL1” and review of other projects in our lab and others has indicated that there may be an intrinsic problem with this particular clone of cells. Therefore, we have retargeted R1 ES cells (which we have used successfully on many occasions) and we are screening clones now by Southern blotting to determine if proper recombination has occurred. Once correctly targeted R1 clones are

identified, they will be promptly injected into blastocysts to create chimeras and then germline transmission will be assessed. We anticipate that this will proceed smoothly over the course of the next year.

Research Infrastructure Project 8: Project Title and Purpose

Vivarium – Research Infrastructure (Construction Phase III) - The purpose of this vivarium construction is to breed and study specialized mice and other rodent models which have specific genes deleted, added or altered. Mouse models are our most important tool to study diseases with a genetic component and to understand normal body processes which may be altered to result in disease. Mice are the ideal model because they are mammals and share many similarities in basic body functions to humans, while they are small, breed rapidly and have been a major focus of scientific study. This project is a multi-year project including scope development, design, contract awards, construction and commissioning. This infrastructure project is a continuation of a project supported by prior formula grants.

Duration of Project

1/1/2007 - 6/30/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 9: Project Title and Purpose

Novel Technologies for System Level Analysis of Complex Human Diseases - The purpose of this project is to develop new technologies to aid the understanding of complex genomic bases of human diseases. The new technologies to be developed include computational analysis procedures for genome-scale datasets and high-throughput screening technologies for molecular, chemical, and morphological measurements. The results of the project are expected to assist researchers in developing detailed models of genome-specific risk profiles and predictions of therapeutic efficacy and side effects.

Duration of Project

1/1/2007 – 3/31/2010

Project Overview

Many common human diseases from hyper-tension to malignant tumor are influenced by complex genetic factors. Prior to recent development in genomics, our understanding of genetic contributions to disease risk, treatment efficacy, and therapeutic side-effects were restricted to simple single factors. The advent of genome sciences in the last ten years have brought forth new

technologies such as massively parallel sequencing, extremely large-scale genotyping, genome-scale expression analysis, and other technologies that have allowed us to begin to understand the system level mechanisms underlying complex diseases. These methods have enabled some of the first glances at the complex inner workings of system-level molecular processes; and, a key finding has been that each genomic variant is likely to have specific processes that affect both normal functions as well as disease related dysfunctions. Thus, an individual will have his or her specific risk profiles for diseases as well as specific responses to therapeutics. We need to understand these processes in detail-- especially how different variants of the components work together, to develop genome-specific therapeutic strategies. The key to this kind of understanding is to develop high-throughput measurement technologies for biological processes at all levels, from molecular profiles, to metabolic processes, to cellular morphologies, to anatomical measurements, and behavioral processes; and to develop the proper computational analysis strategies and high-performance tools to construct a system-level model of biological function. The goal of this project is to develop the technologies for system-level integrated high-throughput analysis of human diseases.

Aim 1: Develop computational infrastructure for integrated analysis of complex diseases

Aim 2: Develop high-throughput measurement technologies for integrated assays of molecular, chemical, and morphological profiles.

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Expected Research Outcomes and Benefits

Complex human diseases involve multi-factor genetic susceptibility causes as well as non-conventional treatment strategies. The proposed project will develop new genetic mapping strategies for such diseases as well as for developing novel treatment strategies that leverage genomics technologies.

Summary of Research Completed

In the final year of this grant, we continued to refine the development of novel measurement tools.

Real-time sizing/mass measurement and multi-channel detection of protein molecules extracted from electroporated live cells.

In the last year, we have worked in two parallel directions that help advance each other: first, to push the frontiers of traditional translocation experiments, and second, to make nanoelectrodes and perform translocation experiments with them.

The first direction involved improving the signal-to-noise in translocation experiments by locally thinning silicon nitride membranes down to only a few nanometers, which increased the signal-to-noise by about 50 times compared to the current technology. We are now employing these devices to detect very small and short molecules and test their ultimate sensitivity. Using nanopores we have also shown the possibility to detect hydroxymethylated cytosines with nanopores. Figure 1 shows ultrathin SiN membranes produced by our procedure.

The second direction involves the advancement of nanoelectrode translocation techniques. In the last year, we have developed the necessary procedures to make devices compatible with salt solutions. In particular, this involved electrically insulating nanoelectodes from the salt solution, while leaving small exposed electrode tips to sense the electrical current. So far, we ran several experiments on nanoelectrodes to try to slow down the translocating molecules and to detect the transverse electrical signal from them, which was then correlated to the macroelectrode signal. The device fabrication currently involves about 10 fabrication steps and the yield of working devices has been about 20%. We have not detected any significant structure in nanoelectrode signals. We are working on optimizing all the measurement parameters, such as minimizing the nanopore size and applying larger voltages to achieve the proposed results within the next few months. Figure 2 shows the results of our experiments showing improved DNA translocation through 4 nm pores.

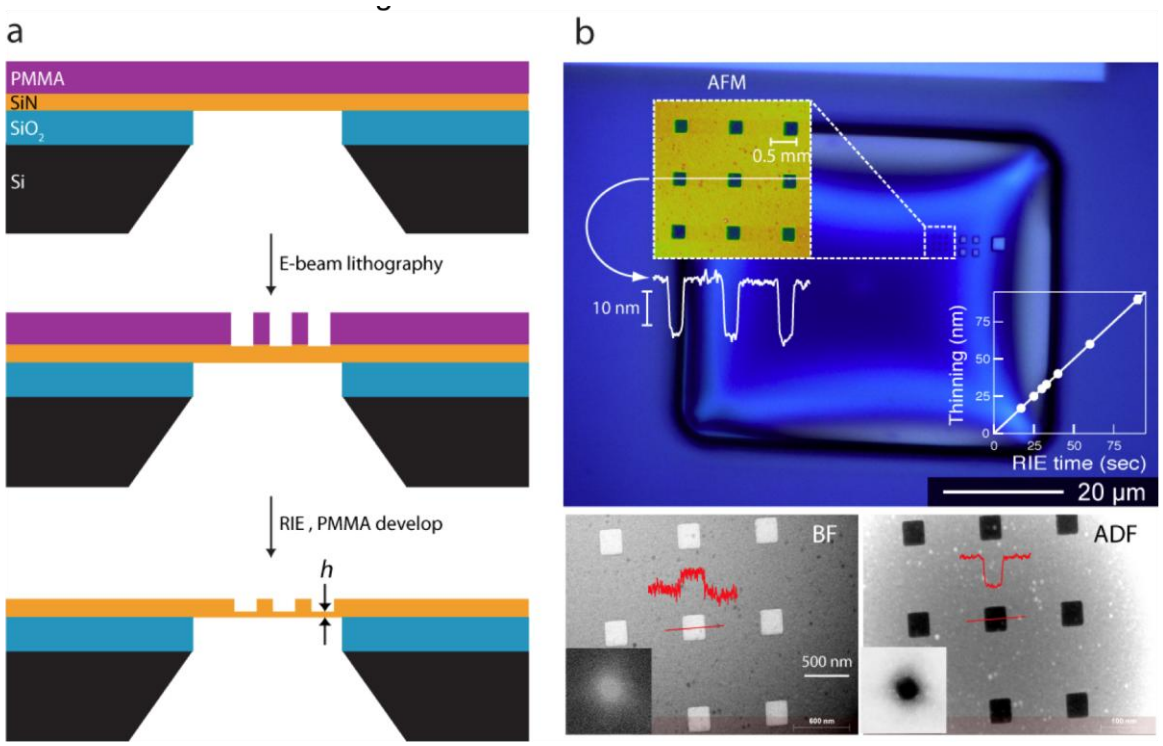

Figure 1. Ultrathin SiN membranes. (a) Process for local thinning of solid-state membranes for improving the nanopore resolution. (b) Optical microscope image of a pattern of squares used for thinning experiments. Following RIE process and lift-off, AFM image of the 250 nm squares is shown in the inset (17 nm thinning). The thinning was checked for different RIE times and shows excellent linearity (slope: 1 nm/sec). The bottom shows bright-field (BF) and annular dark field (ADF) images of the etched membranes, as well as corresponding images for a 10 nm pore (inset). The intensity profiles shown in red highlight the exceptional contrast of ADF imaging, useful for accurate thickness determination.

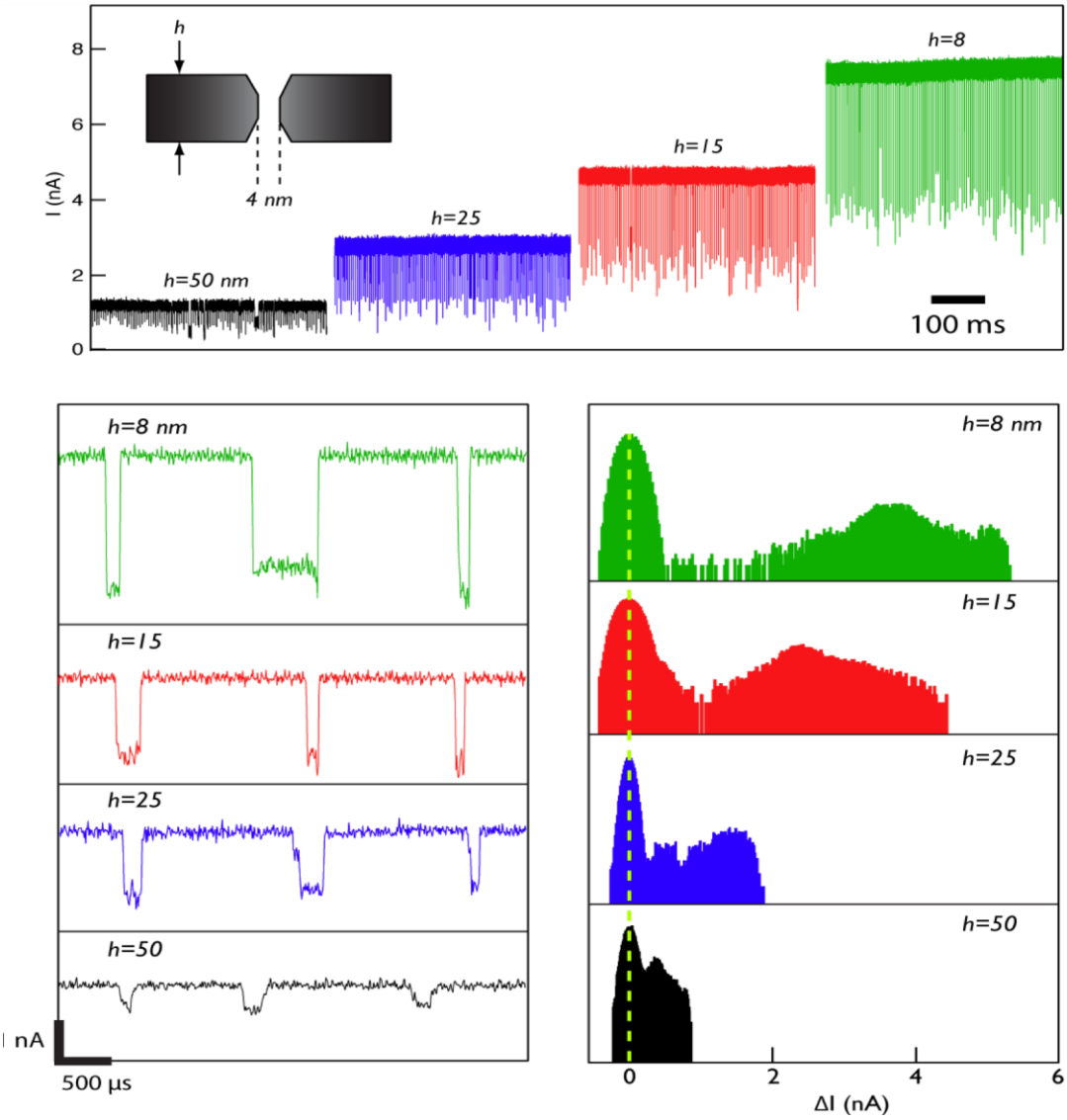

Figure 2**.** DNA translocation through 4 nm pores in SiN membranes of various thickness values. Top: Concatenated events for 3 kbp dsDNA translocations, as a function of membrane thickness (h) (300 mV, 21°C, 1M KCl, pH 8). Note: except for h=50 nm (10 kHz lpf), all data are maximum bandwidth (the rollover frequency of our amplifier is 70 kHz). Bottom left: expanded events from top traces. Bottom right: Semi-log all-point current histograms from events shown on top, demonstrating the improvement in signal-to-noise for decreasing nanopore thickness values.

Research Project 10: Project Title and Purpose

Expansion of Biosafety Research Capacity – Research Infrastructure - The purpose of this research infrastructure project is to expand the University of Pennsylvania's biosafety laboratory capacity, both Biosafety Level 3 (BSL3) and Animal Biosafety Level 3 (ABSL3) to accommodate the rapid growth in NIH-funded research for emerging infectious diseases and biodefense that supports the work of a large number of Penn investigators. While both research and vivarium space have been greatly expanded in recent years, construction of new BSL3 and

ABSL3 facilities have not kept pace with the unexpectedly rapid growth in research and research training on emerging infectious diseases and HIV at the University. At present, research at Penn on emerging infectious diseases and, to a lesser extent HIV, is greatly constrained by limited BSL3 and ABSL3 capacity.

Anticipated Duration of Project

7/1/2008 - 12/31/2010

Project Overview

The University of Pennsylvania is playing an increasingly major role in biodefense and emerging infectious diseases research. Unfortunately, there is only a single, common use BSL3 laboratory at the School of Medicine that itself is in need of renovation. In addition, there is not a single animal BSL3 (ABSL3) facility at Penn. The overall goal of this project is to increase biosafety research capacity.

Specifically, this project aims to:
1. Renovate the central 612 net sq. ft. BSL3 facility to accommodate and further facilitate the dramatic growth in emerging infectious disease, biodefense and HIV research at the School of Medicine, and to provide surge capacity for clinical pathology diagnostic services in the event of a public health emergency. We propose a two-step renovation project that will first convert a lab adjoining the current BSL3 into a new BSL3 facility, after which the wall separating the two rooms will be removed, resulting in a single, modern BSL3 suite containing five individual workrooms as opposed to one, with eight associated biosafety cabinets (BSCs) as opposed to four.
2. Construct a new 500 net sq. ft. BSL3 facility. In addition to greatly expanding biosafety research capacity, the new facility will provide laboratory space for researchers during renovations of existing facilities.
3. Convert the existing ABSL2 facility to a 3,882 net sq. ft. ABSL3 facility, making it possible for Penn investigators to perform BSL3 studies in small animals. The ABSL2 facility, designed more then 25 years ago to support work on infectious agents, will be renovated and brought to BSL3 standards.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

Outcomes: The success of this infrastructure project will be determined by the number of researchers able to utilize the newly renovated and or constructed BSL3 and ABSL3 facilities. In addition, the number of projects supported and NIH and other funds awarded to Penn investigators for biodefense and infectious diseases research will be other viable measures of success.

The existing 612 net sq. ft. BSL3 facility, which contains an anteroom and a containment room with four biological safety cabinets, is used by multiple investigators, including three who study SARS. This facility opened in 2000, and has been overwhelmed by large numbers of users. Since work with SARS requires the use of a respirator, this facility is effectively rendered off-limits to other investigators when even a single individual working with SARS is using the BSL3 suite. In addition, as there is not a single animal ABSL3 facility at Penn, investigators cannot perform small animal studies with BSL3 agents. Instead, this work is done on a contractual basis with private firms, or at other institutions via academic collaborations.

Summary of Research Completed

During the period of July 1, 2009 through June 30, 2010, the design and contract documents were completed and the project was bid and awarded to a contractor. The final design consolidated the project areas of specific aims one and two into a single enhanced facility. Construction of the BSL3 facility on Johnson Pavilion 3rd floor is currently in progress and is expected to be commissioned in August 2010.
Construction of the ABSL2 facility on Stemmler Hall 5th floor is currently ongoing and is expected to be commissioned in December 2010.

The Johnson BSL3 facility consists of three new multi-purpose cell culture rooms, one select agent cell culture room, one equipment/procedure room, a fully renovated cell culture room, and a renovated support lab adjacent to the BSL3 containment suite. All rooms within the containment facility are connected by an interior, connecting corridor accessible to the public corridor through entrance and egress airlocks.

The Stemmler ABSL3 facility consists of multiple suites within a larger containment area. The barrier suite is accessible from the surrounding vivarium via an entrance airlock. Once inside the containment barrier, access is provided to either the multi-purpose animal procedure/holding suite, or the Select Agent suite, which includes two animal holding rooms. Both areas share an aerosol/procedure room, and a cage rack/equipment decontamination chamber. Both suites exit through a dirty corridor, which leads to either the bulk pass-thru autoclave, or the degowning/exit rooms. Animal husbandry staff support areas are located outside the containment barrier.