University of Pennsylvania

Annual Progress Report: 2006 Formula Grant

Reporting Period

July 1, 2010 – December 31, 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.

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.

Principal Investigator

David A. Asch, MD, MBA
Leonard Davis Institute of Health Economics
University of Pennsylvania
Colonial Penn Center – 210
3641 Locust Walk
Philadelphia, PA 19104

Other Participating Researchers

Katrina Armstrong, MD, MSCE (Project Director 1/1/2007—9/30/2007) - employed by University of Pennsylvania
NEW – Peter W. Groeneveld, MD, MS (Project Director 10/1/2007—12/31/2010) - employed by University of Pennsylvania

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

Health Services Research Data Center (HSRDC) Overview

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 2010, 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 has provided 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 and it has encouraged numerous collaborations on increasingly interdisciplinary research that includes investigators from the Wharton School and the Schools of Medicine, Nursing, and Dentistry.

HSRDC Personnel Update

The Data Center Coordinator (Gina Pugliano, 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. Pugliano 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 high-performance server with four 2.4 GHz E7330 Xeon quad-processors and with a 90 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. In 2010, 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.10) 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 74 researchers are active on the HSRDC server, an increase of 22 users, or 42%, 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 Penn's 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 (five new this year, eight ongoing); five additional studies completed during the past year. Included below are brief descriptions of all new projects that have used the HSRDC server for data storage and statistical analysis during FY2011 (Table A):

New Projects

- *Measuring Quality of Care in U.S. Hospitals*. Led by Rachel Werner, MD, PhD, this study is designed to examine quality of care in U.S. hospitals. The specific objectives are: 1) to measure quality of care of U.S. hospitals, 2) examine the relationship between different measures of hospital quality of care, 3) examine how hospital quality of care varies with patient- and area-level factors, and 4) examine whether hospital quality of care changes with quality improvement initiatives.
- *Evaluating Pay-for-Performance and its Design: Evidence form Nursing Homes.* Led by Dr. Werner, this study is designed to evaluate whether pay for performance improves nursing home quality and how the design of pay-for performance programs affects quality improvement. Our specific aims are 1) To examine the impact of pay-for-performance on quality of care, 2) To examine how the design of pay-for-performance affects improvements in quality of care, and 3) To examine the impact of pay-for-performance on disparities in care between providers
- *Comparing VA Health Disparities to Health Disparities outside of the VA.* Led by Kevin Volpp, MD, PhD, this study is designed to determine if the lower mortality often observed in teaching-intensive hospitals is because of lower complication rates or lower death rates after complications (failure to rescue) and whether the benefits at these hospitals accrue equally to white and black patients, since black patients receive a disproportionate share of their care at teaching-intensive hospitals.
- *Impact on Resident Work Hour Rules on Errors and Quality.* Led by Dr. Volpp, this study is designed to determine whether the change in duty hour regulations was associated with relative changes in quality of care among Medicare patients in hospitals of different teaching intensity. Funded by the NHLBI.
- *Breast Cancer Risk Stratification*. Led by Katrina Armstrong, MD, MSCE, the goal of this project is to determine if incorporating pre-test risk improves mammography risk stratification. The project simulates a cohort of women undergoing screening mammography, and calculating pre-test risks based on genetic and standard risk factors. Bayes probability rules are then used to determine post-test risk.

Ongoing Projects

- *Improving the Value of Pay-for-Performance.* An Evaluation in Hospitals. Led by Co-Investigators Dr. Werner and Dan Polsky, PhD, this study has been funded by the Agency for Healthcare Research and Quality (AHRQ). It is focusing on the impact of P4P on the value of health care, examining which providers are the most likely to show value improvements under P4P, and testing 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.
- *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 Dr. Volpp was awarded a subcontract. Dr. Volpp's research is providing technical assistance to hospitals and other stakeholders in order to reduce avoidable re-hospitalizations, and creating a robust learning community of medical providers to improve quality of care.
- *Do Nursing Home Report Cards Improve the Quality of Care for Persons with Dementia?* Investigator R. Tamara Konetzka, Ph.D 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 is examining in detail how new technology increases health care costs, and it is investigating how cost growth due to new technology is influenced by conditions in local health care markets as well as Medicare's coverage policies.
- *Influence of Obesity on Quality of Care.* The research of Virginia Chang, MD, PhD, funded by Robert Wood Johnson Foundation (RWJF), focuses on the potential influence of patient weight status on quality of care and provider-patient communication. Dr. Chang is also investigating the extent to which reported racial disparities in care can be explained by differences in body weight.
- *Implications of Cardiovascular Technology Diffusion among Medicare Beneficiaries.* Funded by the National Heart, Lung, and Blood Institute (NIH), Dr. Groeneveld's ongoing research measures diffusion, utilization, outcomes, and costs of three implantable cardiovascular devices. The goals of the project are to determine if racial disparities, CMS reimbursement policies, or hospital characteristics can predict early adoption of new cardiovascular devices, and if variations in outcomes yielded by these technologies are relative to increases in cost of care.
- *Impact of Treatment Profitability on Hospital Responses to Financial Stress.* Led by Dr. Volpp, this project assesses differences in profitability among hospital service lines, and the relationship of these differences to hospital outcomes. Dr. Volpp's project is supported by the RWJF - Health Care Financing and Organization.
- *Market Competition and the Quality of Home Health Services.* Led by Dr. Polsky, the goal of this project is to establish the relationship between competition and quality in the home health industry, and the relationship between competition and the market-based reform of the Home Health Quality Initiative (HHQI). This project will test whether competition improves quality,

whether HHQI improves quality, and whether HHQI is more likely to improve quality in more competitive markets. Funding has been provided by the NHLBI (NIH).

Projects Completed During FY10

- *Impact of Advanced Medical Devices and Procedures on Cardiovascular Diseases.* Led by Dr. Groeneveld and funded by the Institute for Health Technology Studies, this project provided a comprehensive cost-benefit analysis of the value of cardiovascular devices. Outcomes were measured in terms of short-term effects of medical technology utilization, the survival benefit of receiving a cardiovascular device/procedure, short-term and long-term utilization and costs of medical care as reimbursed by Medicare, and quality of life among device recipients.
- *Segregation and Racial Disparities in Prostate Cancer.* Co-investigators Dr. Armstrong and Tim Rebbeck, PhD aimed to demystify the complex interaction of biological, clinical, behavioral, and environmental factors predictive of outcomes following a prostate cancer diagnosis, evaluated how these factors might explain ethnic disparities in prostate cancer outcomes, and disseminated this information to at-risk populations and the public health community. The project was funded by the National Cancer Institute (NCI).
- *The Impact of Public Reporting on the Quality of Post-Acute Care.* Dr. Werner's research focused on the impact of quality improvement incentives on health care delivery, health care organization and finances, racial disparities, and overall quality of care. The project examined whether publicly reporting quality information and incentivizing specific aspects of health outcomes is detrimental to the quality of unreported, nontargeted areas of care. This project was financially supported by the Agency for Healthcare Research and Quality.
- *Critical Care Unit Transfers in a Medicare Population.* Funded by the American Thoracic Society (ATS), Theodore Iwashyna, MD, PhD investigated patterns of patient transfers within the critical care network, examining how the transfer of patients from low-performing to high-performing hospitals could improve patient outcomes, and how the existing transfer network could be improved to yield substantial improvements in patient survival rates.
- *Evaluating Performance Measurement in Older Clinically Complex Patients.* Dr. Werner examined the efficacy and appropriateness of performance measurement in assessing the quality of health care provided to older adults. The project investigated the extent to which performance measures improve actual health care quality, versus focusing physician attention on compliance with measured quality indicators and diverting attention from unmeasured aspects of care. Financial support was provided by the Robert Wood Johnson Foundation.

Publications

Use of the HSRDC server for data storage and analysis resulted in 11 scientific publications during FY2011 (Table B).

Health Research Formula Fund FY11 Report Table A

FY 2011 Research Projects Using the HSR Data Center

Projects currently active on HSR Data Center		
Investigator(s)	Project	Funder
Katrina Armstrong, MD, MSCE	Breast Cancer Risk Stratification	National Cancer Institute (NIH)
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)
Peter Groeneveld, MD, MS	The Comparative Effectiveness of Cardiovascular devices and Medicare Cost Growth	Agency for Healthcare Research and Quality (AHRQ)
R. Tamara Konetzka, MD	Do Nursing Home Report Cards Improve the Quality of Care for Persons with Dementia	Illinois Department of Public Health
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)
Kevin Volpp, MD, PhD	Comparing VA Health Disparaties outside of the VA	Department of Veterans Affairs HS R&D service
Kevin Volpp, MD, PhD	Impact on Resident Work Hour Rules on Errors and Quality	National Heart, Lung, and Blood Institute (NIH)
Rachel Werner, MD, PhD and Daniel Polsky, PhD	Improving the Value of Pay-For-Performance	Agency for Healthcare Research and Quality (AHRQ)
Rachel Werner, MD, PhD	Evaluating pay-for-performance and its design: evidence from nursing homes	National Institute of Aging (NIH)
Rachel Werner, MD, PhD	Measuring quality of care in U.S. hospitals	Agency for Healthcare Research and Quality (AHRQ)

Health Research Formula Fund FY11 Report, Table B

Peer-Reviewed Publications of Scientific Work
Conducted on the HSR Data Center, FY 2011

Bekelman JE, Mitra N, Efstathiou J, Liao K, Sunderland R, Yeboa D, Armstrong K. Outcomes after Intensity-Modulated versus Conformal Radiotherapy in Older Men with Non-Metastatic Prostate Cancer. In review, 2011.
Groeneveld PW, Epstein AJ, Yang F, Yang L, Polsky D: Medicare's policy on carotid stents limited use to hospitals meeting quality guidelines yet did not hurt disadvantaged. *Health Affairs*. 2011; 30:312-321
Groeneveld PW, Polsky D, Yang F, Yang L, Epstein AJ: The impact of new

cardiovascular device technology on health care costs. *Arch Intern Med.* In press, 2011.
Handorf E, Bekelman J, Hietjan D, Mitra N. Assessing the sensitivity of treatment effect on cost to unknown confounders. In review, 2011.
Merchant RM, Becker LB, Yang F, Groeneveld PW: Racial disparities in survival after out-of-hospital cardiac arrest. *Am Heart J.* In press, 2011.
Park J, Konetzka RT, Werner RM. Performing well on nursing home report cards: Does it pay off? *Health Services Research*. In press, 2011.
Park J, Werner RM. Changes in the relationship between nursing home financial performance and quality of care under public reporting. *Health Economics*. In press, 2011.
Polsky D. Glick HA. Yang J. Subramaniam GA. Poole SA. Woody GE. "Cost-effectiveness of Extended Buprenorphine-Naloxone Treatment for Opioid-Addicted Youth: Data from a Randomized Trial." *Addiction*. 2010; 105:1616-1624.
Pollack C, Bekelman J, Liao K, Armstrong K. Hospital racial composition and the treatment of localized prostate cancer. In review. 2011.
Werner RM, Bradlow EB. Public reporting on hospital process improvements is linked to better patient outcomes. *Health Affairs*. 2010; 29:1319-1324.
Werner RM, Konetzka RT, Stuart EA, Polsky D. Changes in patient sorting to nursing homes under public reporting: Improved matching or provider gaming? *Health Services Research*. In press, 2011.
Werner RM. Stuart EA. Polsky D**.** "What drives nursing home quality improvement under public reporting? An examination of post-acute care" *Health Affairs*. 2010. 29(9): 1706-1713.
Yeboa D, Guzzo T, Mitra N, Christodouleas J, Haas N, Vapiwala N, Armstrong K, Bekelman J. PSA surveillance among men with clinically localized prostate cancer who do not receive initial treatment. In review. 2011.

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.

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.

Principal Investigator

Lewis A. Chodosh, MD, PhD
University of Pennsylvania
School of Medicine
Department of Cancer Biology
612 BRB II/III, 421 Curie Blvd.
Philadelphia, PA 19104-6160

Other Participating Researchers

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

Determine whether cells with tumorigenic potential are enriched in residual neoplastic lesions.

We sought to determine whether GFP-positive cells from neoplastic lesions are enriched for tumorigenic potential. We reasoned that if the cells that survive oncogene down-regulation have cancer stem cell-like properties, then the tumor initiating cell (TIC) frequency should be higher in GFP-positive cells isolated from residual lesions compared to GFP-positive cells isolated from primary tumors. To address this we employed an assay to determine TIC frequency in which different dilutions of cells are injected into the fat pad of recipient mice and scored for their ability to form tumors.

GFP-labeled primary MTB/TAN tumor cells were injected into the fat pad of recipient mice on doxycycline and tumors allowed to form. Once tumors reached 3 mm, mice were sacrificed and the primary tumors were harvested. Primary tumors were then dissociated and GFP-positive cells isolated by Fluorescence-activated cell sorting (FACS). Limiting numbers of GFP-positive primary tumor cells were then injected into the fats pad of recipient mice on doxycycline to measure TIC frequency. A schematic of this experiment is shown in Figure 1.

We first sought to measure TIC frequency in primary MTB/TAN tumors to confirm our ability to sort GFP-positive cells, inject limiting numbers into recipient mice, and score tumor outgrowth. Primary GFP-labeled MTB/TAN tumors were digested and GFP-positive cells were isolated by FACS. Limiting numbers of cells (50, 200, 500, or 1500) were injected into the fat pad of recipient mice and tumor formation was scored after 13 weeks. The fraction of sites that

developed tumors from each cell number is shown in Table 1. Based upon these proportions we calculate a TIC frequency of 1/35 (1/12 – 1/104) in primary MTB/TAN tumors.

These results demonstrate that a relatively high percentage of cells in MTB/TAN tumors have tumor initiating potential. Importantly, these results also show that we are able to isolate GFP-positive cells from different populations and assess TIC frequency. The finding that primary tumors already have a high percentage of cells with tumorigenic potential could make it less likely that we will be able to detect enrichment in residual lesions for cells with enhanced tumorigenic potential, if it occurs.

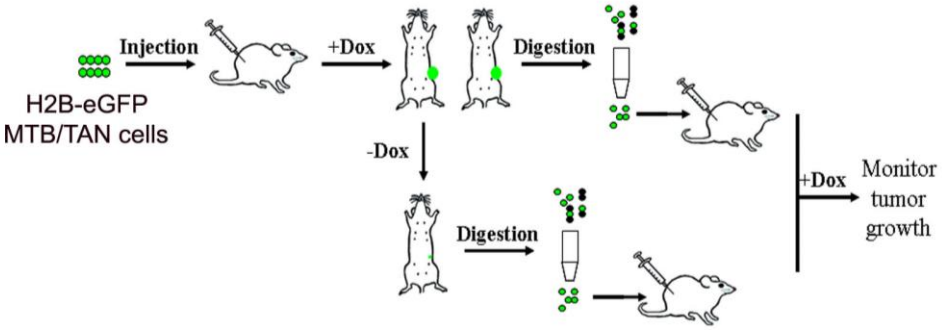


Figure 1. Experimental design to compare TIC frequency of primary tumors and residual lesions. H2B-eGFP-labeled MTB/TAN cells were injected into recipient mice. Following tumor formation, primary tumors were harvested. Primary tumors were dissociated and GFP-positive cells were isolated by FACS. TIC frequency was determined by limiting dilution injections into recipient mice and determination of tumor formation.

# of cells	Host	Cell type	Tumor outgrowths
50	Nude	GFP+PI-	3/4
200	Nude	GFP+PI-	6/6
500	Nude	GFP+PI-	6/6
1.5k	Nude	GFP+PI-	6/6
		TIC frequency: 1/35 (1/12 – 1/104)	

Table 1. Serial dilution transplantation efficiency for tumor outgrowth.

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

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 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

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 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

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 6: Project Title and Purpose

Clinical Trials of Novel Targeted Therapies in Patients with Advanced Cancers - Treatment of most metastatic cancers 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 (angiogenesis) and the abnormal function of enzymes within the tumor cell. Clinical trials will be conducted evaluating novel strategies to inhibit tumor blood vessel formation (antiangiogenic therapy) and, separately, to block the function of abnormal enzymes within tumor cells. In addition, a technology platform will be developed for analyzing/quantifying angiogenic activity in human cancer specimens. This will lay the groundwork for discovery of potential predictive biomarkers of tumor responsiveness to antiangiogenic therapy.

Duration of Project

7/1/2007 - 12/31/2010

Project Overview

Specific Aim 1: To evaluate the effect of 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 (RCC) 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) and its receptors have demonstrated efficacy in patients with metastatic RCC. However, some patients respond to treatment with these inhibitors while others are refractory, and current clinicopathological features do not allow prediction of response. Along with conduct of new clinical trials of antiangiogenic agents in RCC, identification of new predictive biomarkers of therapeutic response will be a goal of this project.

Activating mutations in the serine threonine kinase BRAF have been identified in 7% of all solid tumors and in a majority of malignant melanomas. These mutations 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.

Principal Investigator

William F. M. Lee, M.D., Ph.D.
Abramson Cancer Center
University of Pennsylvania
712 BRB, 421 Curie Blvd.
Philadelphia, PA 19104

Other Participating Researchers

Katherine L. Nathanson, M.D., Abramson Cancer Center of the University of Pennsylvania

Expected Research Outcomes and Benefits

The clinical trials proposed in this project will produce preliminary evidence of therapeutic effect among the patients treated. This data will serve as the basis of 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. And, biomarkers developed in the conduct of this project will be proposed for validation in such trials.

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. Additional regimens that more effectively inhibit this process will likely translate into further benefit for patients with these and other types of cancers. Identification of biomarkers that predict which patients will respond and/or which will not will greatly facilitate the use of these new therapeutics and help personalize cancer care.

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

Antiangiogenic clinical trials A clinical trial of sorafenib (a multikinase inhibitor and antiangiogenic agent) in patients with metastatic RCC was conducted at Penn several years ago. Tumor material from patients in this trial will be used to help identify biomarkers predictive of response to antiangiogenic therapy. An ongoing clinical trial of sunitinib (a multikinase and VEGF receptor kinase inhibitor and antiangiogenic agent) as first line therapy of metastatic ccRCC (UPCC03809) is currently being conducted at Penn. In the past 6 months of the granting period, the latter trial continues to accrue patients. A total of 15 patients have been accrued to date. 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 are being analyzed by histocytometry (see below). The results of histocytometry analysis, the paired DCE-MRI studies (including Ktrans), and clinical parameters of response will be then be correlated to help identify biomarkers predictive of response to antiangiogenic therapy. Finally, the investigator who initiated this project, Dr. Keith Flaherty, is PI of an Eastern Cooperative Oncology Group clinical trial (ECOG 2804) in which patients with metastatic RCC are randomized to treatment with (a) bevacizumab, (b) bevacizumab+sorafenib, (c) bevacizumab+temsirolimus, or (d) sorafenib+temsirolimus to determine the most effective combination therapy.

Development of the histocytometry analysis platform
Currently, there is no method to assess tumor angiogenesis or tumor angiogenic activity – the process that is targeted for inhibition in antiangiogenic cancer therapy. Development of a method to measure/quantify this activity in patient tumors is a goal of this project. Such a method might allow identification of patients most likely to benefit from antiangiogenic therapy and targeted antiangiogenic agents.

Study of mouse tumors treated with selected antiangiogenic agents revealed characteristic changes in endothelial cell signaling and the pharmacodynamics of antiangiogenic agents. With the support of this grant, we have been developing 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 (multiplex immunostaining): Some antigens are for identifying cell types of interest (endothelial

cells and tumor cells), while other antigens reveal the analyte(s) being studied (e.g. biomarkers of cell proliferation [Ki67] or signal transduction activity [phospho(p)-ERK, p-AKT, etc.]). Staining for analyte antigens is usually done by immunohistochemical staining (IHC) using DAB reporter, as this provides maximum sensitivity of detection for these rarer antigens (many are poorly revealed by less sensitive IF staining). On the other hand, staining for cell-typing antigens, which tend to be more abundant structural antigens, is usually performed by immunofluorescent (IF) staining using fluorescent reporters. The multiplex-stained slides are imaged multispectrally in both brightfield and fluorescence mode using a microscope fitted with a multispectral imager (Vectra®, CRi, Woburn MA) to generate brightfield and fluorescent "cubes" of spectral data (each cube represents a stack of images of one microscopy field of view taken at pre-determined wavelength intervals, e.g. 420nm, 440nm, 460nm, etc. up to 720nm). Commercial software (Nuance®, CRi) is then used to "unmix" the image cubes into the component chromogens and fluorochromes present in the images. This results in separation of the individual chromogens and fluorochromes (and the structures and antigens they represent) into individual "channels" of image data. Analysis then proceeds using custom FARSIGHT software. For vessel/endothelial cell and tumor cell analysis in RCC specimens, each slide is typically immunostained for four antigens (CD34, CA IX, SMA plus an analyte of interest) and counterstained with hematoxylin (stains nuclei); unmixing results in 5 channels of stain data.

<u>FARSIGHT image analysis</u> FARSIGHT is the custom image analysis software used to process the information in the different channels of stain information resulting from spectral "unmixing". It processes the data in an ordered sequence. In step 1, the nuclei of cells in images are "segmented" (delineated) using data in the hematoxylin channel. In step 2, the segmented nuclei are classified as belonging to cell types of specific interest (endothelial and tumor cells) based on their proximity to staining for cell-type antigens. Nuclei belonging to RCC tumor cells are typed by their proximity to carbonic anhydrase (CA) IX staining/channel (CA IX is a plasma membrane protein that is upregulated and overexpressed in tumor cells in the majority of RCC). Nuclei belonging to endothelial cells are typed by their proximity to CD34 staining/channel and their lack of association with SMA and CA IX staining/channels; the latter eliminates nuclei of pericytes and tumor cells, respectively, which may be situated near vessels and CD34 staining. Subsequently, the cell boundaries of individual tumor cells may be segmented, if desired, using the pattern of staining in the CA IX channel. In step 3, analyte staining is referenced to the nuclei/cells segmented and classified in the prior two steps. The result is identification of individual tumor and endothelial cells in images of RCC histopathology specimens and quantification of analyte expression (e.g. Ki67, p-ERK, p-STAT3 and/or p-AKT) in each of these cells. 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.

In the past year, numerous software improvements were made in order to address the special needs of endothelial cell and tumor cell analysis in clinical RCC specimens – more automatic operations and reduced need for human input. Fundamental improvements were made resulting in core segmentation algorithms that (1) produce more accurate results faster, (2) additional cell

features specifically designed to help identify vascular endothelial cells and to separate them from pericytes, (3) 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, and (4) advanced editing functions. These have been tested and shown to function well. In the past 6 months, FARSIGHT has been developed to permit (5) submission of multiple images for analysis at one time (i.e. in batches of tens to hundreds of images), rather than submission of one image for analysis at a time, and (6) presentation of results on endothelial and tumor cell expression of analytes of interest in easily interpretable, tabulated format. The histocytometry platform is currently being tested and validated on clinical RCC samples from patients enrolled in past and present antiangiogenic clinical trials. Tumors from 4 patients in the sorafenib RCC clinical trial and from 8 patients in the sunitinib RCC clinical trial conducted at Penn have been studied to date.

Specific Aim 2 Results of clinical trials of PLX4032 have been reported and application for approval of this agent for treatment of patients with metastatic melanoma (Plexxicon/Genentech-Roche) is underway.

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.

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 mice (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.

Principal Investigator

Jonathan Epstein, MD
Chair, Department of Cell and Developmental Biology
University of Pennsylvania
1154 BRB II
421 Curie Blvd.
Philadelphia, PA 19104-6058
(215) 898-8731

Other Participating Researchers

Gwenn Danet, PhD, Kurt Engleka, Qiaohong Wang, Jun Li, Nicole Antonucci – employed by University of Pennsylvania

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.

Currently, our maximum capacity is 6 x 140 cages = 840 cages (4200 mice). Of note, the NSG colony has been so successful that at the end of the grant period, we have initiated plans to buy additional microisolator racks in order to expand the colony.

In prior years, we focused on developing use of the Xenogen Imaging System. The Xenogen Imaging System is being used routinely to study tumor samples.

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

Previously we expanded the NSG breeding. Last year we produced an average of 40 pups per week (approximately 2000 animals per year). In the calendar year, 2010, this number was increased to 50-60 pups per week. Thus, the NSG colony is being widely used by Penn researchers for studies of HIV, cancer, and stem cell biology.

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)

In the previous grant period, we were able to determine that we get consistent high engraftment using $2x10^5$ CD34 cells per mouse. This is now our standard methodology.In addition to studies described below for UCB stem cell experiments, we now routinely use engraftment of NSG mice with human CD34 cells to generate "humanized" NSG mice with human lymphoid cells. These mice are used for studies of immunotherapy of cancer and HIV.

<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.*

Progress on the trial has been slow but promising. Four patients have now been treated on this trial. Preliminary results suggest that in fact engraftment is hastened in patients receiving ex vivo expanded T cells compared to historical controls but further work is necessary to demonstrate that this result is statistically robust. Note that funding for the clinical trial is NOT from this grant.

Previously we 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 the 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. Initial studies of this work in the end of 2010 are quite promising. In particular, the incubation of CD34 cells with lithium and rapamycin maintained stem cells as viable SCID engrafting cells for 4 days in culture, unlike control conditions. Work on this Aim is still being replicated in collaboration with Dr. Klein.

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

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 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.

Duration of Project

7 /1/2009 – 12/31/2010

Project Overview

The University of Pennsylvania is playing an increasingly important 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.

Principal Investigator

Glen N. Gaulton, PhD
Office of the Executive Vice Dean
Chief Scientific Officer
University of Pennsylvania Medicine
354 Biomedical Research Building II/III
421 Curie Blvd.
Philadelphia, PA 19104-6160

Other Participating Researchers

None

Expected Research Outcomes and Benefits

Outcomes: The design and operation of these new facilities meet the objective of enhancing the overall ABSL3/BSL3 programs of the University of Pennsylvania School of Medicine. This project represents an important phase in the development of our research resources and physical infrastructure improvement, and will make it possible for Penn investigators to continue to expand their work on emerging infectious diseases and HIV.

Now that the BSL3 is open and the ABSL3 will open shortly, Penn investigators can submit grants on infectious agents that require high-level biocontainment. With the completion of the project, Penn investigators are able to launch studies on a variety of emerging infectious diseases including SARS, West Nile, Dengue, Coxiella, hantaviruses and alphaviruses. This facility will be open to all investigators who need BSL3 level containment. Importantly, having these new, state-of-the-art facilities will make it possible for Penn to 1) recruit new faculty whose work is centered on emerging infectious agents, and 2) secure extramural funding to advance research in this area.

Summary of Research Completed

During the period of July 1, 2010 through June 30, 2010, construction continued on both the BSL3 and ABSL3 sites. The final design consolidated the project into two locations, rather than three as originally proposed. Specific Aim 2 — the development of a discrete BLS3 laboratory through the renovation of an existing, unoccupied tissue culture room — was eliminated through the expansion of the BSL3 and ABSL3 program areas in Specific Aims 1 and 3. Modifications to the tissue culture room to accommodate the safe handling of HIV diminished the original intent of placing the 500 net sq. ft. BSL3 laboratory in this area. Indeed, although we eliminated one site, the overall area increased from 4,994NSF proposed in our original specific aims to 6,039NSF in the final project. Thus, the plan as implemented meets the specific intent of the HRFF grant and needs of our institution.

Construction of BSL3 facility was completed during this reporting period and the new 1,860 NSF BSL3 facility was fully commissioned and available for occupancy in January 2011. This 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.

Construction of the ABSL3 facility is ongoing with completion expected in the first quarter of 2011. HRFF funds were expended prior to 12/31/2010 and institutional funds are being used to complete the project. Due to the more extensive scope of work and unexpected delays, the construction of the 4,179 NSF ABSL3 facility is projected to be complete in February with commissioning completed in April 2011. This new 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.

The University of Pennsylvania School of Medicine, with this essential award from the PA Department of Health HRFF program, combined with funding from the NIH and institutional resources, has built a state-of-the-art BSL3 Suite and an ABSL3 Suite within the School's ten-building, interconnected, main campus in West Philadelphia. The successful completion of this project provides essential support for the current and future needs of our scientific community.

Research Project 11: Project Title and Purpose

Prevention of Nosocomial Infections – The purpose of this project is to develop proof-of-concept technology for preventing the transmission of antibiotic resistant bacterial infections in medical facilities. This technology is based on the antimicrobial activity of nitric oxide, and it will be embodied in a device that treats and disinfects the hands of staff and visitors before and after contact with patients. The efficacy and safety of nitric oxide disinfection has already been established in human studies. The first stage of this project will yield a prototype device that can deliver nitric oxide in a manner that is acceptable to potential users, and that enables us to determine optimal operating parameters for the device. Human testing of its efficacy against bacteria will occur in a subsequent stage of development and will be supported by a different source of funding.

Duration of Project

7/1/2010 – 12/31/2010

Project Overview

Objectives and aims: Nosocomial infections are most often caused by bacteria that are transmitted from patient to patient by hospital staff and visitors. Over the 5 ½ month duration of this project, we aim to design and fabricate a prototype proof-of-concept device for killing disease-causing causing bacteria that superficially adhere to human skin and cause nosocomial infections. The device will be designed for deployment in hospitals and other medical centers, and used by staff and visitors to disinfect their hands before and after contact with patients. It will employ nitric oxide, which kills disease-causing bacteria, and is non-irritating to the skin. The two main alternatives at present – handwashing and alcohol-based hand sanitizers – are ineffective because of inconsistent personal technique. Moreover, compliance is poor because they are inconvenient and irritating to the skin. The proposed device would replace them with a simple, passive, quick, dry, safe, inexpensive, and non-irritating procedure that would not rely on personal technique to be effective.

Design and Methods: The efficacy of nitric oxide as a topical disinfectant and its safety for human skin is well documented; however there are several key variables that must be considered when applying it in a healthcare setting. This project will focus on the development of a prototype device that will enable us to adjust and optimize these parameters. These parameters include: (1) size, shape, and access to the compartment in which treatment will occur. We will design a compartment that accommodates hands of various sizes, and people of various dimensions – including children. Access to the compartment will be controlled with inflatable cuffs that retain gas within the compartment during treatment. The device will be built at the University of Pennsylvania in the Biomedical Research Instrumentation Shop by personnel with whom the PI has had a longstanding relationship. (2) Duration of treatment and gas concentration. The antimicrobial efficacy of nitric oxide follows a classic time×concentration product, meaning that treatment time may be reduced by a factor of 2 if gas concentration is increased by the same factor. User acceptance and compliance is likely to increase as the treatment time is reduced. Therefore, we will aim for treatments no longer than 15 sec in duration, and determine the gas concentration required to kill bacteria with treatments of this duration. (3) Gas composition. The efficacy of nitric oxide against bacteria also depends on the oxygen concentration and humidity. Experiments will be conducted to determine how they affect the gas concentrations required to complete an effective treatment with the treatment duration goal of 15 sec.

Principal Investigator

Paul H. Axelsen, MD
University of Pennsylvania
105 Johnson Pavilion
3610 Hamilton Walk
Philadelphia, PA 19104-6084

Other Participating Researchers

None

Expected Research Outcomes and Benefits

Outcomes: This project will yield a proof-of-concept prototype device ready for clinical trials. Its design will be optimized for user acceptance, and for accessibility to persons with different hand and body sizes, including children. Its operating parameters will be optimized to shorten treatment durations as much as possible and thereby increase compliance.

Benefits: This project addresses a problem of immense medical and economic significance: the spread of highly resistant bacterial infections in hospitals and other medical centers. These infections are common – every medical center currently struggles to control them, and often fails. These infections are expensive – the cost of treating one infection usually exceeds the entire infection control budget of an average-sized hospital. These infections are also deadly – most hospitals now encounter several infections each year for which there are no effective treatments. The most effective means to control the spread of these infections within hospitals is simple handwashing. However, handwashing fails because it is not done effectively, there are an insufficient number of places to wash hands in most hospitals, and human skin does not tolerate as many washings per day as are necessary. Hand sanitizers have been offered as an alternative, but they have the same drawbacks.

This project will provide an alternative to alcohol-based hand sanitizers and handwashing that is superior in efficacy and user compliance. The device is simple, passive, quick, dry, safe, inexpensive, non-irritating to the skin on repeated use, and it does not rely on personal technique to be effective. These characteristics will improve efficacy by improving compliance.

Summary of Research Completed

This project yielded a working prototype that delivers nitric oxide in a manner that is acceptable to potential users, and that enabled us to determine optimal operating parameters for the device.

The project has been enormously successful. The prototype device not only works, but works fast enough to assure low risk and high user acceptance. Precise details cannot be provided at this point because a formal disclosure has only just been initiated through the Technology Transfer Office at Penn. However, in keeping with the research plan as proposed in our application, the prototype delivers a low concentration of nitric oxide gas in a manner that consistently kills >99% of bacteria on a surface. The experimental results are controlled, quantitative, reproducible, and statistically sound. After suitable protections are in place for the intellectual property, the results will be suitable for publication.

<u>Research Project 12: Project Title and Purpose</u>

Development of a Sleeve Introducer Device for Rapid, Safe and Easier Placement of Naso Gastric Tubes – Nasoenteral tubes were originally designed to deliver therapy and are now also used for diagnosis, decompression, and feeding. Large bore enteral tubes are more often placed for decompression and function via a double lumen sump mechanism, while small bore tubes are softer single lumen tubes that are typically used for feeding and delivery of therapy. Despite a

long history of nasoenteral tubes, placement remains difficult and even dangerous for certain patients. Difficulty is frequently described for the placement of large bore tubes under general anesthesia, while some patients can get bleeding and infections from the placement of this semi rigid tube. Rarely the tube can end up infecting or even perforating the lung. Placement in the lung occurs almost one time in fifty. This project evaluates a new sleeve introducer device designed to make this process safer, faster and less dangerous for the patient.

Duration of Project

7/1/2010 – 12/31/2010

Project Overview

The product is an inexpensive, small, disposable hollow introducer, about 15cm long and one-half cm wide, made of polymeric material that will allow for safer and rapid placement of nasoenteral tubes in awake and comatose (or anesthetized) patients. To achieve this, this project will develop initial prototypes, evaluate them, and make necessary refinements on the design. The prototypes will initially be evaluated by testing in cadavers (that will probably occur after this grant period). The final prototype/s and resulting data will be very useful and desirable information to potential companies interested in the development, marketing and sale of this product for clinical use.

Principal Investigator

Ashish C Sinha, MD, PhD
University of Pennsylvania
3400 Spruce Street
680 Dulles
Philadelphia, Pa 19104-4293

Other Participating Researchers

Kelly Malloy, MD – employed by University of Pennsylvania

Expected Research Outcomes and Benefits

In certain medical conditions a patient needs to have their stomach decompressed, for example when a patient swallows a dangerous chemical. At other times the intestines are not working properly or are obstructed, and the patient may need to have a tube placed in the stomach to empty it of the fluids that continue to be secreted. In the absence of this decompression, a patient can get nauseous, violently sick, and vomit.

To treat any of these conditions, a semi rigid tube, called a naso gastric tube (NGT) is placed into the stomach of the patient via the nose. Medical professionals in Emergency Rooms, on the patient floors and even in the Intensive Care Unit (ICU) and the Operating Room face this challenging task every day. NGTs are placed hundreds of time a day in a large hospital and

millions of times a year in the US alone. The reason this is a challenge is that the patient has to have a tube forced down the nostril, through the tender parts of the nasal mucosa, via the back of the throat into the stomach. In non-anesthetized patients, as done majority of the time, this process is painful and traumatic and probably stressful for the physician or nurse involved. Brute force, crying, screaming, luck and multiple attempts qualify this process. In children and patients under anesthesia the challenge is even greater. The current NGT appropriately serves the purposes of either placing things in or emptying the stomach; what is inadequately addressed or designed, is the act of actually placing it. This tube is essentially blindly pushed into the nose risking injury to the tender and vascular nasal lining, frequently causing bleeding and increasing sinus and other infections. Placement in the lung happens almost 1 time in 50, which is difficult to detect without an X-ray of the lung. In certain cases placement in the brain has also been reported. The overall complication rate is up to 80%; complications generally range from a minor nose bleed to pneumonia.

This project will test a device that has been partially designed to seriously decrease or even eliminate many of these challenges. Subsequently it may decrease the cost of X-rays and decrease radiation exposure from it.

Summary of Research Completed

Multiple prototypes were developed as proposed, during the grant period. This was done by computer assisted designing and using 3D- printing.

The nasoenteral introducer device was discussed with persons in the School of Medicine and in the School of Engineering at University of Pennsylvania. The faculty Otorhinolaryngology expert physician (ENT surgeon) helped with anatomical dimensions. Graduate students from the School of Engineering were identified who had interest and expertise in Computer assisted design (CAD) systems. After multiple meetings and provisional designs, a 3D printer company was identified and asked to make a prototype. This prototype was then further modified to suit physiological functions and confirm to anatomical limitations. New CAD files were drawn up and submitted to the 3D printer company. Thereafter new more acceptable multiple prototypes were developed.

These were tested for shape and size in anatomical models and seem to be feasible for cadaveric studies. Confidential development continues in refining the design based on gender, age and weight. Further discussion is difficult at this time in order to allow pursuance of further patent and commercial activities.

Currently, a patient safety initiative, at the Hospital of the University of Pennsylvania, is considering this prototype for trials.

<u>Research Project 13: Project Title and Purpose</u>

RS Rearrangement Frequency: A Clinical Lab Test for Autoimmunity – Systemic lupus erythematosus (SLE) is a debilitating and sometimes lethal autoimmune rheumatic disease that afflicts over five million individuals worldwide. Current medical therapy is given on a trial and

error basis because the underlying immune defects are not known for individual patients. However, one clue is that nearly everyone with SLE makes antibodies that bind to self (this is due to aberrant function of B cells, white blood cells that produce antibodies). The purpose of this study is to develop a clinical lab test called RS rearrangement frequency that identifies B cells that have defective early development. Identifying the developmental timing of the B cell defect in SLE will lead to improved therapy, in which specific B cell subsets can be targeted.

Duration of Project

7/1/2010 – 12/31/2010

Project Overview

Using flow cytometry and quantitative PCR, we have previously shown that approximately one-third of patients with lupus (SLE) have low levels of RS gene rearrangement (a type of antibody light chain gene rearrangement that occurs in early stage B cells). A low RS frequency is consistent with the presence of a defect during early B cell development. There are drugs in clinical use that are known to target particular B cell subsets or developmental stages. Thus, we hope that in the future this assay will guide clinicians to target their treatment to specific B cell subsets in individual patients with SLE. *In this project we have one overarching goal: to convert our research assay into a clinical lab test that can be performed on a routine blood sample.* In order to achieve this goal we will:

Aim 1: convert the RS rearrangement assay to a whole blood assay so that it can be performed without the need for B cell purification. In the whole blood assays we will use molecular calibrators (quantitative PCR assays) to determine the B cell fraction.

Aim 2: analysis of pre-analytical variables on whole blood assay performance. Determine if hemolysis, room temperature storage or frozen storage of blood alter the results of the RS assay.

Aim 3: preliminary validation of the whole blood assay. We will use the research method as a gold standard for comparison to the whole blood assay method. We will also perform the new whole blood assay in 20 SLE patients versus 20 demographically matched healthy control subjects to establish preliminary reference ranges for the assay.

The whole blood assay will be less expensive and less difficult to perform than the research assay. The ability to perform this assay in whole blood is essential for converting the assay into a test that can be performed in a clinical laboratory instead of a research laboratory. This clinical lab test can then be used to evaluate and potentially guide therapy for larger numbers of patients with autoimmune or inflammatory conditions. These conditions include not only SLE but also type 1 diabetes (where we have also observed a decreased RS frequency in one-third of patients), rheumatoid arthritis, Sjogren's syndrome, immune thrombocytopenia, autoimmune hemolytic anemia, multiple sclerosis, asthma and others.

Principal Investigator

Eline T. Luning Prak, MD, PhD
University of Pennsylvania
405B Stellar Chance Labs
422 Curie Blvd.
Philadelphia, PA 19104

Other Participating Researchers

Jonathan Dunham, MD – employed by University of Pennsylvania

Expected Research Outcomes and Benefits

Current therapy for lupus is by trial and error because physicians do not know the underlying immune defects in individual patients. There are over one hundred different mouse models of lupus and there is evidence to suggest that human lupus is similarly diverse; many different immune defects can give rise to lupus. The current laboratory tests for lupus focus on antibodies, proteins that are secreted by B cells (circulating white blood cells that are important for immune defense). But antibodies represent the final end-product of a mature B cell-- their presence does not reveal at which stage of B cell development things got off-track in an individual patient with lupus. The RS rearrangement frequency lab test that we propose to validate in this project is different from any other lab test for autoimmunity because it will allow doctors to determine if early B cell development is abnormal in their patients with lupus and other autoimmune conditions. There are multiple drugs that can target different types of B cells. A drug of particular interest with respect to early stage B cells is imatinib. Imatinib (a.k.a. Gleevec®) is FDA-approved to treat chronic myelogenous leukemia, but it also targets pre-B cells (which are the cells undergoing antibody rearrangement, including RS rearrangement). Imatinib ameliorates lupus nephritis in mouse models of lupus, including the MRL/lpr mouse, in which we have shown an abnormal RS rearrangement frequency. *In sum, the conversion of the RS assay into a clinical lab test will potentially allow physicians to target therapy towards particular B cell subsets in some of their lupus patients. In addition, the RS assay may predict which patients are susceptible to the future development of autoimmunity.*

Summary of Research Completed

<u>Aim 1: convert the RS rearrangement assay to a whole blood assay</u>.
We attempted to perform the original RS quantitative PCR assay on two different types of samples from ~5 healthy control subjects: whole blood and CD19 enriched B cells (the latter comprised approximately 50% of PMBC and over 90% of lymphocytes using positive selection with anti-CD19 magnetic beads, Miltenyi). We found that the RS quantitative PCR assay was not sufficiently sensitive to be performed on whole blood. But it did work well on CD19 enriched B cells. However, the challenge in using CD19 enriched, rather than flow cytometrically separated B cell preparations, was that the B cell fraction varied from one subject to the next. To control for the B cell content in different samples, we devised a "ratio PCR method." We reasoned that by measuring the ratio of proximal to distal Vκ-Jκ rearrangements,

we could deduce an index of the overall extent of rearrangement. Since both proximal (i.e. Vκ-Jκ1) and distal (Vκ-Jκ5 or Vκ-RS) PCRs specifically amplified antibody gene rearrangements in B cells, the use of a proximal-to-distal ratio would normalize for the B cell content between samples.

To set up this assay, we validated the following sets of PCR primers by amplifying and sequencing Vκ-Jκ and Vκ-RS rearrangements in genomic DNA from whole blood of 1-2 healthy subjects. The PCR primers and PCR conditions are summarized in **Table 1**. Table 1 shows that we have PCR assays with sequence-validated products up and running for nearly all of the major human Vκ gene families. In addition, we have cloned amplicons that will be used for absolute quantitation in qPCR experiments as well as for titration controls to compare assay sensitivities.

In addition to conventional PCR assays (summarized in Table 1, discussed above) we have developed semi-quantitative and quantitative PCR assays with a subset of Vκ-Jκ and Vκ-RS primers. The semi-quantitative method utilizes CDR3 spectratyping. In the spectratyping assay, one of the two primers is labeled with a fluorophore and the amplified products are separated by capillary electrophoresis. The spectratyping assays are summarized in **Table 2**. Because Vκ-Jκ and Vκ-RS rearrangements exhibit junctional diversity, there is variation in the sizes of the junctions between the recombining gene segments (due to P-addition and/or exonucleolytic nibbling by non-homologous end joining enzymes). As a result, different sized amplicons can be recovered using various PCR primer sets. **Table 3** summarizes the primers, probes and assay conditions for our quantitative PCR assays.

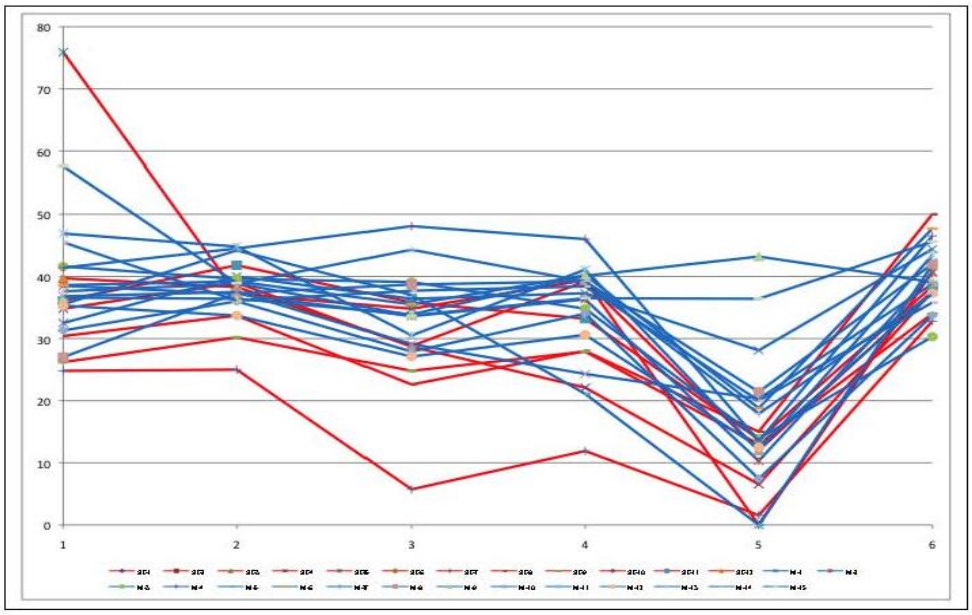
<u>Aim 2: analysis of pre-analytical variables on whole blood assay performance</u>.
We have performed preliminary experiments to determine if pre-analytical factors influence qPCR assay performance. As discussed for aim 1, the whole blood assay was not sufficiently sensitive, even under optimal conditions of sample quality. Therefore, these experiments were restricted to the analysis of CD19-enriched cells. The DNA quality and yield, as judged by spectroscopy was reduced only slightly (~5-10% reduction in yield, minimal differences in A_{260}:A_{280} ratio) when samples were stored over night at room temperature (fresh vs. 24 hrs). When qPCR was performed for β-actin, slightly lower yields (less than one cycle difference in Cp, corresponding to less than a 2-fold change in template concentration) were observed. Hemolysis had minimal effect on the sample quality and these experiments and the freezing experiments were discontinued when we realized that most of the samples we routinely processed for PCR were obtained from flow cytometry preparations. For our flow cytometry preparations, red blood cell lysis is already performed maximally.

<u>Aim 3: preliminary validation of the whole blood assay</u>.
To establish reference ranges for the new assay, we proposed to analyze genomic DNA from whole blood and CD19 enriched B cells from patients with SLE (n=20) vs. healthy control subjects (n=20). In parallel we would perform flow cytometry to determine the B cell fraction and κ/λ light chain ratio.

To date, we have recruited 12 SLE patients and 15 healthy control subjects. The number of subjects is 68% of our recruitment target of 40 subjects.

We have performed immunophenotyping, B cell separations by magnetic bead enrichment and genomic DNA extractions from CD19 enriched cells from all 25 subjects. The immunophenotyping data were notable for statistically shifts in average percentages of CD95 (Fas), IgD, lambda, the fractions of class-switched memory B cells and the proportion of TACI (CD267) expressing memory B cells ($p<0.05$ by Mann Whitney non-parametric test). The differences are statistically significant even when we correct for multiple comparisons. These data are fascinating because they suggest that there are multiple stages of B cell development that are altered in the setting of lupus, not just the early B cell stages (which the RS assay specifically measures). The lower frequency of lambda B cells, coupled in some patients with an elevated frequency of early stage B cells (transitional and naive mature) could reflect an early B cell tolerance checkpoint defect (**Fig. 1**). . Conversely, alterations in the memory B cell compartment could reflect problems later in B cell maturation (**Fig. 2**).

% lambda



B cell subset (see legend)

Fig. 1 Legend. SLE patients are shown in red, normal controls in blue. Each line respresents a separate subject. The y-axis depicts the fraction of the B cells in a given subset that expresses lambda light chains. The x-axis depicts the different B cell subsets as follows: 1= transitional (CD38++, CD27-); 2= naive mature (CD38+, CD27-); 3= mature activated (CD38+, CD27+); 4= resting memory (CD38-, CD27+); 5=plasmablasts (CD27++, CD38++) and 6 = possibly anergic (CD27-, CD38-).

Fig. 2. Alterations in TACI (CD267), Fas (CD95) and IgM expression in mature activated B cells of SLE patients.

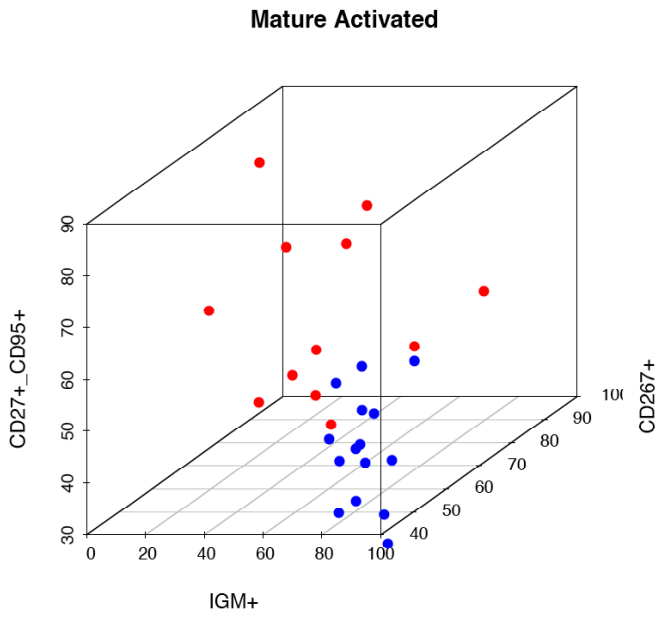


Fig. 2 Legend. Lymphocytes were gated on B cells using CD19 expression. B cells were further sub-setted into mature activated cells on the basis of CD27+ and CD38+ expression. Plotted are the percentages of mature activated B cells that express IgM (x-axis), Fas (CD95, y-axis) and TACI (CD267, z-axis). Each patient is represented by a dot. Red dots represent SLE patients and blue dots represent normal controls. The SLE patients have higher proportions of Fas and TACI expression and lower proportions of IgM+ cells. These changes could reflect a difference in memory B cell survival or activation, with a skewing towards more class switched B cells.

Table 1: PCR primers and reaction conditions

Forward Primer Name	Reverse Primer Name	Foreword primer sequence	Reverse primer sequence	Tm (C)	Amplicon size (bp)	CLONED (X-YES)
Vk1f/6#2	Jk1 Intron#2	5'-gttcagcggcagtggatctg-3'	5'-tggccaaaagtgtacacacaatg-3'	61	506	X
Vk1/4#1	Jk1-Intron#2	5'-cctgcagcctgaagatttg-3'	5'-tggccaaaagtgtacacacaatg-3'	61	420	-
Vk2f	Jk1-Intron#2	5'-ggcctccatctcctgcaggtctagtc-3'	5'-tggccaaaagtgtacacacaatg-3'	60	618	X
Vk3f	Jk1-Intron#2	5'-cccaggctcctcatctatgatgcatcc-3'	5'-tggccaaaagtgtacacacaatg-3'	64	534	-
Vk4	Jk1-Intron#2	5'-caactgcaagtccagccagagtgtttt-3'	5'-tggccaaaagtgtacacacaatg-3'	66	620	X
Vk5	Jk1-Intron#2	5'-cctgcaaagccagccaagacattgat-3'	5'-tggccaaaagtgtacacacaatg-3'	66	599	X
Vk7	Jk1-Intron#2	5'-gaccgatttcaccctcacaattaatcc-3'	5'-tggccaaaagtgtacacacaatg-3'	62	461	X
Vk1f/6#2	Jk5-Intron#2	5'-gttcagcggcagtggatctg-3'	5'-agtcaatactggccatcagacc-3'	64	222	-
Vk2f	Jk5-Intron#2	5'-ggcctccatctcctgcaggtctagtc-3'	5'-agtcaatactggccatcagacc-3'	60	342	X
Vk3f	Jk5-Intron#2	5'-cccaggctcctcatctatgatgcatcc-3'	5'-agtcaatactggccatcagacc-3'	64	248	X
Vk4	Jk5-Intron#2	5'-caactgcaagtccagccagagtgtttt-3'	5'-agtcaatactggccatcagacc-3'	66	337	X
Vk5	Jk5-Intron#2	5'-cctgcaaagccagccaagacattgat-3'	5'-agtcaatactggccatcagacc-3'	64	317	X
Vk7	Jk5-Intron#2	5'-gaccgatttcaccctcacaattaatcc-3'	5'-agtcaatactggccatcagacc-3'	62	175	X
Vk1f/6#2	KDEQR1	5'-gttcagcggcagtggatctg-3'	5'-aggcttcctagggaggtcag-3'	64	210	X
Vk2f	KDEQR1	5'-ggcctccatctcctgcaggtctagtc-3'	5'-aggcttcctagggaggtcag-3'	64	344	X
Vk3f	KDEQR1	5'-cccaggctcctcatctatgatgcatcc-3'	5'-aggcttcctagggaggtcag-3'	64	251	X
Vk4	KDEQR1	5'-caactgcaagtccagccagagtgtttt-3'	5'-aggcttcctagggaggtcag-3'	64	329	X
Vk5	KDEQR1	5'-cctgcaaagccagccaagacattgat-3'	5'-aggcttcctagggaggtcag-3'	64	317	X
Vk7	KDEQR1	5'-gaccgatttcaccctcacaattaatcc-3'	5'-aggcttcctagggaggtcag-3'	64	173	X

Table 1 Legend: Shown are the PCR primer names, nucleic acid sequences, melting temperatures, amplicon sizes (in base pairs), amplicon and a summary of cloned PCR products. The reverse primers are Jκ1 (for proximal or early stage κ rearrangements), Jκ5 (for distal Jκ rearrangements) and KDE (kappa deleting element or RS for late stage κ rearrangements).

Table 2: Spectratyping PCR Assays.

Forward Primer Names	Reverse Primer Names	Foreword primer sequence	Reverse primer sequence	Tm (C)	Amplicon size (bp)	GENE AMPLIFIED
Vk1f/6#2	Jk1-Intron#2-FAM	5'-gttcagcggcagtggatctg-3'	5'-tggccaaaagtgtacacacaatg-3'	62	506	Vk1Jk1
Vk1/4#1	Jk1-Intron#2-FAM	5'-cctgcagcctgaagatttg-3'	5'-tggccaaaagtgtacacacaatg-3'	61	420	Vk1Jk1
Vk1f/6#2	KDEQR1-FAM	5'-gttcagcggcagtggatctg-3'	5'-aggcttcctagggaggtcag-3'	61	210	Vk1KDE

Table 2 Legend: Shown are the PCR primer names, nucleic acid sequences, melting temperatures, amplicon sizes and genes amplified. FAM refers to 6-carboxyfluoresceine, which is used to label one of the two PCR primers for spectratyping. Amplicons are separated by capillary electrophoresis.

Table 3: Quantitative PCR assays.

Forward Primer Names	Reverse Primer Names	Foreword primer sequence	Reverse primer sequence	Tm (C)	Amplicon size (bp)	GENE AMPLIFIED
Vk1/4#1 Jk1/4-Vk-PROBE	Jk1-Intron#4	5'-cctgcagcctgaagattttg-3' 5'-TTTGATCTCCACCTTGGTC-3'	5'-cacagacaactgaggaagc-3'	64	133	Vk1Jk1
Vk1f/6#2	Jk1-Intron#2 Jk1/6-Vk-Probe	5'-gttcagcggcagtggatctg-3' 5'-TTCTGGACAGAGCCTTGGTC-3'	5'-tggccaaaagtgtacacacaatg-3'	64	506	Vk1Jk1
Vk1f/6#2 Jk5-Vk-Probe	Jk5-Intron#2	5'-gttcagcggcagtggatctg-3' 5'-ACGACTGGAGATTAAACGTA-3'	5'-agtcaatactggccatcagacc-3'	64	222	Vk1Jk5
Vk1f/6#2 KDEQR-Probe	KDEQR1	5'-gttcagcggcagtggatctg-3' 5'-TCTGCAGCTGCATTTTTGCCA-3'	5'-aggcttcctagggaggtcag-3'	60	210	Vk1KDE
HubactQfor1 HubactQ-Probe1	HubactQrev2	5'-cccagcacaatgaagatcaa-3' 5'-TGCCTGAGCTGACCTGGGCA-3'	5'-agtacttgcgctcaggagga-3'	61-64	180	**Beta actin**

Table 3 Legend. Shown are the primer and probe names, nucleic acid sequences, melting temperatures, amplicon sizes and genes amplified. Beta actin is used as a standard to control for sample DNA content.

Research Project 14: Project Title and Purpose

Vibrotactile and Auditory Feedback for Robotic Minimally Invasive Surgery – The purpose of this project is to determine whether tactile and/or auditory feedback of tool contact vibrations will improve the usefulness of minimally invasive robotic surgery systems. Commercial surgical robots such as the Intuitive da Vinci system enable the surgeon to see and manipulate structures deep within the body via tiny incisions. Though the robotic tools mimic one's hand motions, the surgeon cannot feel what the tools are touching, a striking contrast to non-robotic approaches. We have developed a new method for partially restoring this lost sense of touch; our VerroTouch system measures the vibrations caused by tool contact and immediately recreates them on the robot handles for the surgeon to feel and hear. This project's human subject study will measure how this additional feedback affects novice and expert surgeons during manual task performance.

Duration of Project

7/1/2010 – 12/31/2010

Project Overview

The broad objective of this project is to improve the state of the art in minimally invasive robotic surgery. This technology-mediated surgical method is now widespread and highly popular with both patients and surgeons. Long, thin instruments are inserted into the patient's body through tiny incisions, the surgeon sees a high-resolution stereoscopic image of the operating scene, and the surgical tools are robotically controlled to precisely follow the surgeon's hand motions in real time. Although minimally invasive robotic surgery systems (such as the Intuitive da Vinci S) provide excellent visualization and tool dexterity, they suffer from a notable shortcoming: the

surgeon cannot feel or hear the effects of contact between the instruments and the surgical environment, yielding an intense reliance on visual feedback. We hypothesize that providing tactile and auditory feedback of the tools' contact vibrations will significantly improve the usefulness and usability of minimally invasive robotic surgery systems. This project's specific aims all relate to testing this hypothesis.

The key to this project is VerroTouch, a fully functional vibrotactile and auditory feedback system that has already been created by the research team. VerroTouch consists of two vibration sensors, two vibration actuators, connecting circuitry, speakers, and a vibration gain knob. It attaches to an Intuitive da Vinci S surgical system very easily and does not encumber the surgeon's hand movements. When turned on, VerroTouch constantly measures the vibrations occurring at the surgical tools, which might stem from tissue cutting or tool collisions, and recreates them at the hands of the operator, so that the surgeon can naturally feel and hear what the tools are experiencing. This project will test the VerroTouch system in a controlled human subject study to determine the qualitative and quantitative effects of this additional sensory feedback on user performance.

The study's subject pool will include novice surgeons and expert robotic surgeons. After giving informed consent, each participant will be introduced to the da Vinci S surgical robot and have time to practice using it. The experimenter will then explain the set of manual tasks being used in the study (e.g., object transfer, knot tying). Each subject will complete this set of tasks under four different sensory feedback conditions: unaugmented system, with VerroTouch auditory feedback, with VerroTouch tactile feedback, and with both auditory and tactile feedback. The subject will complete a survey at the end of each condition and at the conclusion of the experiment, and we will also examine task completion times, number of errors, and applied force levels.

Principal Investigator

Katherine J. Kuchenbecker, PhD
University of Pennsylvania
224 Towne Building
220 South 33rd Street
Philadelphia, PA 19104

Other Participating Researchers

David I. Lee, MD – employed by University of Pennsylvania

Expected Research Outcomes and Benefits

This project will allow us to discover whether additional sensory feedback, namely vibrotactile and auditory cues of tool contact, can improve the usefulness and usability of minimally invasive robotic surgery systems. Surgeons use these systems every day, all around the world, to treat thousands of patients, most commonly for urological problems such as prostate cancer. At present, robotic surgeons must rely exclusively on visual feedback to guide the motions of the

tools, which is a strikingly contrast to open surgery. The planned study will scientifically show how the feedback provided by VerroTouch affects users of the da Vinci surgical system. These results will be widely published in the scholarly literature for surgery and robotics, and they will be shared with the public through online and video press releases by the research team's institution.

When we analyze the data collected in the study, we may discover that this additional sensory feedback helps surgeons perform tasks more quickly, more accurately, or more confidently. We might also discover that it helps novice surgeons achieve task performance levels closer to those of expert surgeons, implying that it could reduce the learning curve for this method of surgery, which might help prevent surgical errors. We might also discover that our innovation has no obvious benefits to surgeons; though we don't anticipate such an outcome, this discovery would help the research community know how to focus our efforts on other promising ideas.

The intellectual property behind the VerroTouch system has already been protected via a provisional patent. If the results of this project are encouraging, we expect to be able to license the idea to a company that makes minimally invasive surgical robots or to another company in the area of surgical devices. If successful, we will seek a quick transfer of this technology to industry, so that surgeons and patients can begin benefiting from this new approach to sensory feedback in minimally invasive robotic surgery as soon as possible.

Summary of Research Completed

During the period of this project, the research team designed, conducted, analyzed, and published a human-subject study testing the impact of vibrotactile and auditory feedback on surgeon performance of in vitro manipulation tasks in robotic minimally invasive surgery. The system that delivers this additional sensory feedback is called VerroTouch. It uses acceleration sensors mounted close to the surgical instruments to measure the vibrations caused by tool contact, and it uses speakers and voice coil actuators to auditorily and tactilely present these vibration signals to the surgeon in real time. The PI and the other engineers on the research team previously developed this system in close collaboration with Dr. David I. Lee, an expert urologic surgeon who uses the Intuitive da Vinci robot extensively in his clinical practice. While the VerroTouch system had previously been shown to function correctly in an engineering sense, the current project is the first study to test its performance with surgeons who are not members of the research team. As the device seeks to improve the user interface for robotic surgery, such evaluations are essential for testing the feasibility of the chosen approach and understanding the strengths and drawbacks of this new technology. The bullets below list the essential steps that were accomplished in this research.

- <u>Create materials for several candidate tasks that could be included in the study.</u> At the outset, we were not sure which tasks would be most appropriate for use in the study, as the sensory feedback provided by VerroTouch depends strongly on the actions the surgeon is executing. We began by searching the surgical training literature, reviewing the Fundamentals of Laparoscopic Surgery (FLS) Curriculum, and consulting with Dr. Lee. We then obtained and/or created the materials necessary to test six different in vitro manipulation tasks. The tasks marked with asterisks were eventually chosen for use in the study:

1. *FLS Peg Transfer: Six colored plastic objects are arrayed on a board that contains twelve vertical posts. The surgeon uses two graspers to transfer the objects to and from the other six posts, always handing the objects from one hand to the other.
2. FLS Circle Cutting: A square of gauze marked with a circle is stretched by a set of clips. The surgeon uses one scissors tool and one grasper tool to cut out the circle in the gauze, trying to follow the marked line as closely as possible.
3. *Needle Pass: A vertical piece of plastic is perforated with a set of evenly spaced holes. The surgeon uses two needle drivers to pass a suture needle with attached suture through neighboring holes to create a running stitch. This is a custom task.
4. *Intuitive Surgical, Inc. (ISI) Suturing: A round sample of synthetic skin contains several straight-sided incisions. The surgeon uses two needle drivers and a surgical needle with attached suture to tie square knots across the incisions. This task is from the ISI training curriculum.
5. ISI Dissection: A round sample of synthetic tissue contains several embedded synthetic arteries. The surgeon uses two tools to remove the overlaying tissue to expose the synthetic arteries, attempting to minimize damage to the surrounding material. This task is from the ISI training curriculum.
6. Occluded Texture Localization: A piece of plastic etched with rough textured patches is submerged in liquid that has been dyed red, to imitate blood. The surgeon uses a pair of needle drivers to explore the plastic surface and attempt to find the patches of rough texture. This is a custom task.

- <u>Run one pilot subject to select tasks and refine experimental protocol.</u> A surgical resident known to the research team participated as a pilot subject to help finalize the design of the experiment. He did all six of the proposed tasks under three experimental conditions, which was found to require an excessive amount of time. Examination of this pilot subject's experience and associated data showed that three of the tasks could be eliminated to speed up the experiment without compromising its value. Though it creates nice vibration cues, we eliminated circle cutting because it requires tool changes, which are slow and use up the lifetime of the tools. We eliminated the dissection task because the very soft synthetic tissue in our samples did not cause vibrations that could easily be felt. Lastly, we removed the occluded texture localization task because it was dangerous to the tools being used: the subject could not perform the task without vibration feedback, so they pushed far too hard on the plastic surface, damaged the tools, and sprayed red liquid across the operating room. Keeping just FLS peg transfer, needle pass, and ISI suturing allowed us to use the same two tools (both needle drivers) throughout the experiment for efficiency.
- <u>Run another pilot subject to refine experimental protocol.</u> The second pilot subject did just the three chosen tasks, which kept the experiment to a more reasonable time (less than two hours). For this second pilot subject, we increased the number of experimental conditions to four, doing the full combination of with and without auditory feedback and with and without haptic (tactile) feedback of tool vibrations. The first subject had not done the tactile-only condition, but we decided we were interested in testing this condition against the others so we could better understand the effects of the two different sensory modalities. This choice gives four feedback conditions for the study: visual only (V), with audio (VA), with haptic (VH), and with both audio and haptic (VAH).

- Set up data collection system and build instrumented task board. We wanted to be able to make quantitative measurements of the actions done by subjects during the study, so we set up an extensive data collection system. One core element is the instrumented task board shown in Figure 1: it interposes a high fidelity force/torque sensor (ATI Mini40) between the task materials and the operating table so that we may record the forces and torques the tools exert on the task materials. The data from this sensor was fed into a National Instruments USB data acquisition interface (NI USB-6259) connected to a laptop running custom data recording code. The outputs of VerroTouch's two tool-mounted accelerometers were also connected to this data collection system. Lastly, we used two DVD recorders with wireless lapel microphones to capture the left and right camera views and the words spoken by the subject and the main experimenter.
- Run a final pilot subject to test data collection and experiment design. Co-PI Lee served as the final test subject, running through the study exactly as planned for real subjects.
- Secure final IRB approval. The updated study design was submitted to Penn's Institutional Review Board to gain human subjects research approval under protocol 811721.
- Recruit subjects. We sent recruitment emails to all expert da Vinci surgeons in the greater Philadelphia area as well as all of the surgical residents at the Hospital of the University of Pennsylvania. Participation required the subject to drive to and from the Glenolden Research Facility (30 minutes each way), with approximately 90 minutes of time actually participating in the study. All available test times were on weekends and evenings due to limited availability of the Glenolden da Vinci S robot. Despite these challenges, we were pleased to be able to recruit eleven surgeon subjects, including three expert robotic surgeons and eight surgeons with no significant prior experience in robotic surgery.
- Conduct study. A team of three experimenters met each subject at the test site at the agreed-upon time. The study instructions were conveyed using a standard video, the subject was introduced to the robot, the subject practiced the three tasks, and then the subject performed all three tasks four times – once in each experimental condition, done in random order. Subjects also completed surveys after each task, after each condition, and at the end. Figure 2 shows the experimental setup in the Glenolden operating room.
- Analyze data. All sources of study data were examined, condensed, and statistically analyzed. Most importantly, the addition of a subject's favorite modality of tool vibration feedback was found to have a *significant positive impact* on their preferences between the conditions. Figure 3 shows a box plot of these ratings. Notably, no subject rated the standard da Vinci (V, visual feedback only) highest. This finding strongly indicates that surgeons appreciate the feedback provided by VerroTouch. Interestingly, none of the quantitative performance indicators (task completion time, errors, forces applied, accelerations generated) were found to be improved or diminished by the addition of tool vibration feedback. We are encouraged to know VerroTouch does not impede surgeons in performing these very standard operative tasks. The benefits of such feedback seem to depend strongly on the chosen tasks. Surgeon comments also help elucidate the subjects' perception of the system: "liked the sensory feedback of the posts hitting the graspers," "feedback helped me in determining needle position when it's close to the object," and "when suture pops, there is good feedback to the surgeon."
- Write abstract. The research team wrote a brief abstract summarizing the results of the study described above. Entitled "VerroTouch: Vibrotactile Feedback for Robotic Minimally Invasive Surgery," this abstract was submitted to the 2011 Annual Meeting of the American

Urological Association (AUA), a prestigious scholarly meeting for urologists. The abstract was accepted for presentation in a moderated poster session on "Technology & Instruments: Robotics/Laparoscopy." Numbered MP31, this moderated poster session took place on Monday, May 16, 2011 in Washington, D.C

- Write manuscript. The research team wrote a journal article that describes the way VerroTouch functions and reports the results from the study described above. Entitled "Tool Contact Acceleration Feedback for Telerobotic Surgery," this paper was submitted to the IEEE Transactions on Haptics, the premiere journal in the field of haptic (touch-based) technology. The paper was very well reviewed, and the associate editor asked for minor revisions before publication. The current version of this manuscript (incorporating all requested changes) is pending final approval by the reviewers and the associate editor.
- Apply for follow-on funding. Encouraged by the results of the current study, the PI submitted a pre-proposal to the Coulter Translational Research Awards in Biomedical Engineering program in late December, 2010. As described on the Coulter Foundation website (http://www.whcf.org/research-award/overview), this program is "directed at promising technologies with the goal of progressing toward commercial development and entering clinical practice." The PI recently heard that the project was selected for submission of a full proposal, which is due on February 27, 2011.
- File patent. Penn filed the formal patent applications for VerroTouch on February 8, 2011.

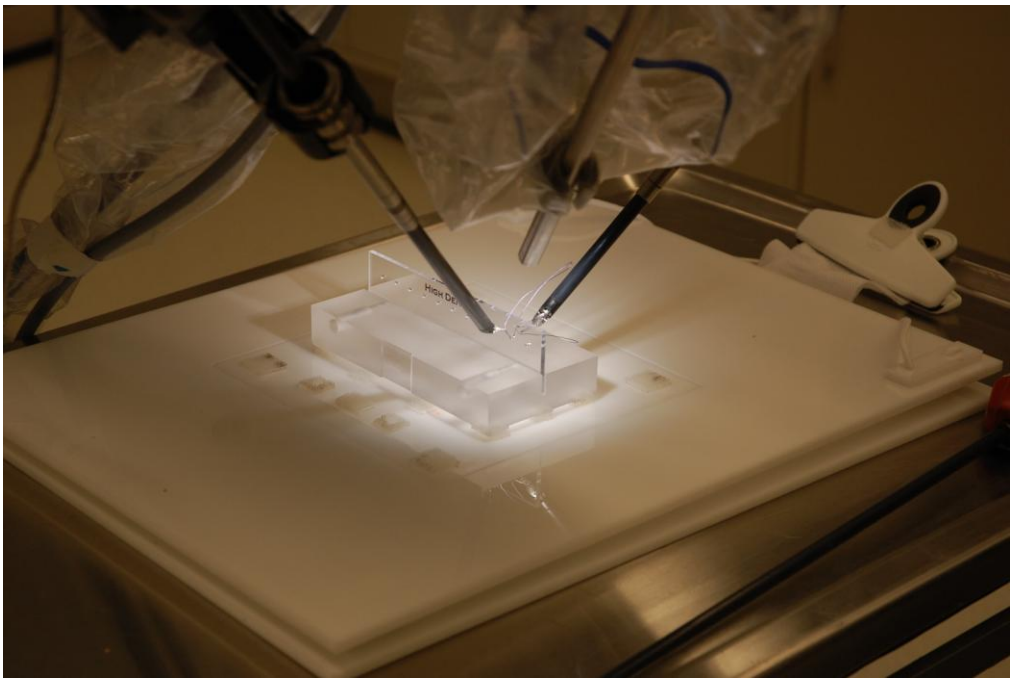


Figure 1: Instrumented task board created for use in study. The task materials are attached to the center area using repositionable fasteners similar to Velcro. The task shown is an early version of needle pass, where the surgeon threads a running stitch through holes in a piece of clear plastic. A force/torque sensor is located between the center area of the top plate and the base plate so that the forces applied to the task materials can be measured.

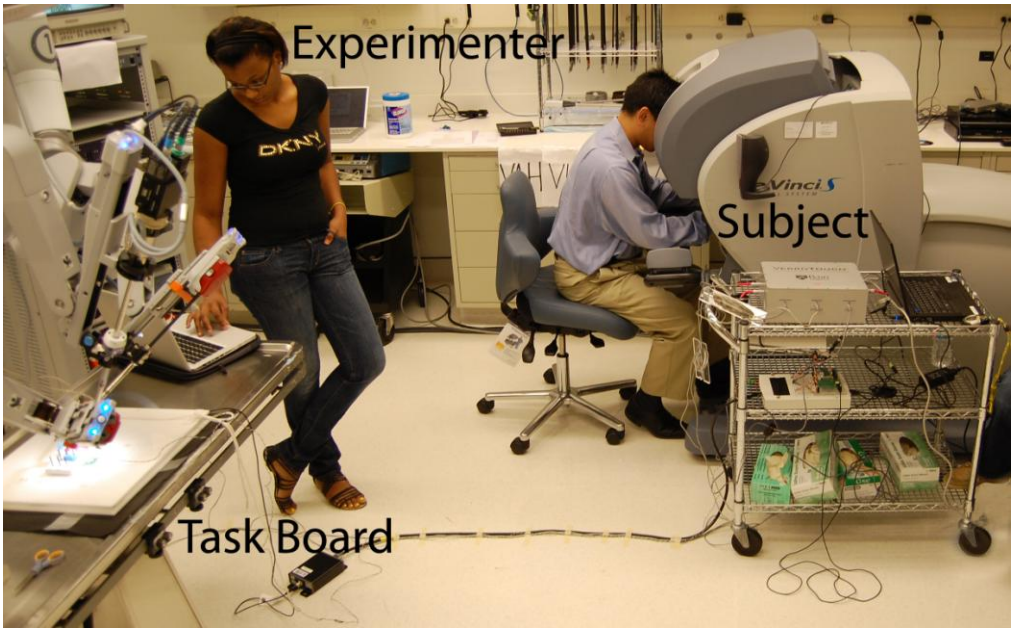


Figure 2: The experiment setup. The subject uses the console to control the da Vinci S robot to perform the assigned tasks. The pictured experimenter coordinates the subject's completion of surveys. One unpictured experimenter changes the task materials, and another experimenter sits at the black laptop on the right to control the data recording system. VerroTouch is visible as the orange attachments to the tools, the wires on the floor, and the gray box on the cart.

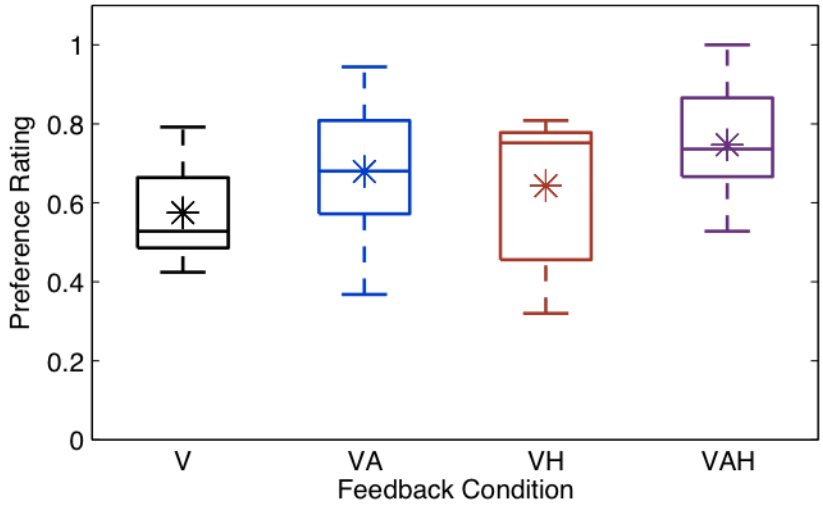


Figure 3: Box plot of surgeon preference data, taken from the survey each subject completed at the end of the study. Higher values indicate a stronger preference. Individuals vary in their preferences, giving wide ranges, but the surgeons agree that feedback about tool vibrations (present in VA, VH, and VAH conditions) is preferable to visual feedback alone (V). Statistical analysis shows a significant difference between the preferences for V and each subject's favorite condition among VA, VH, and VAH ($p = 0.002$).