

# University of Pennsylvania

## Annual Progress Report: 2007 Formula Grant

### Reporting Period

July 1, 2010 – June 30, 2011

### Formula Grant Overview

The University of Pennsylvania received \$8,868,580 in formula funds for the grant award period January 1, 2008 through December 31, 2011. Accomplishments for the reporting period are described below.

### **Research Infrastructure Project 1: Project Title and Purpose**

*Research Infrastructure: Construction of Life Sciences Building, Phase II* - The purpose of this infrastructure project is to provide state-of-the-art laboratory space for investigators in the Departments of Psychology and Biology in the School of Arts and Sciences. Most importantly, it will enable realization of one of the key elements of the strategic plan of the School of Arts and Sciences, entitled “Genes to Brains to Behavior.” This thrust emphasizes the growing importance of the biological underpinning, including the genetic basis, of even the most complex behaviors. This vitally important space will help Penn to attract the high-quality faculty, students, and postdoctoral researchers who are the essential critical success factor in modern research in the life sciences. These researchers, from a wide variety of fields, will stimulate each other and their students to greater research success in this cutting-edge field.

### **Summary of Research Completed**

This project was dropped prior to the expenditure of any grant funds.

### **Research Infrastructure Project 2: Project Title and Purpose**

*Research Infrastructure: Renovation of Lab Space for the Fundamental Physics Techniques to Biomedicine* - The purpose of this project is to upgrade and renovate 4750 square feet of laboratory space to standards appropriate for a 21<sup>st</sup> century laboratory performing research at the interface between the physical and biological sciences. The renovations include outfitting the laboratory space with sophisticated instrumentation, including a two-photon microscope and a high-powered laser light source. The renovated space will be occupied by investigators from the Department of Physics & Astronomy, whose research has implications for biomedicine, including work at the frontiers of diagnostic imaging using infrared light as well as the use of innovative techniques to make measurements of unprecedented precision aimed at understanding how genes guide basic process of development of an organism.

## **Summary of Research Completed**

This project was dropped prior to the expenditure of any grant funds.

### **Research Infrastructure Project 3: Project Title and Purpose**

*SVM Research Infrastructure—Behavioral Testing Laboratory* - The purpose of this infrastructure project is to upgrade and renovate approximately 225 net square feet of laboratory space in the Hill Pavilion Vivarium Barrier Facility to provide necessary mouse behavior testing space for conducting extramurally funded research projects related to understanding the role stress plays in the development of obesity and depression. The renovated space will allow researchers to produce high quality, reproducible and consistent results. As rodent behavior is highly dependent on the testing environment, it is imperative for these studies that we have designated testing space that is soundproof and not utilized for any invasive procedures. The space will be temperature controlled and free from outside disruption and noise, and able to be controlled by testing investigators.

### **Duration of Project**

1/1/2008 - 6/2/2008

## **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. 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**

*Financial Incentives for Smoking Cessation* - Despite progress in recent decades in helping people to quit smoking, about 65 million Americans still smoke cigarettes. Tobacco addiction is the leading cause of preventable mortality in the United States, and even though 70% of smokers report wanting to quit, only about 3% of smokers succeed annually. The purpose of this proposal is to provide supplemental funding to complete a CDC-funded trial of financial incentives for smoking cessation. In this randomized controlled trial, we have completed follow-up of subjects at 12 months with highly significant differences in quit rates for subjects in the incentive (14.7%) and control groups (5.0). At 18 months post enrollment incentive group subjects still had significantly higher rates smoking cessation (9.4% vs. 3.6%,  $p < 0.001$ ). Funding is requested to complete analyses of cost effectiveness and process evaluation.

### **Duration of Project**

7/1/2008 - 12/27/2010

## Project Overview

The objective of this project is to complete long-term follow-ups on a randomized controlled trial of financial incentives for smoking cessation. Previous efforts to test the effectiveness of financial incentives for smoking cessation in the workplace have generally used incentives of small magnitude and have been statistically underpowered. In partnership with General Electric (GE), we designed a scalable financial incentives intervention that could be broadly utilized by employers if effective in increasing smoking cessation rates.

878 GE employees including subjects from Erie and Grove City (GE Rail), Trevose (GE Water) and Hatfield (GE Energy), were enrolled in a randomized controlled trial, in which subjects were randomized to receive either information about smoking cessation programs within 20 miles of their workplace or the same information plus a package of financial incentives worth \$750. The randomization was stratified by worksite, income, and degree of nicotine dependence. Intervention arm participants were eligible to receive \$100 for completing a smoking cessation program, \$250 for quitting smoking anytime in the first 6 months of study enrollment, and \$400 for continuous abstinence between the 6 month and 12 month follow-up visits. Self-reported cessation was regarded as valid only if confirmed by a negative cotinine test. Quit and relapse rates were compared between groups using a two-sided chi-square test. Results were analyzed using intention to treat, with any subjects lost to follow-up assumed to have resumed smoking.

Due to greater than expected effectiveness of financial incentives in achieving both short- and long-term quit rates which induced budget shortfalls, supplemental funding was requested in 2008 to fulfill the following specific aims:

- 1.) Complete 18 month follow-ups on all subjects to determine long-term effectiveness without incentives;
- 2.) Examine cost effectiveness of this incentive program in achieving long-term tobacco cessation.

All subjects have now completed 12-month and 18-month follow-up visits. During the first 6 months following study enrollment, 14.9% of the incentive group completed a smoking cessation program vs. 6.8% of the control group (p-value<0.001). Quit rates in the first 6 months were significantly higher in the incentive group compared to the control group 20.9% vs 11.8%, p-value=0.001). At 12 months, long-term quit rates were 14.7% in the incentive group vs. 5.0% in the control group (p-value<0.001), and at 18 months, long-term quit rates were 9.4% in the incentive group vs. 3.6% in the control group (p-value <0.001).

The relative differences in quit rates observed at 9/12 months were largely sustained 6 months later after cessation of the incentives, as the ratio of 2.6 of quit rates between incentive and control groups at 15/18 months was only slightly lower than the ratio of 2.9 observed at 9/12 months.

## Principal Investigator

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## Other Participating Researchers

None

## Expected Research Outcomes and Benefits

This study is one of the only studies to have shown a large and significant difference in long-term tobacco cessation rates in an employer setting. Optimizing the design of such an intervention and determining the long-term cost effectiveness is essential to realize the full potential benefits of this intervention in reducing the burden of smoking-related illnesses such as cardiovascular disease and cancer among the millions of Pennsylvanians and other Americans who smoke. Based on the results of this study, GE has publicly committed to offering this program or one resembling it to all 152,000 employees domestically starting in January of 2010.

## Summary of Research Completed

### Methods.

Process Evaluation and RE-AIM Analysis. Data from the main trial was used to explore why financial incentives were not effective at influencing some smokers to quit. The public health impact of the intervention was assessed using the RE-AIM framework. Data from the main trial was used to analyze reach and efficacy dimensions. Focus groups were conducted with employees, and telephone interviews were conducted with worksite staff to explore barriers and facilitators to intervention adoption, implementation, and maintenance.

We examined incentive-group participants' awareness of and perceptions about financial incentives. Awareness about the incentive program was assessed by the question, "Did you know that you would receive money for quitting smoking?" Perception about the incentive was assessed by the following questions: (1) "How important was the monetary incentive in motivating you to try to quit?" (four-point Likert scale from *not at all helpful* to *extremely helpful*) and (2) Why were incentives helpful/not helpful in motivating you to quit? (open-ended). Nonquitters were asked whether they would have quit if paid a larger incentive, and if so, what amount would be necessary. Quitters were asked whether they would have quit for less money, and if so, what is the minimum amount they would have quit for. We compared the minimum incentive amount reported to motivate cessation attempts across subgroups of participants categorized by number of quit attempts in the past year, Prochaska's Stage of Change and the Fagerstrom Test for Nicotine Dependence (described more fully in manuscript). Quitters were also asked how they planned on utilizing the incentives (eg, nicotine replacement therapy, other cessation products, paying bills, product to reward self for quitting, or other). The

primary endpoint was long-term smoking cessation rates 9 to 12 months after enrollment. Participants were defined as *long-term quitters* if their self-reports of quitting were biochemically confirmed by a negative saliva or urine cotinine test at both the short-term and long-term follow-ups.

Multiple data sources were used to examine all five dimensions of the RE-AIM framework. The RE-AIM dimensions were operationalized as follows:

- Reach: the proportion of eligible smokers who expressed interest and enrolled in the study; the representativeness of study participants when compared to the demographics of the target population
- Efficacy: short-term and long-term quit rates defined as biochemical validation of smoking cessation
- Adoption: barriers and facilitators to intervention participation by employees
- Implementation: barriers and facilitators to intervention implementation by worksite staff
- Maintenance: barriers and facilitators to sustaining/institutionalizing intervention long-term

Data for reach and efficacy were analyzed from the main trial. Representativeness of study participants was estimated using data from the Behavioral Risk Factor Surveillance System (BRFSS). Qualitative data for adoption, implementation, and maintenance were gathered from focus groups with employees and in-depth telephone interviews with worksite staff. These interviews and focus groups allowed us to explore factors that may have impeded or facilitated program delivery, determine why the intervention was more successful in some sites than others, and how future interventions can be improved.

Cost-Effectiveness Analysis. Data from the main trial were used to examine the cost effectiveness of this intervention in achieving short- and long-term cessation rates. Measured and derived costs included program administration, incentives, smoking cessation, and other health care costs. The final endpoint was the employer's 1-year cost per quitter.

*Cost measurement:* We measured the costs of the incentives (program enrollment costs, direct payments, payment administration costs, and abstinence confirmation costs via oral or urine cotinine test), smoking cessation costs (nicotine replacement therapy, prescription drugs, and smoking cessation programs), and other health care costs. Incentive payments were recorded when they were made to individual study participants. Program enrollment and payment administration costs were measured by use of staff self-time assessment and wage data. Cost of abstinence confirmation was derived from bills from Examination Management Services (Scottsdale, AZ) (sample collection) and the Clinical Pharmacology Laboratory at the University of California, San Francisco) (sample analysis). Costs were allocated to individual participants based on the number of services utilized (e.g., incentive payments made, confirmatory tests conducted, etc.).

Smoking cessation costs and other health care costs were derived by multiplying patient self-reported service use times estimates of cost per service. For nicotine replacement therapy, the cost per service was derived from the National Cancer Institute (<http://www.smokefree.gov/pubs/MythsaboutNRTFactSheet.pdf>; last accessed, 12/24/2010); for prescription drugs, cost was derived from the Redbook; for smoking cessation programs, it was derived from national

sources. Participants were asked to report the number of times they visited the physician, the number of times they visited the emergency room, and the number of days they spent in the hospital. The cost for a physician visit, an emergency room visit, and a day in the hospital were derived from the Medical Expenditure Panel Survey and the Health Care Utilization Project National Inpatient Sample (<http://hcupnet.ahrq.gov/HCUPnet.jsp?Id=BCA3534C7A0C1C28&Form=SelLAY&JS=Y&Action=%3E%3ENext%3E%3E&LAY=Researcher>, last accessed, 12/24/10).

*Effectiveness measurement:* The primary clinical effectiveness measure was self-reported continuous abstinence at either 3 and 9 months or 6 and 12 months which was biochemically confirmed by use of a cotinine test performed on a saliva or urine sample. The primary economic endpoint was the quality-adjusted life year (QALY) which captures both duration of life and the quality of life. Because no one died during the study, this measure reflects the quality of life as measured by the EQ-5D, which was measured at baseline and the 2 follow-up visits. The preference-weighted EQ-5D produces a score between -0.594 (one of a number of states worse than death) and 1 (fully functional health).

*Cost-effectiveness:* Our primary cost-effectiveness outcome was measured from the perspective of the employer: 1 year direct medical cost per quitter. Other reported 1-year cost-effectiveness ratios included the direct medical cost per QALY, the incentive cost per quitter, and the smoking cessation cost per quitter. The same 4 ratios were also calculated for the 6-month outcomes.

### Results.

Process Evaluation and RE-AIM Analysis. Despite high rates of program awareness, financial incentives are not universally effective in motivating some smokers to quit. Readiness to quit needs to be sufficiently high for incentives to be effective. Reward amounts need to be higher for participants with lower levels of motivation to achieve successful cessation, with a third of smokers who did not quit reporting that they would have quit if they received a reward in excess of \$3400, the annual cost to employers for having a smoker versus a nonsmoker employee. RE-AIM analyses suggest the intervention was not uniformly successful across GE business types.

Cost-Effectiveness Analysis. The 1-year incremental direct medical cost per incremental quitter was \$2102 (95% CI, -2451 to 5264; see Table 1 below). We estimated a short-term (1-year) cost per quality-adjusted life year (QALY) ratio of \$16,345, but we could not be 95% confident that incentive payments are cost-effective, no matter what the value of our willingness to pay (see Table 2 below).

### Discussion.

Financial incentives are increasingly utilized by employers and insurers as a cost-effective strategy to improve consumer health. Results from the process evaluation suggest incentives likely are best targeted at smokers who are at least partly inclined to quit. Public health professionals should consider how incentives can be best optimized for the right target population. Even though a financial incentive intervention is an easy to administer, minimally invasive health promotion strategy, given limited public visibility, substantial effort is needed to gain buy-in from staff and garner interest and participation among eligible employees.

While we didn't have enough statistical power to make definitive statements about the cost effectiveness of this intervention, we can conclude that it appears based on the effectiveness of the program and the observed costs that this was similar in cost effectiveness to other widely used smoking cessation modalities such as smoking cessation counseling and nicotine replacement therapy. The current study indicates that the intervention was not any more costly, and provided a similar level of benefit relative to these other widely used interventions. Adoption of the smoking cessation intervention implemented in this study would be more likely had the analysis shown clear evidence of cost-effectiveness. However, employers and insurers are seeking efficacious strategies to maximize the health of their populations, and incentives may be more convenient, less costly and more popular than other interventions in certain contexts (in fact, General Electric rolled out an intervention based on this approach among its entire employee population of 152,000 employees in the United States in January of 2010).

*Publications, Presentations, and Media Coverage.*

1. Kim AE, Towers A, Renaud J, Shea J, Zhu J, and Volpp K (in preparation). Beyond efficacy: evaluating the public health impact of a financial incentive program for smoking cessation using the RE-AIM framework.
2. Kim AE, Kamyab K, Zhu J, and Volpp K (In press). Why are Financial Incentives not Effective at Influencing Some Smokers to Quit? Results of a Process Evaluation of a Worksite Trial Assessing the Efficacy of Financial Incentives for Smoking Cessation. *2011 Jan;53(1): 62-67.*
3. Kim, A., Kamyab, K., Zhu, J., & Volpp, K. (2009, June). *Why are financial incentives not effective at influencing some smokers to quit? Results of a process evaluation.* Presented at the Academy Health Annual Research Meeting, Chicago, IL.
4. Kim, A., Kamyab, K., Zhu, J., & Volpp, K. (2009, May). *Why are financial incentives not effective at influencing some smokers to quit?* Presented at the Society of General Internal Medicine, Miami, FL.
5. Volpp KG, Das, A., "Comparative Effectiveness — Thinking beyond Medication A Versus Medication B," *New England Journal of Medicine.* 2009; 361(4):331-333.

Table 1. 1-Year and 6-Month Costs and Outcomes

	1-Year				6-Months			
	Incentive	Control	Diff	P-Value	Incentive	Control	Diff	P-Value
Smoking Cessation								
Incentive	219.53	0.00	219.53	<0.0001	116.36	0.00	116.36	<0.0001
NRT	91.78	83.26	8.52	0.63	43.52	38.52	5.00	0.59
Zyban	63.05	44.02	19.03	0.18	23.55	15.69	7.87	0.18
Program cost	2.36	3.05	-.6909	0.76	1.18	1.53	-.35	0.76
Other Health Care	1494.22	1535.06	-40.83	0.89	760.59	781.92	-21.33	0.89
Total Health Care	1870.94	1665.38	205.55	0.50	945.20	837.65	107.55	0.49
%Quit	.1473	.0496	.0978	<0.0001	.2098	.1169	.0929	<0.0001
QALY	.8395	.8270	.0126	0.20	.4217	.4154	.0063	0.19
Wage	48,568.96	48519.5	49.46	0.96	24284.48	24259.75	24.73	0.96
Net Benefit	46,698.02	46,854.11	-156.10	0.88	23,339.28	23422.10	-82.83	0.88

Table 2. 1-Year and 6-Month Cost-Effectiveness Ratios

Outcome	1-Year		6-Months	
	Ratio	Confidence interval	Ratio	Confidence interval
Direct medical cost per quitter	2102.24	-4425 to 8779	1157.14	-2451 to 5264
Direct medical cost per QALY	16,344.98	Undefined	18,461.98	Undefined
Incentive cost per quitter	2245.15	1749 to 3344	1251.89	904 to 2293
Cessation cost per quitter	2519.85	1807 to 3918	1386.57	943 to 2598

### **Research Infrastructure Project 5: Project Title and Purpose**

*Center for Genetics and Complex Traits – Research Infrastructure* - The purpose of this research infrastructure project is to renovate space on the second floor of Blockley Hall in support of the Center for Genetics and Complex Traits. This new center is a major initiative of the University of Pennsylvania School of Medicine's Research Strategic Plan and addresses three key needs that are shared by Penn, the NIH Roadmap, and the Commonwealth of Pennsylvania: Advancing new pathways of discovery (bioinformatics); Building research teams of the future (molecular epidemiology-statistical genetics-bioinformatics); and Re-engineering the clinical research enterprise (enhancing translational research). Renovation of space in Blockley Hall is essential to Penn's efforts to create an interactive environment that both promotes the Center's translational outreach initiatives and enables the recruitment of new faculty with expertise in statistical genetics and informatics. With these new collaborators and resources in a position to succeed, Penn's Center will create new studies that lead genetics/genomics research into the future for the benefit of the citizens of the Commonwealth.

#### **Duration of Project**

7/1/2008 - 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 Annual C.U.R.E. 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**

*Integrated Approaches to Genome-based Therapeutics* - It is now widely appreciated that the genotype plays a significant role in outcomes of disease treatment. For example, the toxicity of a particular drug can be modulated by the patient's genome-wide genotype—leading to the recent idea of "Personalized Medicine." Furthermore, about 2000 human diseases such as Parkinson's, diabetes, and others are thought to arise from defects of genomic function, suggesting new therapeutic strategies that might use RNA/proteins/small molecules to affect system-level function. The purpose of this project is to identify strategies for genome-based therapeutics, develop an understanding of the interaction between individual genomes and intervention, RNA/protein based therapeutics, delivery systems for new therapeutics, and computational analyses to aid therapeutic design and system-level understanding of genomic dysfunction.

#### **Anticipated Duration of Project**

1/1/2008 - 12/31/2011

## **Project Overview**

Therapeutic drug treatment strategies for human diseases have relied on two assumptions: (1) that the disease can be treated by targeting a single molecule that is the keystone to the dysfunction that led to the disease; and (2) that all treated patients will respond in a similar manner (except for gross physiological factors such as body weight). Despite more than fifteen years of development, only recently has genome-scale data been studied in relation to human diseases and therapeutic strategies. The nascent data has revealed that an individual's response to particular therapeutics, in terms of efficacy and toxicity, may depend to a surprising degree on various genomic factors. Thus, a treatment that might not be effective in one group of individuals might be effective in another group that has a different genomic composition; similar genome-dependence might be found for toxic side effects. An important direction for the future of therapeutic strategies is to develop an understanding of the interaction between individual genomes and specific interventions.

Another recent development has been the realization that many of these diseases arise from 'systems level' dysfunction, where more than one defect leads to the same or similar observable phenotype. For example, spectrum disorders often manifest in the same clinical outcomes, e.g. dementia, and causes, neuronal cell death, but the path to generating these symptoms is different. As noted above, the classic model is to seek a key target whose inhibition or induction might lead to amelioration or even reversal of the dysfunction. However it is becoming increasingly clear that therapeutic strategies for more complex diseases will require multi-parameter manipulation of genomic function through RNA or protein-based agents.

Given the above background, the aims of this project are the following:

Aim 1: Develop an understanding between therapeutic strategies and human diseases through the use of genomic information and clinical/intervention outcome data in humans and model organisms.

Aim 2: Develop novel therapeutic strategies including RNA and protein agents that modulate whole-genome function.

Aim 3: Develop computational analysis tools for system-level modeling of genomic dysfunction and manipulation by therapeutic agents.

## **Principal Investigator**

Junhyong Kim, PhD  
Co-Director, Penn Genome Frontiers Institute  
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## **Other Participating Researchers**

Jim Eberwine, PhD, Vijay Kumar, PhD, Hongzhe Li, PhD, David Meaney, PhD, Brian Gregory, PhD - employed by University of Pennsylvania

## **Expected Research Outcomes and Benefits**

The first expected outcome of this research is increased ability to better match therapeutic strategies to individual patients, increasing drug efficacy and ameliorating or predicting adverse side effects. The second expected outcome and benefit is to obtain new tools and basic knowledge to develop new classes of therapeutic agents, RNA and protein molecules, which target whole genomic function manipulation. The expected knowledge to be generated includes technologies for RNA/protein delivery, genomic effects monitoring including technologies for in vivo assays, and computational models for system-level genomic function vis-à-vis genomic interventions. The new research from this project is expected to pioneer a whole new class of therapeutics as well as develop a better understanding of the interactions of the genome with intervention agents.

## **Summary of Research Completed**

In the current reporting period, we concentrated on additional progress in achieving Aim 1. In Aim 1, our goal is to develop an understanding between therapeutic strategies and human diseases through the use of genomic information.

For Aim 1, we were able to leverage a new High-Throughput Sequencing (HTS) facility created in the Penn Genome Frontiers Institute to take a HTS approach into the inflammatory properties of the cyclooxygenase (COX) in mice system. The COX isozymes are the targets of the nonsteroidal antiinflammatory drugs (NSAIDs). The personalization of therapy with NSAIDs will require the precise understanding of sources of variation in pharmacokinetics and -dynamics of these drugs; our first project focuses on pharmacodynamics. COX-2 plays a dual role in inflammatory pain hypersensitivity. It is expressed both at the site of inflammation (e.g. in macrophages) and in the pain signaling pathways in the CNS. Here, we study the relative contribution of peripheral COX-2 to inflammatory pain hypersensitivity using a mouse model in which COX-2 was specifically deleted in macrophages (LysM-Cre/COX-2<sup>-/-</sup>).

Male and female LysM-Cre/COX-2<sup>F/F</sup> mice and male and female wildtype littermates were subjected to the Freund adjuvants (CFA) paw injection model and phenotyped (paw volume, heat and mechanical sensitivity) regularly over a total time period of 3 weeks. We observed a significant reduction in paw edema and thermal and mechanical hyperalgesia in LysM-Cre/COX-2<sup>F/F</sup> mice (compared to wild type) one hour after the injury, which was sustained until 2 weeks and had resolved almost completely 3 weeks after injury (Figure 1).

Since the pain phenotype associated tightly with the degree of inflammation in the paw (paw edema), we focus on the mechanics of the inflammation in the paw and how it might be affected by macrophage COX deletion using RNA seq.

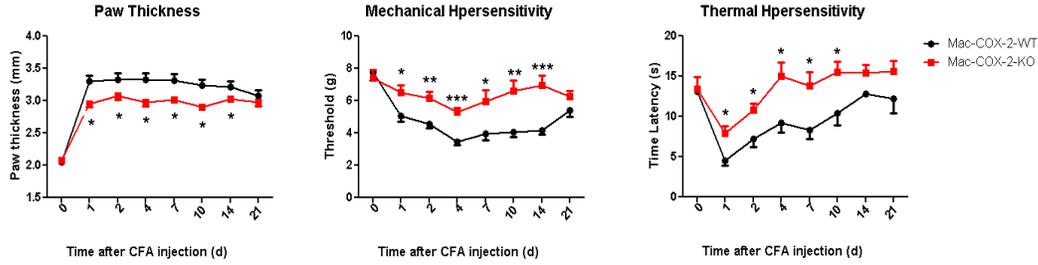
Samples for RNAseq analysis of the inflammatory lesion in the paw were prepared from parallel experiments. Again, the CFA-induced paw edema model was performed on WT and Macrophage-COX-2-KO mice (n=2 for each group). 24 hours after CFA injection, the mice were sacrificed by asphyxiation with CO<sub>2</sub>. The paw tissue of uninjected (control) and CFA-injected mice were snap frozen in liquid nitrogen for RNA isolation. (Spinal cord (L3-L5) tissues was also collected but has not yet been worked up for RNAseq.) Total RNA was extracted and RNA with an Integrity Number (RIN) value greater than 8 was used for library preparation. Four samples were run (WT (n=2) vs. Mac-COX2-KO (n=2)) in two technical replicates; thus a total of 8 data sets were generated and aligned using RUM on the PGFI cluster. Data quality and alignment were excellent: For example, lane 1 generated 136,663,667 reads of which 97.3% of all fragments mapped and 83.94% of them uniquely. Both forward and reverse mapping occurred 88.9% of the time. Given the size of the data sets, the analysis was computationally intense: it took 25 nodes 32 hours (for one lane) to get to the post-processing step, which then took roughly another day.

Hierarchical clustering analysis of the 8 lanes showed that technical replicates were most similar (Figure 2). The WT samples clustered together, the variability in the knockout samples was higher; one KO sample (1a, 1b) was more similar to wildtype than to the other KO sample (2a, 2b).

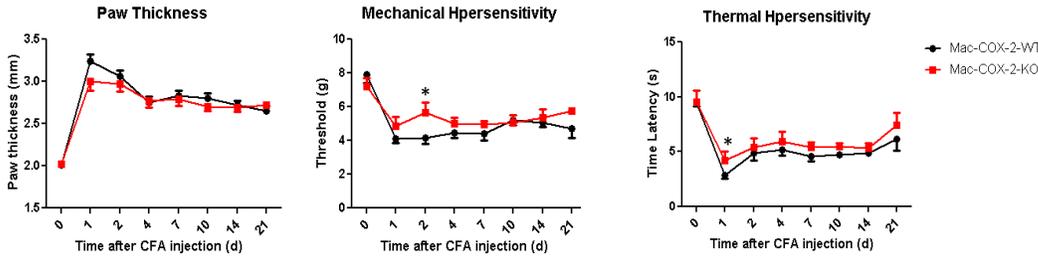
We identified a total of 30854 genes on which analysis for differential regulation was performed:  
T-stat  $\geq 0.5$  & *conf.level*  $\geq 0.5$ : 4344 genes (807 upregulated, 3537 downregulated)  
T-stat  $\geq 0.5$  & *conf.level*  $\geq 0.5$  &  $\geq 1.5$  fold change: 1026 genes (628 upregulated, 398 downregulated)  
T-stat  $\geq 0.5$  & *conf.level*  $\geq 0.5$  &  $\geq 2$  fold change: 431 genes (382 upregulated, 49 downregulated)

Several key players of the COX pathway were among the differentially expressed genes. These data are currently being further analyzed. The next experiments will include the analysis of the spinal cord samples, and the analysis of isolated COX-2 deficient macrophages under various experimental conditions in vitro. We also are in the process of generating clinical study samples for which we have prospectively obtained consent for various HTS approaches. We are also in the process of obtaining permission retrospectively to use samples of previous studies.

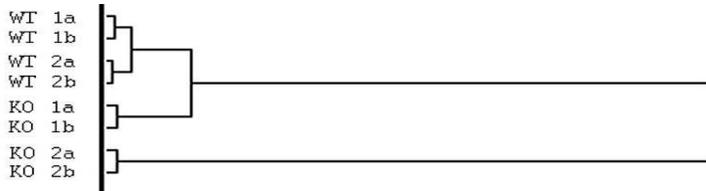
**Male**



**Female**



**Figure 1.** Inflammation and inflammatory pain in Mac-COX2-KO mice (Male, \* p<0.05 , \*\* p<0.01, \*\*\* p<0.001 vs. WT For paw thickness and the mechanical hypersensitivity, n=12 per group. For the thermal hypersensitivity, n=4 per group. **Female**, n=8 per group \* p<0.05 vs. WT



**Figure 2.** Hierarchical cluster analysis by sample.

## **Research Project 7: Project Title and Purpose**

*Isolation and Characterization of Lung Cancer Stem Cells* - Evidence from leukemia researchers have shown that the capacity of the tumor to grow, propagate, and resist therapy may be dependent on a small subset of cells, termed “cancer stem cells.” Although rare, they could regenerate a tumor identical in appearance to the parent cancer. The identification of cancer stem cells in solid tumors was first shown in 2003 in primary human breast cancer. If such cancer stem cells exist in other cancer types, like lung cancer, isolating them could have numerous implications in our understanding of tumor biology (i.e. interactions with the microenvironment) and in designing new therapies (biochemical, molecular, and immunologic). Our goal is to isolate lung cancer stem cells.

### **Duration of Project**

7/1/2008 - 12/31/2010

### **Project Overview**

Our long-term objective is to develop new therapies for lung cancer that are based on the unique biological characteristics of this malignancy. Our working hypothesis is that lung cancers contain tumor initiating stem cell populations that self-renew and give rise to the heterogeneous populations making this tumor so exceptionally therapy resistant.

The goals of this project are to establish operating procedures to isolate cancer stem cells using lung cancer stem cells as a paradigm. Once identified, these cells will then be analyzed using genomic and proteomic approaches to identify potential therapeutic targets for biochemical or immunologic strategies. To accomplish this, we propose the following specific aims.

1. Establish the infrastructure to collect lung specimens in a sterile fashion suitable for cell isolation.
2. Use a variety of approaches (established by our collaborator, Dr. Meenhard Herlyn) to isolate lung cancer stem cells from these tumor specimens and/or primary lung cancer cell lines.
3. Compare the genomic and proteomic characteristics of lung cancer stem cells to “traditionally isolated” lung cancer cell lines or lung cancer cells within tumors with a focus on new therapeutic targets.

In addition to obtaining cancer stem cells, we should also be able to transfer this expertise to a Penn Cancer Stem Cell Core lab so that it can be extended to other tumor types within the cancer center.

### **Principal Investigator**

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Vice Chief and Director, Lung Research

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### **Other Participating Researchers**

John Kucharczuk, MD, M. Cecilia Crisanti, MD - employed by University of Pennsylvania  
Meenhard Herlyn, VMP, PhD - employed by Wistar Institute

### **Expected Research Outcomes and Benefits**

Lung cancer is the most common cancer killer. Treatment remains very poor, with only 15% of patients cured (all those by surgery). Chemotherapy can extend survival by only a few months and is not curative. We need new ideas and approaches. Based on studies in leukemia, there is a new idea that only a very small number of very resistant cancer cells (called cancer stem cells) are responsible for keeping cancers going, even after >95% of “regular” cancer cells have been killed by chemotherapy. Investigators think they have identified such stem cells in brain tumors and breast cancer. This has not yet been done for lung cancer. If we can identify lung cancer stem cells, we could analyze them carefully to look for specific ways to attack them. This might give us a way to use existing drugs or design new targeted chemotherapy drugs or activate the immune system to specifically kill those cells.

### **Summary of Research Completed**

We have made progress on our project, although in directions that we had not anticipated.

Our approach of isolating lung stem cells had focused on taking advantage of the purported resistance of these cells to chemotherapy. As reported two years ago, by exposing lung cancer cell lines to moderate doses of chemotherapy (esp. doxorubicin), we were able to find marked increases in the message levels (by RT-PCR) of known key stem cell genes, such as Oct4a, Nanog, and Sox 2. As described in last year’s report, we discovered that the cells we had isolated were not stem cells, but instead were senescent cells- cells that remained alive for months, yet did not divide. One interesting finding was that the Oct4a was actually in the cytoplasm, not nucleus, of the cells. In the last 6 months of this project, we have continued to pursue these findings and have made the following observations.

1. Other forms of oxidative stress (i.e. radiation or hydrogen peroxide) could also induce cytoplasmic Oct4a in lung cancer cells (see Fig 1 below).
2. We could see increased Oct4a staining within tumor xenografts that had been treated with chemotherapy.
3. We spent time trying to determine the exact molecular form of Oct4a that we were dealing with. This involved designing PCR probes to identify each known isoform and pseudogene of Oct4a. We also used high fidelity PCR to amplify the Oct4a message in the Doxo-treated tumor cells, cloned the PCR into bacteria, selected clones and then did

sequencing. We found that the only species detected was the bone fide full length Oct4a gene with no mutations that might explain why it was located in the cytoplasm.

4. We also did immunoprecipitation of Oct4a from our Doxo-treated cells. We detected a faint band at the size corresponding to Oct4a, but two much more intense bands at around 50kDa that we speculate might be binding to Oct4a. Mass Spec analysis was done and we have a few interesting candidate genes. Our lead candidate is the elongation factor 1-alpha.
5. We have also discovered that oxidative stress can upregulate Oct4a in normal bronchial epithelial cells.

Given our previous data showing that knockdown of Oct4a could induce cell death in senescent cells; we conclude that Oct4a (like some other transcription factors) can have multiple functions. In the nucleus it functions to maintain “stemness”. However, after oxidative or genotoxic stress, we hypothesize that cytoplasmic Oct4a is released and that it functions to protect the cells from stress-induced death. How this happens and what the implications might be for normal bronchial epithelial cells remains an area of active interest and potential grant applications.

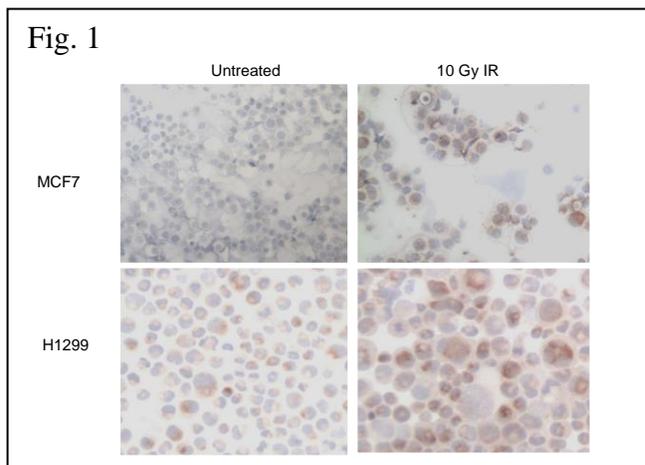


Figure 1. Exposure of a breast cancer or lung cancer cell line to 10 gy of irradiation induces strong upregulation of cytoplasmic Oct4a staining.

### **Research Project 8: Project Title and Purpose**

*Phenotyping the PI3 Kinase-AKT Pathway in Human Breast Cancers by Immunohistology* - We propose to develop an accurate and convenient method for determining the activity of a critical signaling pathway in breast cancer cells, the phosphatidylinositol-3-kinase (PI3K)-AKT pathway. The PI3K-AKT pathway is activated in many human breast cancers by mutations affecting a number of different genes. These mutations and activation of the PI3K-AKT pathway influence clinical behavior of breast cancers and response to drug treatment. Detection of these mutations currently requires performance of multiple genetic tests, most of which are available only on a research basis. Development of a test based on antibody staining of tumor tissue obtained at the time of initial diagnosis or surgery will allow PI3K-AKT pathway activity to be determined routinely and factored into the care of patients with breast cancer.

**Duration of Project**

1/1/2008 - 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 Annual C.U.R.E. 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**

*Neural Substrates of Varenicline (Chantix®) Efficacy for Smoking Cessation* - This project aims to identify the neural mechanisms that underlie varenicline effects on early nicotine abstinence symptoms, specifically, smoking urges and deficits in emotional and cognitive (working memory) processing.

**Duration of Project**

7/1/2008 – 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 Annual C.U.R.E. 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**

*Combination Immunotherapy Targeting K-ras in Adenocarcinoma of the Pancreas* - Pancreatic cancer is the fourth leading cause of cancer mortality in both men and women. Despite a deeper understanding of cause of pancreatic cancer, extensive clinical research has yielded only modest improvements in the outcome of this devastating malignancy. Pancreatic cancer cells are naturally resistant to current chemotherapy and radiation therapy. Based on recent evidence that vaccines have promise in patients with surgically incurable pancreatic cancer, this research will test a new therapy involving a customized patient-specific vaccine in combination with chemotherapy, radiation therapy and when possible, surgery.

**Anticipated Duration of Project**

1/1/2008 - 12/31/2011

## **Project Overview**

The broad objectives of this research are to 1) assemble the infrastructure and expertise required to evaluate new therapies such as biologic therapies and targeted therapies for advanced pancreatic cancer, and 2) conduct a pilot test of a novel tumor specific K-ras vaccine in combination with chemotherapy and radiation therapy to determine if lymphocytes can kill tumor cells, and whether this impacts on K-ras expression and signaling in the tumor microenvironment.

The three specific aims are (1) Assemble a multidisciplinary team of scientists and physicians to conduct novel trials of biologics and targeted therapeutics for pancreatic cancer; (2) Conduct a pilot study of safety and feasibility of an inactivated recombinant *Saccharomyces cerevisiae* vaccine expressing mutant K-ras protein combined with adoptive T cell transfer and chemoradiotherapy in locally advanced pancreatic cancer and (3) To analyze the specimens from the pilot study to determine the effects on host immune reconstitution, ras tumor immunity and the tumor microenvironment.

The research design and methods are designed to test the hypothesis that combining a patient and tumor specific *Saccharomyces cerevisiae* based K-ras vaccination with adoptive transfer of ex vivo-activated and vaccine-primed autologous T cells will overcome immune tolerance induced by pancreatic cancer, thereby restoring immune surveillance, as demonstrated by induction of T cell infiltrates in the tumor microenvironment and decreased expression of K-ras in resected tumor specimens. A pilot study to test the susceptibility of pancreatic tumor cells to killing by T cells to establish the feasibility of this approach will be carried out at the Abramson Cancer Center of the University of Pennsylvania.

## **Principal Investigator**

Carl H. June, MD  
Professor, Dept. of Pathology and Laboratory Medicine  
University of Pennsylvania  
Abramson Cancer Center  
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Philadelphia, PA 19104-6160

## **Other Participating Researchers**

Peter J. O'Dwyer, MD, Wafik El-Deiry, MD, PhD, Benjamin Musher, MD, Richard Carroll, PhD – all employed by the Hospital of the University of Pennsylvania.

## **Expected Research Outcomes and Benefits**

This translational project will determine whether a potent cancer vaccine that is customized to the patient's tumor generates a tumor immune response in patients with locally advanced pancreatic cancer. In addition, the study will determine if it is safe and feasible to integrate the cancer vaccine into the standard chemotherapy and radiation therapy that is currently given to

patients with pancreatic cancer. This approach is a form of ‘personalized medicine’ that in theory should have less toxicity, while providing more specific benefit than currently available treatments for this presently incurable form of cancer.

In addition to determining the safety and feasibility of this new approach, the research will assemble a team of experienced physicians, surgeons and basic scientists that will be available to carry out translational research on other new biologic and targeted therapies that are being developed by bench scientists engaged in pancreatic cancer research in the field. Related to the above, this project will serve as a training vehicle for junior physician scientists who are interested in careers of translational medicine in the area of pancreatic cancer, which remains as a significant unmet medical need. Finally, research in pancreatic cancer is under funded by the NIH relative to other cancer sites. Thus, there are a range of scientific and society health benefits that can be expected from the successful completion of this study, from new insights into cancer biology to the possibility of new drugs being developed as a result of this project.

### **Summary of Research Completed**

The following progress was made during the reporting period 7/1/2010 to 6/30/2011 on Aim 1 and Aim 2:

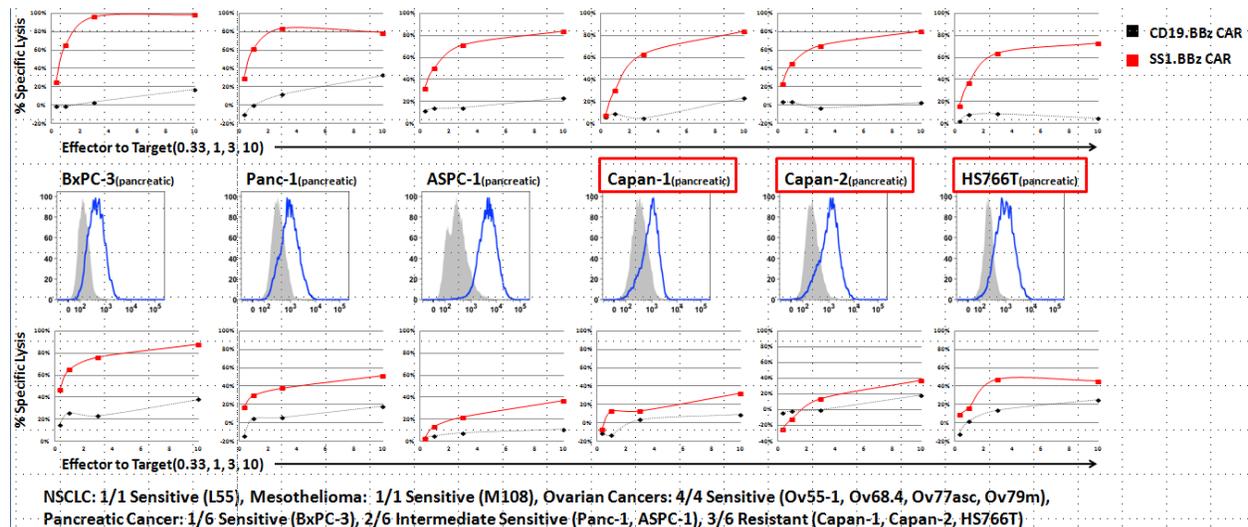
*Aim 1. Assemble a multidisciplinary team of scientists and physicians to conduct novel trials of biologics and targeted therapeutics for pancreatic cancer.*

Preclinical studies were completed testing a mesothelin based targeting strategy to determine if anti-mesothelin chimeric antigen receptor T cells could target pancreatic cancer. A variety of pancreatic tumor cell lines were tested in vitro to determine if the CAR T cells could kill pancreatic cancer cell, and the results are summarized in **Table 1** below. The lines tested were BxPc-3, Panc-1, ASPC-1, Capan-1, Capan-2, and HS766T. At low effector to target ratios between 1:0.5 to 1:10, the CAR T cells killed 10 to 55% of the pancreatic tumor cells. A graphical summary of these results is shown in Figure 1, which depicts the range of mesothelin expression on the pancreatic tumor cells lines and the cytotoxicity over a range of E:T ratios. These studies were conducted by Dr. Carroll, and more recently by Ms. McGettigan in the laboratory.

**Table 1**

Type	Cell Line	R/IS/S	ΔBackground - 4hr Chromium	ΔBackground - 18hr Flow
Mesothelioma	M108	S		>80%
Lung Cancer	L55	S		>80%
Pancreatic Cancer	BxPC-3	S		40-55%
Pancreatic Cancer	Panc-1	IS		10-20%
Pancreatic Cancer	ASPC-1	IS		10-35%
Pancreatic Cancer	Capan-1	R		20-30%
Pancreatic Cancer	Capan-2	R		10-30%
Pancreatic Cancer	HS766T	R		10-20%

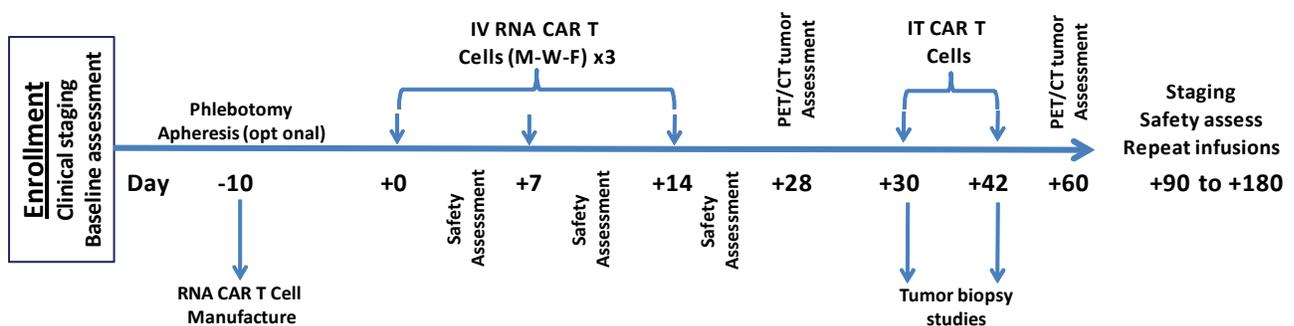
Figure 1. The expression of mesothelin for each pancreatic tumor cell line is shown on the top row, and below, is the cytotoxicity, depicting the cytotoxicity (% specific lysis) for each pancreatic cancer line (bottom row).



We have now assembled a complete team of basic scientists, translational scientists and clinicians to conduct a pilot trial testing T cell immunotherapies for pancreatic cancer. Given the above pre-clinical data, and the development of a protocol, Aim 1 is now considered completed.

*Aim 2. Conduct a pilot study of safety and feasibility of an inactivated recombinant Saccharomyces cerevisiae vaccine expressing mutant K-ras protein combined with adoptive T cell transfer and chemoradiotherapy in locally advanced pancreatic cancer.*

Based on the above pre-clinical data targeting pancreatic cancer cells using CAR T cells that was supported by this grant, and on promising human pilot clinical trial data from patients with leukemia treated with anti-CD19 CARs funded by other sources, we have developed a protocol entitled “Compassionate Use of Chimeric Antigen Receptor Modified Cells Administered by Intravenous or Intratumoral Injection in Metastatic Pancreatic Cancer”. The protocol is UPCC 814373. The principal investigator is Gregory Beatty, MD, PhD, and the subinvestigators are Michael Soulen MD, Peter O’Dwyer, MD, Weijing Sun MD at the Hospital of the University of Pennsylvania. The laboratory investigators are Bruce L. Levine, Ph.D., Anne Chew, Ph.D., Michael Kalos, Ph.D., Yangbing Zhao, M.D., Ph.D., and Robert Vonderheide, MD, DPhil, all from the Abramson Cancer Center. We expect the first patient to be treated in October, 2011, and if this pilot protocol is successful as far as safety and feasibility, then a full protocol will be developed to treat ~12 additional patients. The mesothelin CAR T cells will be manufactured under IND 14595, Sponsored by Dr. June.



The outline of the protocol is shown in the schematic figure above. This compassionate use pilot study will determine the safety, tolerability and engraftment potential of mesothelin specific T cells in a patient with metastatic pancreatic cancer. The safety and feasibility of IT and IV routes of administration will be identified in this protocol. The objective of this pilot protocol is to generate data to support a full phase I protocol.

### **Research Project 11: Project Title and Purpose**

*WISER Sister Pilot Study* - The WISER Sister pilot study will recruit 10 women aged 18-35 with elevated breast cancer risk to build to 60 minutes of daily exercise over 5 months and assess the effects of this intervention on factors that affect or reflect breast mitotic activity (including estrogens, MRI breast imaging, other sex steroid hormones, adipokines, and body composition). An ongoing parallel trial with 320 low risk women (the WISER study) will allow for novel comparisons of levels of commonly accepted risk factors for breast cancer.

## **Duration of Project**

1/1/2008 – 5/28/2010

## **Summary of Research Completed**

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

## **Research Project 12: Project Title and Purpose**

*Stem Cell Niche and Epithelial Tumorigenesis* - Stem cells rely on the surrounding microenvironment for their extensive proliferation and pluripotency. Recent evidence suggests that these distinct properties of stem cells are not autonomously achieved but are regulated by a level of external control. Transcription factor p63, a homolog of tumor suppressor p53, has been shown to serve as a master determinant of epithelial stemness in an epithelial-cell autonomous manner. However, the microenvironment controlling these stem cells has not been identified. In this project, factors provided by stem cell microenvironment will be explored. In addition, these factors will be analyzed in the context of epithelial tumorigenesis.

## **Duration of Project**

1/1/2008 - 12/31/2011

## **Project Overview**

In general, stem cells require specialized culture conditions such as growth factors and a feeder layer for their long-term proliferation. Mouse embryonic stem (ES) cells, for instance, require a layer of mouse embryonic fibroblasts (MEFs) as well as lymphocyte inhibitory factor (LIF). Likewise, human ES cells also require fibroblasts and certain growth factors. Although researchers do not fully understand the importance of fibroblasts in these systems, these cells are absolutely required to maintain the integrity of stem cells. Therefore, it is important to identify the factors provided by these fibroblasts to better understand the microenvironment surrounding the stem cells. Recently, researchers found that insulin-like growth factor (IGF)-II is expressed from the surrounding fibroblasts and alone is sufficient to maintain the pluripotency of human ES cells. This demonstration strongly argues that distinctive properties of stem cells are not autonomously achieved but are maintained by the factors produced by the stem cell environment.

Similar to ES cells, epithelial stem cells can also be cultured on specialized fibroblasts called 3T3-J2. These 3T3-J2 cells were established from normal MEFs based on their natural ability to support long term proliferation of epithelial stem cells. In this project, we will identify the factors provided by these 3T3-J2 cells to maintain the integrity of epithelial stem cells by using microarray and proteomics approaches. It is widely believed that abrogation of stem cell's environment might trigger tumorigenesis due to overproduction of cells with high proliferative potential. This project will also demonstrate this point by overexpressing the identified factors in

fibroblasts to see whether uncontrolled epithelial stem cells can generate cancers. In addition these engineered mice will be crossed with well-characterized epithelial tumor mouse models to see if tumor grade and onset of tumorigenesis will be altered. Overall, this project is anticipated to bring important information about the stem cell microenvironment and their contribution to epithelial cancers.

### **Principal Investigator**

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Philadelphia, PA 19104

### **Other Participating Researchers**

Ji-Kang Fang, PhD - employed by University of Pennsylvania

### **Expected Research Outcomes and Benefits**

Recent advances in stem cell research suggest that distinctive properties of stem cells are not autonomously achieved but are maintained by the stem cell environment called “stem cell niche”. In the case of epithelial stem cells, the master regulator of stemness is a transcription factor p63, a homolog of the tumor suppressor p53. However, essential factors provided by epithelial stem cell microenvironment are not fully investigated. Thus, this project will lead us to better understand the cellular society of stem cell biology.

Stem cells are tightly controlled for their number, differentiation status and proliferation. Abrogation of these controls might cause cancers because stem cells have extraordinary proliferative potential. This project will also address this point by overexpressing the stem cell environment factors in mice in a tissue-specific manner in fibroblasts. These mouse models will be unique tools to study the tumorigenesis with altered stem cell microenvironment.

In all, this project will bring significant information about the regulation of epithelial stem cell niche and its contribution to cancers.

### **Summary of Research Completed**

Recent evidence suggests that the properties of stem cells are not autonomously achieved but are regulated by a level of external control called “stem cell niche” that intimately links to the cell-intrinsic regulation of stem cells. We have shown previously that transcription factor p63, a homolog of the tumor suppressor p53, serves as a master determinant of epithelial stem cell self-

renewal. We hypothesize that the level of p63 is crucial for the maintenance of epithelial stem cells and that both stem cell niche and cell-intrinsic regulation play an important role to sustain high levels of p63 in epithelial stem cells. It has been shown that p63 is a phosphoprotein and its phosphorylation is increased upon epidermal cell differentiation *in vitro*, leading to the proteasome-mediated degradation of p63. However, it is not yet clear whether p63 phosphorylation occurs during epithelial stem cell differentiation *in vivo*. In this funding period, we used the epidermis as a model to investigate the potential involvement of p63 phosphorylation during early stages of epithelial stem cell differentiation into transit-amplifying (TA) cells with more limited proliferative potential than stem cells.

The basal and the suprabasal layers in the epidermis provide an excellent *in vivo* model to study the transition of stem cells to TA cells. Because p63 is phosphorylated upon differentiation of epidermal cells *in vitro*, we postulated that p63 phosphorylation also accompanies the early transition of stem cells to TA cells *in vivo*. To address this issue, we first stained adult mouse skin sections with anti-p63 and anti-phosphorylated p63 antibodies and quantified total and phosphorylated p63 levels (Figure 1).

Consistent with current models that the basal layer of the epidermis contains a heterogeneous population of cells with stem cell and early to late TA cell functions, p63 expression was variable within the basal layer. However, cells with high p63 levels were observed not only in the basal layer but also in the first suprabasal layers, suggesting that high levels of p63 are not restricted to stem cells. As we postulated that p63 phosphorylation accompanies differentiation, we next determined if high p63-positive cells in the first suprabasal layers could be distinguished from those in the basal layer by their p63 phosphorylation levels. To focus on these early differentiative states, we assessed phosphorylated p63 levels in epidermal cells expressing the highest levels of p63 (the top 5%). These high p63-positive cells were then further subdivided according to their phosphorylation status; high p63-phosphorylation (top half) and low p63-phosphorylation (lower half).

Among the high p63-positive cells analyzed, approximately one fourth of the cells were located in the first suprabasal layers and the levels of total p63 expression were statistically similar between the basal and the first suprabasal cells. Notably, we found that high p63-positive cells in the first suprabasal layers as well as in the top half of the basal layer were all highly phosphorylated. In contrast, cells with a high p63- and low-phosphorylation phenotype were exclusively preserved in the basal layer. Because stem cells are confined to the basal layer while TA cells are found in both the basal and suprabasal layers, cells with a high p63 and high-phosphorylation phenotype likely represent early TA cells, while a high p63 and low-phosphorylation phenotype is characteristic of stem cells. These results indicate that levels of p63 phosphorylation are variable among high p63-positive cells and increase during early steps of stem cell differentiation to TA cells before significant decline in p63 expression is observed.

Next, we sought to determine whether the phosphorylation status of p63 reflects proliferative potential of epidermal cells. To address this issue, we utilized clonogenic cultures of primary human epidermal keratinocytes. In these cultures, single epidermal cells are grown at clonal density on a feeder layer, which provides essential elements of the epidermal stem cell niche. These clonal epidermal cells form three types of clones; *holoclones*, *meroclones* and *paraclones*

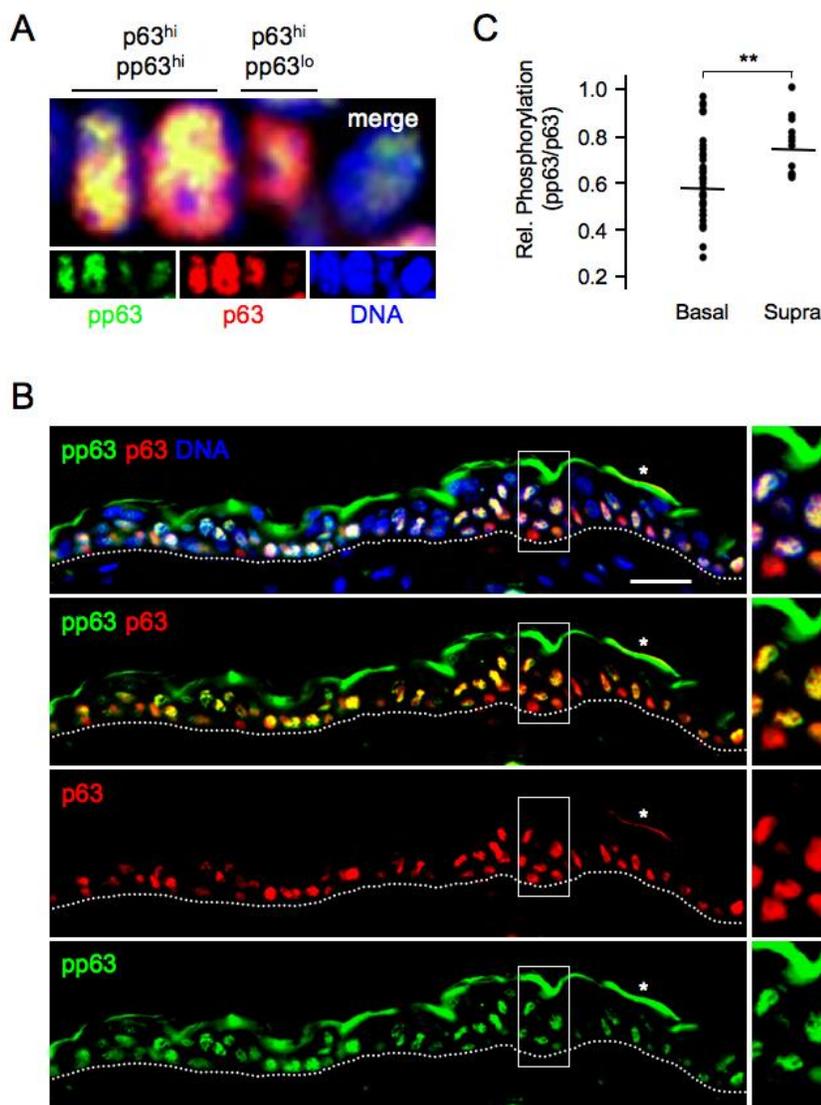
in decreasing order of both proliferative potential and p63 expression and increasing order of differentiation. Holoclones consist primarily of stem cells with high p63 expression throughout the clone and individual cells are morphologically immature and indistinguishable throughout the clones. Longer term culture of holoclones eventually leads to the generation of meroclone-producing cells, which are thought to represent TA cells. As such, these cultures are ideal for studying early changes in epidermal stem cells.

Notably, we found that although total p63 expression was similarly high throughout a holoclone, cells located near the center of the clone showed increased levels of p63 phosphorylation compared with those near the clone periphery (Figure 2). This suggests that within a holoclone, cells can be subdivided into two major populations of cells based on p63 phosphorylation levels. We predict that cells in the clone center are descendants of high p63-positive cells located near the clone periphery. The differential distribution of immature cells and more mature cells within the same clones is well supported by observations in meroclones, where cells in the periphery express moderate levels of p63 and look relatively immature but cells in the clone center appear morphologically more mature and express terminal differentiation markers such as involucrin.

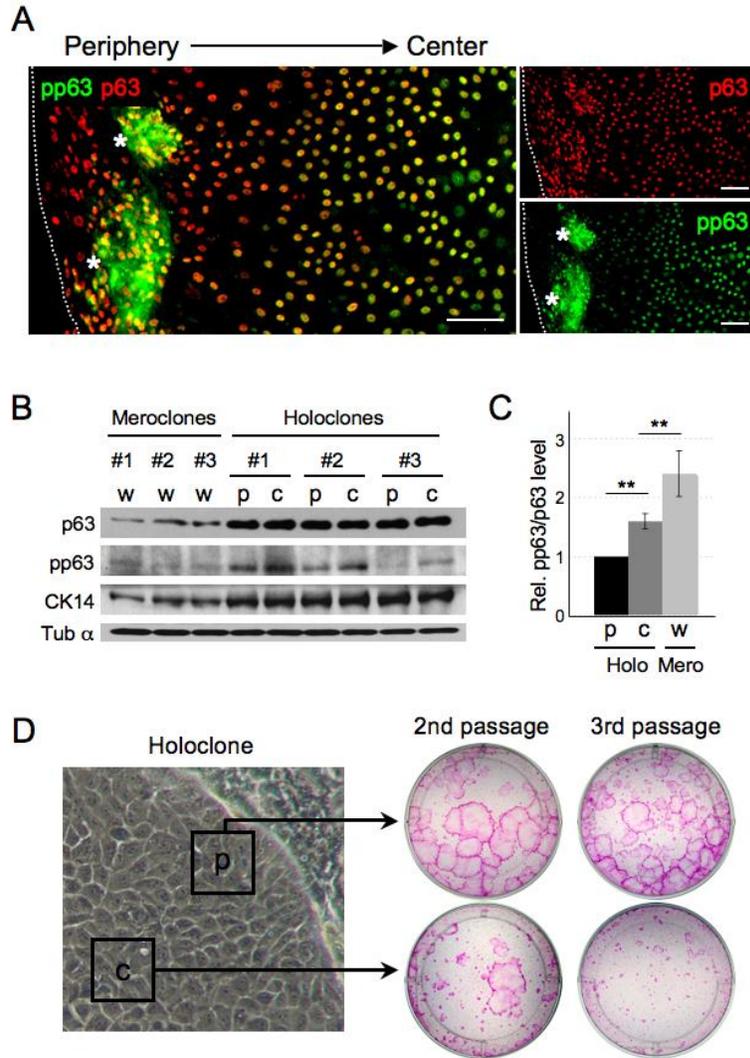
In order to quantify total p63 expression and phosphorylation levels of p63, cells were isolated from the periphery and the center of holoclones and total and phosphorylated levels of p63 were examined by western blot analysis. Consistent with immunofluorescence analysis, p63 expression was similarly high in the periphery and the center of holoclones while relative levels of p63 phosphorylation in the cells of the clone center were 1.5-fold higher than those in the periphery. As a comparison, we also analyzed meroclones and found that while p63 expression was much lower, relative levels of p63 phosphorylation were significantly higher than those in holoclones. Detection by an anti-cytokeratin-14 (CK14) antibody, a marker for immature epidermal cells, showed that cells in the periphery and center of holoclones expressed similarly high levels of CK14, while its expression was significantly decreased in meroclone-forming cells. These data suggest that high p63-positive cells in holoclones produce daughter cells with variable levels of p63 phosphorylation and that these cells can be further distinguished by their relative locations within the clones.

Having established a system to isolate high p63-positive cells with relatively low and high levels of p63 phosphorylation from holoclones, we performed clonogenic analysis of these cells to compare their proliferative potential. The isolated cells were cultured at clonal density and serially passaged. Our data demonstrate that while cells harvested from the holoclone periphery showed consistently high holoclone-forming efficiency through multiple passages, the proliferative potential of cells derived from the holoclone center declined upon serial passages. These data clearly indicate that phosphorylation of p63 inversely correlates with the proliferative potential of epidermal cells.

Using this clonogenic culture system, we will identify and investigate the function of p63-regulatory kinases in future studies. As stem cells are likely regulated by the niche functions, we will also investigate how niche contributes to the regulation of such kinase activities. As dysregulation of such control may lead to cancer due to mis-regulated self-renewal capacity, elucidation of p63-regulatory mechanisms will be important to understand physiology and pathophysiology of epithelial stem cells.



**Figure 1. Phosphorylation of p63 in adult mouse skin.** (A) Shown are representative images of the basal layer of four month-old mouse epidermis, stained with anti-p63 (red) and anti-Ser66/68 (green, *pp63*) antibodies, and counterstained with Hoechst 33342 (blue). Note only  $p63^{hi}pp63^{hi}$  and  $p63^{hi}pp63^{lo}$  cells are highlighted. (B) Representative images of four week-old mouse epidermis with enlarged views of the boxed area in right panels. Dotted lines indicate the epidermal-dermal border. Asterisks indicate high background of keratinized epithelium. Bar, 50 $\mu$ m. (C) Relative levels of p63 phosphorylation among  $p63^{hi}$  cells in the basal (n=38) and the first suprabasal layers (n=13) of the epidermis based on the fluorescence intensity of each  $p63^{hi}$  cells. An unpaired *t* test was used to estimate the *P* value: \*\* *P* < 0.01.



**Figure 2. Phosphorylation of p63 inversely correlates with the proliferative potential of p63<sup>hi</sup> cells.** (A) A representative image of a holoclone (stem cell clone) stained with anti-p63 (red) and anti-Ser66/68 (green, *pp63*) antibodies. Asterisks and the dotted line indicate high background of staining due to clustering of feeder cells and the clone border, respectively. Bars, 50 $\mu$ m. (B) Western blot analysis of p63 expression and pp63 levels in holoclone center (c), holoclone periphery (p) and meroclone-forming cells as a whole (w). Anti-CK14 and anti-tubulin  $\alpha$  antibodies were used as an immature epidermal cell marker and as a loading control, respectively. Three independent clones are shown for both holoclonal and meroclonal. (C) Quantification of (B) showing that relative levels of pp63 increase in the order of holoclone periphery, holoclone center and meroclone. A paired and an unpaired t test were used to estimate the *P* values for holoclone periphery (n=5) versus holoclone center (n=5), and holoclone center (n=5) versus meroclone (n=3), respectively. Error bars correspond to SD. \*\* *P* < 0.05. (D) Proliferative potential of cells in the periphery and clone center of a holoclone. Shown are representative images of the second and the third passages from three independent experiments with similar results.

## **Research Project 13: Project Title and Purpose**

*Targeting Aberrant BRCA-1 in Breast and Ovarian Carcinomas* - The purpose of this study is to determine whether a process called methylation contributes to the inactivation of the BRCA-1 gene in breast and ovarian cancers, even in those patients who do not have mutations of the gene. Our goal is to characterize patients and tumors in which methylation of the BRCA-1 gene occurs, in order to identify patients whose tumors might respond to a new class of drugs called PARP inhibitors. In addition, we will examine whether methylation is associated with an inflammatory response that might be used as a basis for immune-based therapies in this patient population. These strategies are aimed at developing personalized treatment approaches for patients with breast and ovarian cancer.

### **Anticipated Duration of Project**

12/15/2008 - 12/31/2011

### **Project Overview**

As our understanding of the genetic basis for breast cancer behavior and susceptibility have increased, the opportunity for individualized, or personalized therapy has become a reality. It is now possible to identify patients at risk for developing breast cancer based upon *BRCA-1* germline mutation status, as well as test tumors for genetic alterations likely to result in sensitivity to specific therapies, including Her2-based and hormonal therapies for tumors overexpressing Her2/neu or expressing ER and/or PR receptors, respectively. Similarly, advances in understanding the biologic basis of *BRCA-1* mutation-induced tumorigenesis have resulted in specific therapy for *BRCA-1* mutated tumors, based upon inhibition of Poly (ADP-ribose) polymerase-1 (PARP-1). The purpose of this study is to determine whether an epigenetic phenomenon, hypermethylation of *BRCA-1*, contributes to the inactivation of the *BRCA-1* gene in breast and ovarian cancers, even in those patients who do not have mutations of the gene. Our goal is to characterize patients and tumors in which this occurs, in order to identify an additional group of patients whose tumors might respond to PARP inhibitors. In addition, we will examine whether methylation is associated with an inflammatory response that might be used as a basis for immune-based therapies in this patient population. These strategies are aimed at developing personalized treatment approaches for patients with breast and ovarian cancer in whom aberrant BRCA-1 is the major mechanism of growth and progression.

Specific Aim 1: To perform a cohort study to determine the status of *BRCA-1* gene mutation, *BRCA-1* promoter hypermethylation and BRCA-1 protein expression in breast and ovarian cancer patients treated at the Abramson Cancer Center.

Specific Aim 2: To compare the clinico-pathologic characteristics and outcomes in breast and ovarian cancer patients with *BRCA-1* mutations vs. promoter hypermethylation.

Specific Aim 3: To determine whether presence of T-cell infiltration (TIL) is associated with *BRCA-1* mutation or promoter hypermethylation in this cohort of patients.

## **Principal Investigator**

Angela DeMichele, MD, MSCE  
Principal Investigator  
University of Pennsylvania  
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## **Other Participating Researchers**

George Coukos, MD, PhD, Phyllis Gimotty, PhD, Katherine Nathanson, MD, Susan Domchek, MD – employed by the University of Pennsylvania

## **Expected Research Outcomes and Benefits**

This project is expected to yield several important outcomes that will specifically benefit patients with breast and ovarian cancer. First, we will identify a group of patients who have tumors harboring a specific abnormality (methylation of BRCA-1) that has previously not been well-characterized or understood. We will screen for this abnormality in ovarian cancer patients, as well as in breast cancer patients with one of three characteristics associated with aggressive tumors, 1) young women (<40), 2) African Americans, and 3) those with triple negative (ER-negative, PR-negative, Her2-negative) tumors, for whom better treatments are clearly needed. We will determine whether patients with BRCA-1 methylation also have inflammation associated with their tumors. Ultimately, identifying, characterizing and understanding the biological mechanism of cancer growth and progression in these patients will lead to the personalized therapy of these tumors using both PARP inhibition and immunotherapeutic approaches.

## **Summary of Research Completed**

### Breast Cancer Patients:

Since the last progress report, we have continued to enroll subjects from the existing cohort study, UPCC 19102, “Molecular and Genetic Determinants of Bone Loss in Premenopausal Breast Cancer Survivors Who Received Adjuvant Chemotherapy (“BMD”)” a previously-funded study through Commonwealth funds (PI: Angela DeMichele). From the BMD project, we have enrolled 64 subjects and 26 subjects had verbally agreed to complete the study questionnaire but have not yet returned the signed consent form. After a minimum of 3 follow-up calls and e-mails to those who had originally agreed to join the study, a decision was made to track all of the patients’ next appointments to the the hospital and complete the questionnaire in person.

However, due to the considerable amount of time of disease free survival, many members of this cohort did not schedule or keep their appointment times with their oncologist and thus we were not able to follow-up with in person. Due to this difficulty of getting a hold of subjects from the

BMD study, a modification was made to the protocol to enroll subjects from another existing cohort study. This modification was to expand inclusion criteria to include breast cancer patients who participated in the UPCC 14108 study, “Predictors of Ovarian Insufficiency through Serial Exams in young breast cancer patients” (POISE). The POISE Study has the same inclusion criteria as UPCC 19102.

- (1) Stage I, II and III breast cancer patients who were premenopausal at the time of diagnosis
- (2) Age > 18
- (3) Blood DNA available for study
- (4) Primary tumor specimen available for study
- (5) Able to understand and provide informed consent

The total target enrollment number remained the same.

To date, a total of 100 patients have now been enrolled into the study. The following tables summarize the enrollment activity from both cohorts thus far.

<b>BMD Study Participants</b>	<b>Total Eligible For REPAIR</b>	<b>Enrolled to REPAIR</b>	<b>Agreed/Pending Return of Consent/Questionnaire</b>	<b>No response</b>	<b>Declined</b>
128	108	64	26	18	1

<b>POISE Study Participants</b>	<b>Total Approached For REPAIR</b>	<b>Enrolled to REPAIR</b>	<b>Agreed/Pending Return of Consent/Questionnaire</b>	<b>No response</b>	<b>Declined</b>
87	36	36	0	0	0

Since the previous continuing review and approval by the IRB on 7/21/10, there has only been one small modification, involving the inclusion of subjects from the POISE Study, that was submitted and approved by the expedited review process. The most recent continuing review was submitted on July 7, 2011.

Progress has also started on refining the necessary methylation assays in breast cancer specimens. The laboratory technician has been trained by the CHOP pathology core in basic immunohistochemistry techniques and Laser Capture Microdissection (LCM). LCM will be used to isolate only malignant cells for downstream DNA extraction. Tumor blocks from subjects with breast cancer have been obtained from the Cooperative Human Tissue Network for use on trial runs of LCM to obtain optimal cell quantity for DNA extraction. Tumor blocks were cut, stained, underwent LCM, and subsequent DNA extraction. Extracted DNA underwent quality and quantitative control analyses to assess integrity for downstream methylation assays. For methylation assays, extracted tumor DNA was bisulfite converted, changing all unmethylated cytosines to uracil, allowing for visibility in genomic sequencing. Wild-type, methylation specific, and non-methylation specific nested and non-nested PCR primers were designed and optimized for quality and quantity of PCR product. Bisulfite converted DNA underwent PCR amplification utilizing nested and non-nested wild-type, methylation specific, and non-methylation specific PCR primers. Results were visualized on an agarose gel and showed evidence of methylation. The technician is currently working on performing genomic sequencing of PCR amplified DNA to confirm evidence and amount of methylation.

Thus we have started obtaining the primary tumor specimens of the enrolled subjects. 57 of the enrolled subjects have their primary breast tumor at the University of Pennsylvania and 43 are at outside hospitals. For subjects whose tumor block is not obtainable we are requesting unstained slides. Three primary tumor blocks have been received from outside hospitals for methylation assays.

### **Research Project 14: Project Title and Purpose**

*Understanding the Role of Autophagy Inhibition in Cancer Therapy* - Many existing cancer therapies can induce cancer cell death in laboratory cell lines but have modest effects when used in cancer patients. Autophagy is a process of “self-eating” that has been observed in human cancer cell lines deficient in the ability to undergo cell death in response to a variety of cancer therapies. Recent evidence suggests therapy-induced autophagy promotes tumor cell survival and resistance to existing therapies by allowing cancer cells to clear the damage caused by these therapies. These results have established autophagy as a therapeutic target in cancer. The proposed clinical trials will determine if high doses of the autophagy inhibitor hydroxychloroquine (HCQ) can be safely combined with existing cancer treatments and if autophagy inhibition can be detected in patients receiving this treatment. In each of these clinical trials, blood and tumor tissue will be collected from patients in order to characterize the relationship between HCQ concentration and changes in autophagy. The results of these studies will provide knowledge that will inform the design of future phase II and phase III trials that will test the hypothesis that autophagy inhibition combined with standard therapies can improve outcomes in a wide variety of cancers.

### **Anticipated Duration of Project**

12/15/2008 - 12/31/2011

### **Project Overview**

Background: While the induction of apoptosis has been the focus of preclinical drug development, the majority of human cancers harbor defects in the pathways that control apoptosis. Autophagy is an intracellular process by which damaged mitochondria and proteins are sequestered in autophagic vesicles and destroyed through fusion with lysosomes. Autophagy has been observed in cancer cell lines deficient in the ability to undergo apoptosis in response to a variety of cancer therapies. Recent evidence suggests that therapy-induced autophagy promotes tumor cell survival and resistance to a number of existing therapies. Preclinical studies have demonstrated that hydroxychloroquine (HCQ) inhibits autophagy by deacidifying lysosomes and therefore promotes the death of cancer cells using autophagy as a survival strategy.

Objective/hypothesis: This project describes specific aims to test the hypothesis that combining autophagy inhibitors with existing therapies is safe, and that autophagy inhibition can be achieved and measured in patients.

Specific Aims: 1) Conduct pharmacokinetic (PK), pharmacodynamic (PD), and genetic studies designed to characterize autophagy in blood and tissue samples from a phase I/II trial of HCQ

with radiation therapy with concomitant and adjuvant temozolomide in patients with newly diagnosed glioblasoma multiforme (GBM), 2) Conduct additional phase I and disease-specific phase II trials of HCQ in combination with autophagy inducers in patients with refractory malignancies.

**Study Design:** The phase I/II trial in patients with GBM will be used to pilot the PK and PD analysis for the clinical trials outlined in specific aim 2. First, a population PK model for HCQ in combination regimens will be developed. A PD assay for autophagy inhibition in peripheral blood mononuclear cells will be used on patient samples. Genotypes of specific genes that regulate autophagy will be obtained in patients with GBM that may predict benefit from HCQ therapy. While this study is accruing patients and preliminary results are obtained from correlative studies, additional phase I trials incorporating the PK and PD endpoints outlined in specific aim 2 will be launched in patients with refractory solid tumor malignancies.

### **Principal Investigator**

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### **Other Participating Researchers**

None

### **Expected Research Outcomes and Benefits**

This proposal describes the first phase I clinical trials that will establish the safety of combining escalating doses of HCQ with other cancer therapies. These trials will characterize the relationship between HCQ concentration and autophagy inhibition. Characterizing tumor responses in these clinical trials will also identify malignancies in which launching phase II trials may yield promising results. Finally, this proposal also describes assays to identify newer more potent and specific autophagy inhibitors that can be tested in future clinical trials. The long-term goals of these studies are to improve the life span of cancer patients with advanced disease, and to identify promising new regimens that could be tested as adjuvant therapy to improve cure rates in patients with early disease.

### **Summary of Research Completed**

Funds from this grant have provided support for a research specialist who has conducted immunoblotting studies focusing on changes in levels of the autophagy protein LC3 in collected peripheral blood mononuclear cells for specific aims 1 and 2. The peripheral blood mononuclear cells (PBMC) assay for autophagy inhibition described in specific aim 1 has demonstrated a clear

HCQ-dose dependent accumulation of autophagic vesicles by electron microscopy (EM), confirmed by LC3 immunoblotting. A population PK model was successfully constructed using a subset of patients from the phase II trial to obtain a significant number of data points. PK-PD analysis has revealed that only patients that achieve an estimated peak concentration of  $> 2000$  mg/mL in their blood are likely to achieve autophagy inhibition as measured by the PBMC assay.

The phase I/II clinical trial of bortezomib and HCQ in patients with refractory multiple myeloma has also completed accrual. No DLT was observed with 1200 mg HCQ which was the highest dose allowed by his protocol for dose escalation. Samples analyzed for autophagy inhibition thus far (800 mg daily HCQ has been analyzed) have not found a consistent blockade of autophagy with this combination. This data will be presented at the ASH annual meeting 2011.

The phase I trial of temozolomide and hydroxychloroquine enrolled 7 patients to the 1000 mg cohort as one patient had grade 3 rash. No additional DLT was observed. Dose escalation continued to the top dose level. Currently 3 patients are being treated in the HCQ 1200 mg cohort. There was one patient with prolonged stable disease at 1200 mg HCQ, but no responses thus far. One patient had a DLT of grade 3 dehydration and nausea and therefore the dose cohort was expanded. A portion of this preliminary data was presented at the AACR 2011 annual meeting.

### **Research Project 15: Project Title and Purpose**

*Pilot Study: Imaging and Modifying Hypoxia in Head and Neck Squamous Cell Tumors* - Head and neck cancer is a devastating disease that kills about 8,000 Americans each year. Many of these cancers are poorly oxygenated (hypoxic) and therefore are harder to kill with radiation therapy and more likely to metastasize. Higher doses of radiation can be given to hypoxic tumors, but this approach results in more toxicity and side effects. Our purpose is to (1) determine the presence and amount of hypoxia in recurrent head and neck cancer using  $^{18}\text{F}$ -EF5 PET scanning and using this method, (2) determine whether Nelfinavir, which has safely been used in many HIV patients in the USA, can make tumors more oxidic. The knowledge gained in this project may allow patients with oxidic tumors to avoid high dose radiation and those with hypoxic tumors to receive a safe drug treatment to make standard radiation therapy more effective.

### **Summary of Research Completed**

This project was dropped prior to the expenditure of any grant funds.

### **Research Project 16: Project Title and Purpose**

*Research Recruitment, HPV, and Cervical Cancer Prevention in Asian American Women* - Cervical cancer is an important public health concern, among minority populations, including Asian American women, who are disproportionately affected by this disease. At the same time, participation by Asian Americans in clinical trials and other biomedical research is low. In this three-step project, we propose to: (Step 1) assess barriers and promoters to research participation

among Chinese- and Vietnamese-American women; (Step 2) test different communication and outreach strategies to maximize recruitment of these women into a study using two methods of data collection: a brief questionnaire and biosampling; and (Step 3) perform a preliminary study of the epidemiology of Human Papillomavirus (HPV) among these women.

### **Duration of Project**

1/1/2009 - 12/31/2010

### **Project Overview**

Objective: To identify effective recruitment strategies to engage Asian American women in cervical cancer prevention research and to broaden the understanding of Human Papillomavirus (HPV) epidemiology in this population.

Specific Aims: The primary aims are (1) to identify effective culturally-appropriate communication strategies to recruit Chinese- and Vietnamese-American women into cancer prevention studies involving biosampling. This will involve randomization of patients to one of two different recruitment strategies, based upon results of elicitation research; (2) to assess if subtypes of HPV in Chinese- and Vietnamese-American women are similar to subtypes seen in the general population and the same as those targeted by HPV vaccines that are currently available and those under development; and (3) to describe knowledge, attitudes, and behaviors with primary prevention of cervical cancer (including HPV vaccination) among Chinese- and Vietnamese-American women.

Design and Methods: The proposed study will require a three-step, qualitative-quantitative design. Step 1 will employ elicitation research methodology and use language-appropriate focus groups with Chinese- and Vietnamese-American women to assess barriers and promoters to participation in biomedical research involving biosampling (pap and blood), as well as knowledge and beliefs about HPV and HPV vaccination. Step 2 will involve creation and testing of two alternative methods of study recruitment with the target populations, using different message-framing techniques based upon results of elicitation research. The outcome of this step will be the collection of biobehavioral data including: responses to a brief questionnaire and collection of biosamples. In Step 3 biosamples will be tested for evidence of cervical dysplasia and the presence of specific high-risk HPV types. Behavioral data will be analyzed to correlate knowledge, attitudes and behaviors with sociodemographic factors and HPV infection.

This project will produce data for a culturally and language appropriate, population-based study of HPV and cervical cancer prevention among the growing Pennsylvania and U.S. population of Asian American women.

### **Principal Investigator**

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### **Other Participating Researchers**

Deborah W. Bruner, PhD, RN; Ellen Giarelli, EdD, CRNP; Joseph Cappella, PhD; Cindy M. McGrath, MD; Wei-Ting Hwang, PhD – employed by the University of Pennsylvania

### **Expected Research Outcomes and Benefits**

The outcomes of this project will be a clearer understanding of recruitment strategies to engage the understudied population of Asian American women into research involving biosampling. In addition, the investigators will begin to understand the epidemiology of high-risk Human Papillomavirus (HPV) infection within this population, as well as knowledge, attitudes, and behaviors related to cervical cancer risk and prevention. The identification of successful study recruitment strategies will be useful for the development of larger epidemiologic studies that will follow.

This project takes a novel approach to the comprehensive study of a population that is at high-risk, understudied and therefore, underserved. While there is a body of published work related to Asian American women and behavioral factors associated with Pap testing, there is limited data on behavioral issues pertaining to HPV and HPV vaccine uptake specifically in this population. Moreover, no large studies have combined such behavioral research with biosampling among this cohort.

With ever-increasing numbers of Asian Americans in the United States (US) and Pennsylvania (PA) in particular, this study comes at an important time with respect to its potential impact on public health. The ultimate goal is to understand the epidemiology of HPV and to reduce cervical cancer rates in this high-risk population. This project will apply strategies proven effective in enhancing human subject recruitment to enhancing health promoting behaviors, such as HPV vaccine uptake. This project will produce a model intervention to promote cancer prevention in underserved populations.

### **Summary of Research Completed**

From July 2010 through November 2010, we approached Asian female patients at Philadelphia District Health Center #2 and invited them to participate in our biosampling study. Recruitment strategy was informed by the formative work accomplished in the earlier stage of our research project (which has been described previously). We randomized our recruitment technique so that some women were invited to participate using a standard recruitment method, while others were invited using an enhanced recruitment method that incorporated strategies identified during the earlier stage of this project.

Twenty-one Asian women were identified as eligible based on inclusion criteria and were approached for participation. Of these 21 patients, 7 women agreed to participate, signed consent

forms, and provided biosamples (31.9%). No adverse events or unanticipated problems occurred in the course of these activities.

All participants provided a Pap and blood sample, both of which were collected by clinical staff at Health Center #2 and then transported back to the University of Pennsylvania. Blood was processed for biosample banking. Pap samples were analyzed by the University of Pennsylvania Molecular Pathology Laboratory. Per protocol, Pap results were sent to the medical personnel at Health Center #2 for appropriate follow-up.

All Pap samples collected yielded normal cytology and were negative for HPV. Therefore, no statistical analysis was performed to identify variables that might influence Pap or HPV results.

The following table describes the women who were approached for biosampling participation.

<b>TOTAL NUMBER OF WOMEN APPROACHED FOR PARTICIPATION = 21</b>		
<b>Language of Recruitment</b>		
	Vietnamese	8 (38%)
	Chinese	9 (43%)
	English	4 (19%)
<b>Other Demographics</b>		
	Born in USA	0 (0%)
	Married	17 (81%)
	Finished 12 yrs school	7 (33%)
	Prior research participation	6 (29%)

The following table describes the characteristics of women who consented to participate, as opposed to women who declined participation

<b>CONSENTED = 7</b>		
<b>Language of Recruitment</b>		
	Vietnamese	2 (29%)
	Chinese	2 (29%)
	English	3 (42%)
	<b>Recruitment using enhanced strategy</b>	4 (57%)
<b>DECLINED PARTICIPATION = 14</b>		
<b>Language of Recruitment</b>		
	Vietnamese	6 (43%)
	Chinese	7 (50%)
	English	1 (7%)
<b>Reasons for Refusal</b>		
	No Time	9 (64%)
	Recent Pap already done	2 (14%)

Other (lack of interest, fear of pain)	3 (21%)
<b>Recruitment using enhanced strategy</b>	<b>9 (64%)</b>

Due to the small sample size we were unable to determine whether enhanced versus standard recruitment strategy had an impact on recruitment success. Among patients approached using the standard method, 37.5% agreed to participate in biosampling. Among patients approached using the enhanced method, 30.8% agreed. This was not a statistically significant ( $p = 0.75$ ), though it is unknown what a much larger sample might have revealed with regard to this question.

On the other hand, prior research participation was a strong predictor of biosampling participation in the current study, even in this small sample (83.3% participation among women with prior research history versus 7.1% among those without prior research history;  $p < 0.1$ ).

Looking at this 2-year project as a whole, we have succeeded in learning (through our initial interviews/focus groups) a great deal about participation in biosampling research among Asian immigrant women, and we have been able to develop a recruitment strategy based upon the mixed qualitative/quantitative research that was performed.

So far, some of these findings have been submitted and accepted for publication in the peer-reviewed literature: Giarelli E, Bruner DW, Nguyen E, Basham S, Marathe P, Dao D, Huynh TN, Cappella J, and Nguyen G (In Press). Research participation among Asian American women at risk for cervical cancer: Exploratory pilot of barriers and enhancers. *J of Immigrant and Minority Health*.

It is yet to be determined the degree (if any) to which our culturally-enhanced recruitment strategy can affect the success of biosampling recruitment, but that will have to be determined in another study with larger sample size.

### **Research Project 17: Project Title and Purpose**

*Individualized Therapy for Advanced Non-small Cell Lung Cancer Based on Clinical and Molecular Typing* - The main purpose is to show that *non-empiric* therapy scientifically chosen on the basis of existing clinical and molecular markers can result in improved progression-free and overall survival (PFS and OS) in good performance status patients with advanced Non-small Cell Lung Cancer (NSCLC), compared to historic controls and contemporaneous subjects who are treated empirically. Subsidiary objectives include (1) the collection of tumor tissue and serum to further elucidate the molecular typing of patients with advanced NSCLC; (2) the development of a broad-based, interactive, collaborative database of advanced NSCLC patients treated at the University of Pennsylvania; and (3) parity or improvement with respect to expected toxicities and adverse events (AEs).

### **Anticipated Duration of Project**

12/15/2008 - 12/31/2011

## **Project Overview**

In this project, we will study how to best implement various diagnostic and molecular pathologic studies to allow the optimal selection of customized therapies or enrollment into specific therapeutic protocols for patients with newly diagnosed advanced (stage IIIB and IV) NSCLC.

Our goals include:

- 1) Determining the feasibility of performing immunohistology and mutational analysis (K-ras, EGFR, etc.) in specimens (paraffin embedded and fresh) obtained by current standard of care methodologies employed for minimally invasive diagnosis. (“Run in”)
- 2) Performing a prospective study to evaluate the impact of the program on various outcomes including: a) feasibility, b) the degree to which therapies are changed by “customization”, c) health care utilization and cost, d) patient acceptance, and e) clinical outcome, including overall survival (OS), progression-free survival (PFS).

After the initial run-in, we will determine patients’ preferences for standard vs customized or protocol therapy and whether or not they have a sufficient biopsy material available for histologic and mutational testing. This project includes the following IRB-approved protocols: UPCC 09506 (phase II trial of erlotinib +/- PF-3512676), UPCC 02508 (randomized phase II of combination docetaxel and carboplatin +/- vandetanib); and UPCC 06508 (phase II study of bevacizumab and erlotinib in elderly patients).

We anticipate enrollment of 210 subjects into this three-year effort; we estimate that 62.5% of enrolled subjects will have sufficient prior tissue available from which histologic or mutational analysis can be performed, and that approximately 80% of these will choose “customized therapy” or go on other treatment protocols. Our goal is to show that customized therapy will result in a 50% increase in overall median survival vs standard therapy (15 vs 10 mos). Assuming 5% are not evaluable, accrual of 200 patients should assure that there is adequate power (>80%) and type I error (<5%) to demonstrate this difference.

## **Principal Investigator**

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## **Other Participating Researchers**

Steven Albelda MD, Anil Vachani MD, Tracey Evans MD, James Stevenson MD, Jared Weiss MD, and Dara Aisner MD– employed by the University of Pennsylvania

## Expected Research Outcomes and Benefits

We anticipate the following:

1. Demonstration that therapy based on molecular correlates and clinical factors is feasible and can be rationally delivered to patients with advanced NSCLC, for whom empiric therapy has previously been the standard
2. In comparison to standard, empiric therapy, customized therapy and/or formal therapeutic protocol therapy will result in improved outcome, including
  - a. Higher response rates to treatment
  - b. Delays in tumor progression
  - c. Increased survival
  - d. Equal or less toxicity
3. The therapeutic platform we develop will enable us to further test tissue and blood with the goal of developing new markers to predict therapeutic benefit as our standard treatments evolve, growing increasingly complex and diverse.

## Summary of Research Completed

Goal 1) Determining the feasibility of performing immunohistology and mutational analysis (K-ras, EGFR, etc.) in specimens (paraffin embedded and fresh) obtained by current standard of care methodologies employed for minimally invasive diagnosis. (“Run in”)

### Accomplishments during the reporting period:

Dr. Christopher Watt, the Assistant Director of Molecular Pathology, has taken over this project and has updated this listing, as delineated below [Table 1]; note the incremental acquisition and interrogation of specimens over time. During this period, the EGFR mutation rate documented at Penn has been 14.8%, in keeping with the 10 to 15% range observed generally in the United States. Data for the first six months of 2011 are still pending. Dr. Watt and his staff continue to extend the work performed by Dr. Aisner previously, examining the feasibility of molecular testing on cytology, as opposed to, histologic specimens.

**Table 1:**

EGFR Mutations	2010		2009		2008		Total	
	N	%	N	%	N	%	N	%
Wildtype	197	83.5%	87	87.0%	34	79.1%	318	83.9%
Exon 19 Mutation Present	16	6.8%	15	15.0%	3	7.0%	34	9.0%
L858R Mutation Present	12	5.1%	5	5.0%	5	11.6%	22	5.8%
Inconclusive	9	3.8%	0	0.0%	0	0.0%	9	2.4%
Indeterminate	2	0.8%	3	3.0%	1	2.3%	6	1.6%
<b>Grand Total</b>	<b>236</b>	<b>100.00%</b>	<b>100</b>	<b>100.00%</b>	<b>43</b>	<b>100.00%</b>	<b>379</b>	<b>100.00%</b>

Until the purchase of the pyrosequencer, specimens for KRAS were sent to outside labs for

assessment. They will now finally be done within the institution. In this regard, Dr. Watt has also provided a provisional update of KRAS data at Penn for the past 15 months (Table 2), and, in short order, at our behest, will break this down formally for both NSCLC and Colorectal cancer. This effort continues.

**Table 2:**

KRAS Mutation	Past 15 months	
	N	%
Wildtype	212	60.9%
Codon 12/13 Mutation Present	117	33.6%
Suboptimal Specimen	19	5.5%
<b>Grand Total</b>	<b>348</b>	<b>100.00%</b>

The results of EGFR and KRAS are reported in tandem, and are now documented in pathology notes in our EPIC electronic medical management system. Routine progress notes in Thoracic Oncology now clearly document EGFR and KRAS status. The incidence of EGFR and KRAS mutations will be tracked and broken down by race, gender, histology, etc.

In addition, with the emergence of EML4/ALK as a viable, “actionable” target since the initiation of this project, we are now prospectively evaluating using break-apart FISH testing virtually all newly diagnosed patients with advanced adenocarcinoma of the lung as well as all “never smokers” with NSCLC for the ALK translocation. Those who prove (+) for this molecular abnormality are candidates for an in-house, Pfizer-sponsored, IRB-approved trial evaluating crizotinib in ALK (+) NSCLC patients previously exposed to cytotoxic therapy. [UPCC 10510: Phase 2, Open-label, Single-arm Study of the Efficacy and Safety of PF-02341066 in Patients With Advanced Non-Small Cell Lung Cancer (NSCLC) Harboring a Translocation or Inversion Involving the Anaplastic Lymphoma Kinase (ALK) Gene Locus]. To date, over 11 patients have been accrued to this effort. It should be noted that phenotypically, these patients are very similar to those whose tumors prove (+) for EGFR mutation, although the abnormalities appear to be mutually exclusive; In addition, the therapeutic implications of testing are quite different depending on the nature of the molecular abnormality identified. EML4/ALK status is also documented in the electronic medical record.

Goal 2) Performing a prospective study to evaluate the impact of the program on various outcomes including: a) feasibility, b) the degree to which therapies are changed by “customization”, c) health care utilization and cost, d) patient acceptance, and e) clinical outcome, including overall survival (OS), progression-free survival (PFS).

Accomplishments during the reporting period:

In collaboration with the Center for Comparative Effectiveness in Genomic Medicine [CEGeM] led by Katrina Armstrong of the Department of Internal Medicine and the Abramson Cancer Center, we have formally evaluated the cost efficacy and of routine institution of EGFR TKIs in

the first line setting in mutation (+) patients, and assessed the potential role of re-biopsy in patients with insufficient tissue or previous equivocal results. Unsurprisingly, most of the costs are driven by the therapeutic agents, not by ancillary treatments or the management of toxicities. Beth Hansdorf PhD has written up the findings and submitted the manuscript to the Journal of Clinical Oncology. Dr. Anil Vachani and Dr. Corey Langer have had major input into this project, using data culled from Research Project 17 to inform the analysis and shape the therapeutic scenarios. They have been meeting with Dr. Beth Hansdorf, Dr. Sean McGellicot, and Dr. David Asch periodically, at least monthly. .

In the past year, two additional trials have opened that depend on tissue acquisition:

1. UPCC 01511: Phase 3, Randomized, Double-Blinded, Placebo-Controlled Study of ARQ 197 Plus Erlotinib Versus Placebo Plus Erlotinib in Previously Treated Subjects with Locally Advanced or Metastatic, Non-Squamous , Non-Small-Cell Lung Cancer (NSCLC)
2. UPCC 10510 Phase 2, Open-label, Single-arm Study of the Efficacy and Safety of PF-02341066 in Patients With Advanced Non-Small Cell Lung Cancer (NSCLC) Harboring a Translocation or Inversion Involving the Anaplastic Lymphoma Kinase (ALK) Gene Locus

### **Research Project 18: Project Title and Purpose**

*Research Infrastructure: Renovation of Space for the Center for Cognitive Neuroscience* - The purpose of this project is to provide office and laboratory space for the Center for Cognitive Neuroscience, whose member faculty are currently scattered across the campus in different locations despite their need for close collaboration, and whose faculty are also currently working in poor-quality facilities. The new space will facilitate the research productivity of the group by enabling them to work more collaboratively and to carry out their research programs with adequate office and dry lab facilities.

### **Anticipated Duration of Project**

5/27/2009 – 12/31/2011

### **Project Overview**

The Center for Cognitive Neuroscience is an interdisciplinary community dedicated to understanding the neural bases of the human mind. Its 13 faculty work in a variety of different aspects of human psychology and its brain mechanisms, including vision, attention, language, creativity, social-emotional functions and the development of these psychological abilities in childhood. Seven of these faculty members, with primary appointments in Neurology and Psychology, will move into the renovated space, along with their students, staff and dry lab equipment (mainly PCs), enabling an unprecedented level of collaboration and resource sharing among these labs.

Hence, the broad objective of this infrastructure project is to enable more and better research to be done in this intensely interdisciplinary field, which addresses questions of fundamental importance for understanding the human mind and whose potential applications touch many areas of health and human capital.

The specific aims are to renovate the 3<sup>rd</sup> and 5<sup>th</sup> floors of the Goddard Laboratories building, which are currently in poor condition. This space has not undergone any significant renovation since the building was erected in 1964, and is currently unsuited for laboratory research and, in some locations, not up to current building code. Existing offices and labs will be demolished to the perimeter walls and the entire space will be reconfigured into state-of-the-art dry lab, office and meeting space. The layout of the building is two towers with each floor divided into nine areas by structural concrete beams that support the floor above. The new layout of the space provides for 4 corner offices in each of the two towers per floor with administrative and support places in between the corner offices and a kitchenette area in the glass walkway between the two towers. This renovation also intends to provide ADA compliant restrooms on each floor.

### **Principal Investigator**

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### **Other Participating Researchers**

None

### **Expected Research Outcomes and Benefits**

One of the 21<sup>st</sup> century's most exciting scientific frontiers is cognitive neuroscience, the science of understanding how a human mind can arise from the biological processing of the human brain. In addition to the enormous intellectual value of research in cognitive neuroscience, this research is also crucial to understanding a number of vitally important issues facing society, including the diagnosis and treatment of mental and brain disorders and the protection and encouragement of human potential. In the first category are mental illnesses, most notably Attention Deficit Hyperactivity Disorder, Dyslexia, Autism, Depression, Schizophrenia and Drug Addiction and neurological disorders, most notably aphasia, amnesia, Alzheimer's disease and fronto-temporal dementia. In the second are educational issues including child development and prevention/intervention with at-risk children.

## **Summary of Research Completed**

During the past year (7/1/10 to 6/30/11) we have completed abatement and construction of the third and fifth floors of the Goddard Building according to the design documents. The space was turned over to the occupants for use at the end of January 2011.