

# University of Pittsburgh

## Annual Progress Report: 2006 Formula Grant

### Reporting Period

July 1, 2009 – June 30, 2010

### Formula Grant Overview

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

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

*Immune Assessment of a Novel Influenza Virus-Like Particle Vaccine* - Public health officials predict that an influenza pandemic can cause more illnesses and deaths over a shorter period of time than any other natural health threat. The influenza virus is easily spread from person to person. Although vaccination is a useful and cost effective way to combat the threat of a flu outbreak, seasonal flu vaccines provide only partial protection, especially in the very young and the elderly. The goals of this project are to gain a deeper understanding of how the immune system responds to influenza and to develop better methods for evaluating the effectiveness of influenza vaccines.

### Anticipated Duration of Project

1/1/2007 - 12/31/2010

### Project Overview

The broad objectives of this research project are to examine immune responses by influenza virus-like particle (VLP)-based vaccines and to develop better methods to test the effectiveness of influenza and other vaccines. This project involves two specific aims. The first aim is to compare immune responses elicited by influenza VLPs to recombinant hemagglutinin (rHA) vaccines in small animals (mice and ferrets). The second aim is to develop improved methods of testing how the immune system responds to influenza infection and influenza vaccination. Advanced technology and special instruments to test immune responses will be used in both aims. The goal is to develop improved assays for assessing influenza immune responses elicited by infection or vaccination.

## **Principal Investigator**

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## **Other Participating Researchers**

Ronald C. Montelaro, PhD (co-PI); Simon Barratt-Boyes, BVSc, PhD; Ted M. Ross, PhD - employed by University of Pittsburgh

## **Expected Research Outcomes and Benefits**

Several broad areas of anticipated benefit will be derived as outcomes of this research, which will be conducted at the University of Pittsburgh Center for Vaccine Research (CVR). First, this research will enhance the understanding of immune system responses elicited by influenza vaccines in healthy adults, including elderly individuals. Second, this information will be used to develop effective influenza vaccines; it will also be used to develop new immune assays and improve existing assays for assessing influenza infection and vaccine efficacy. Third, the knowledge gained and technologies developed as a result of this research can be applied to the development of vaccines against other infectious viral diseases that affect citizens of the Commonwealth. Each of these three areas of benefit will contribute to the improved health status of Pennsylvania's citizens, particularly those most vulnerable to infectious viral diseases, like the very young and the elderly.

## **Summary of Research Completed**

### Serum HAI antibody measurements following virus like particle (VLP) immunizations

Individual recombinant baculoviruses were used to infect Sf9 insect cells that subsequently expressed hemagglutinin (HA), neuraminidase (NA), and matrix (M1) proteins from either NC/99 (H1N1), NY/04 (H3N2), or B/Shang/02 strains of influenza virus to spontaneously form VLPs that were then purified. Immunogenicity of VLPs was examined in BALB/c mice (6-8 weeks) inoculated by intramuscular injection (i.m.) at 0 and 3 weeks with purified monovalent (3 $\mu$ g) or a trivalent mixtures (3 $\mu$ g, 0.6 $\mu$ g, 0.12 $\mu$ g) of the antigen (Fig. 1). Post-vaccination antisera were evaluated for the ability to prevent virus-induced agglutination of turkey red blood cells (RBCs) (Fig. 2). These results were compared to mice vaccinated with the commercial split trivalent inactivated vaccine (TIV) (3 $\mu$ g). At week 3, following the priming dose, 75% of the mice vaccinated with 3 $\mu$ g of the seasonal trivalent VLP vaccine (TVV) had measurable titers of HAI antibodies ( $\geq$ 1:40) against the NC/99 (Fig. 2A) while 47% had HAI titers against NY/04 (Fig. 2C) and 100% of the mice had HAI titers against B/Shang/02 (Fig. 2E). At week 5, following two vaccinations, all mice immunized with the TVV at 3 $\mu$ g had HAI titers against NC/99 (Geometric Mean Titer [GMT] 640), NY/04 (GMT 423) and B/Shang/02 (GMT 640).

Mice that were vaccinated with a 3 $\mu$ g dose of each monovalent VLP vaccine had HAI titers to homologous strains that were statistically similar to the corresponding HAI titer elicited by the TVV at the same dose (Fig. 2B, 2D, and 2F). All mice vaccinated with monovalent VLPs had HAI titers  $\geq$ 1:40. There was little, if any, HAI cross-reactivity against viruses from non-corresponding subtypes (data not shown). In contrast, 70-75% of the mice vaccinated with TIV had HAI titers  $\geq$ 1:40 to one of the three homologous viruses (Fig. 2). In addition, mice vaccinated with TVV had statistically higher HAI GMT compared to mice vaccinated with TIV, regardless of influenza strain tested. For NC/99 and B/Shang/02, HAI titers elicited in mice vaccinated with 0.12 $\mu$ g of TVV were statistically higher than mice vaccinated with 3 $\mu$ g of TIV. Therefore, in general, the TVV was inducing higher HAI titers than the TIV, with broader cross-protection.

### Influenza virus challenge of TVV and TIV vaccinated mice

Mice vaccinated with H1N1 VLPs, the TVV, or the TIV were challenged with a mouse-adapted NC/99 (ms-NC/99) (Fig. 3). We had a limited number of mouse-adapted viruses to choose from for these studies. The NC/99 virus matched the H1N1 component in these vaccines. Human Immunodeficiency Virus (HIV-1) VLP vaccinated mice challenged with virus showed physical signs of infection (ruffled fur, dyspnea, lethargy), and they lost 15-20% of their original body weight between days 4-6 post-challenge (Fig. 3). Sixty percent of those mice died by day 8 post-infection, and the remaining mice recovered. Surprisingly, mice vaccinated with the H1N1 VLP, TVV, or TIV and then challenged with ma-NC/99 virus lost a similar amount of weight as the mock control animals but appeared to recover more quickly (Fig. 3). Even though all vaccinated and mock-vaccinated mice had similar high viral lung titers at day 3 post-challenge, all the mice vaccinated with H1N1 VLP, TVV, or TIV survived challenge. As a control, mice were vaccinated with a mouse-adapted B influenza virus (ma-B/Sich/02), and the monovalent B, TVV, and TIV mice showed no signs of weight loss, no outward signs of disease, and had little or no B/Sich/02 virus detected in the lungs, whereas HIV-1 VLP vaccinated mice died by day 6 post-infection (data not shown).

### Cell-mediated immunity elicited by VLP vaccines

It was an unexpected finding that vaccinated mice, subsequently challenged with the mouse-adapted NC/99 virus, lost ~15% of their original body weight since all the vaccines contained NC/99 HA and they both elicited high HAI titers against NC/99 virus (Fig. 2). Therefore, we tested the antisera against the mouse-adapted NC/99 virus used for challenge. Interestingly, there was very low HAI activity against the mouse-adapted NC/99 virus (Fig. 4) and only 20% of the mice had titers  $\geq$ 1:40, whereas all the mice had high titers against the wild-type human NC/99 virus (Fig. 2A). The vaccines contained the human sequences. The virus was mouse-adapted. The HAI titers elicited by the TVV and TIV were compared to the human strains. We then evaluated whether the vaccines that contained the human NC/99 HA elicited HAI antibodies that recognized mouse-adapted NC/99 HA. There are 12 differences in the mouse adapted HA and the human HA.

Viral infection and subsequent weight loss in vaccinated mice challenged with ma-NC/99 virus provided an opportunity to examine the cellular responses in these mice post-challenge since

vaccinated mice appeared to recover more quickly from NC/99 virus infection than unvaccinated mice (Fig. 3). Splenocytes and lung cells were collected at day 6 post-challenge from mice vaccinated with TVV, TIV, or mock control animals. At 6 days post infection, the T cell response was low unless there was preexisting memory allowing for a quicker recall response. Splenocytes were stimulated *in vitro* in an interferon-gamma (IFN $\gamma$ )–enzyme-linked immunosorbent spot (ELISPOT) assay with 6 pools of overlapping peptides representing the HA protein or with peptides to stimulate CD8<sup>+</sup> T cells specific for the immunodominant epitopes HA533 (IYSTVASSL) or NP147 (TYQRTRALV). In both H1N1 vaccine strains in the TVV and TIV, the HA<sub>533</sub> epitope is conserved in the HA protein and, therefore, allows for direct comparison of the CD8<sup>+</sup> T cell responses. As expected, mice vaccinated with the TIV had high HAI titers against the three homologous viruses in the TIV (data not shown). In contrast, HA-specific cellular responses were elicited by the TVV, but not the TIV (Fig. 5A). T cell responses were directed against epitopes throughout the HA (pools 2, 3, 5, and 6) following vaccination with the TVV. In addition, approximately 3 times the number of HA<sub>533</sub> specific CD8<sup>+</sup> T cells was detected in mice vaccinated with the TVV compared to TIV-vaccinated mice (Fig. 5B). As expected, CD8<sup>+</sup> T cells specific for the NP<sub>147</sub> epitope were only detected above background in TIV vaccinated mice since the VLP vaccines did not contain NP. There were no differences in the number of low cellular responses detected against M1 antigen (data not shown). In mice vaccinated with VLPs, there were approximately twice as many HA<sub>533</sub>-pentamer positive CD8<sup>+</sup> T cells in the lung compared to TIV vaccinated mice, as shown by flow cytometry (Fig. 5B). Intracellular cytokine staining for IFN- $\gamma$  revealed that approximately 6% of CD8<sup>+</sup> T cells in the lungs of VLP vaccinated mice produce IFN $\gamma$  after *in vitro* stimulation with the HA<sub>533</sub> peptide (Fig. 5C), which was significantly higher than in TIV-immunized mice (1%). As expected, control mice had few HA-specific responses 6 days post-challenge.

#### Influenza challenge of VLP vaccinated ferrets

To confirm the effectiveness of these VLP vaccines, ferrets were vaccinated with a 15 $\mu$ g dose of the monovalent H3N2 VLPs, TVV, or TIV. At week 3, all ferrets vaccinated with the TVV had high HAI titers against B/Shang/02, NC/99, and NY/04 albeit lower titers than against the influenza B virus (data not shown). The monovalent H3N2 VLP elicited similar titers after a single vaccination at week 3 as the TVV against NY/04. At week 5, all TVV vaccinated ferrets had HAI titers  $\geq$ 1:40 against all three viruses (NC/99, NY/04, B/Shang/02) in the vaccine (Fig. 6A). The HAI GMT against NC/99 was 1:80, against NY/04 was 1:727, and against B/Shang/02 was 1:1280. Ferrets vaccinated with a 15 $\mu$ g dose of the monovalent H3N2 VLP had HAI titers against NY/04 (1:367) but no cross-reactive HAI antibodies against NC/99 or B/Shang/02 (Fig. 6B). No ferrets vaccinated with lower doses of TVV (3 $\mu$ g or 0.6 $\mu$ g) had HAI titers  $\geq$ 1:40 against NC/99 or NY/04 (data not shown). The TVV elicited little or no cross-reactive HAI antibodies against the closely related SI/06 (H1N1) or B/May/04 viruses (Fig. 6A). However, both the TVV and the monovalent H3N2 VLP elicited HAI antibodies in 50-67% of ferrets against related H3N2 viruses, Pan/99, Fuj/02, Wisc/05, or Bris/10/07.

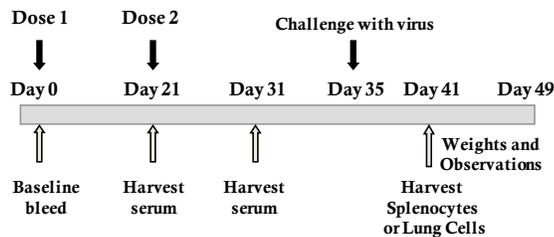
Ferrets were challenged intranasally with the H3N2 NY/04 virus (5x10<sup>5</sup> pfu). HIV-1 VLP vaccinated ferrets showed clinical signs of infection (lethargy), a spike in temperature (~2<sup>o</sup>C) in the first 24 hours post-challenge, and an increase in virus was detected in the nasal wash (8.25x10<sup>5</sup> pfu/ml) (Fig. 7). Similar viral titers were observed in ferrets vaccinated with a 0.6 $\mu$ g

dose of TVV ( $1.57 \times 10^6$  pfu/ml). However, there was a dose-dependent decrease in viral titers that correlated with increasing doses of TVV. Ferrets vaccinated with  $15 \mu\text{g}$  of TVV had similar viral titers in the nasal wash as ferrets vaccinated with  $15 \mu\text{g}$  of the monovalent H3N2 VLP vaccine. In contrast, mice vaccinated with  $15 \mu\text{g}$  of TIV had titers similar to the  $15 \mu\text{g}$  of TVV. Viral titers dropped precipitously by day 3 post-challenge, and no viruses were detected in the nasal wash after day 5 in any of the groups.

Ferrets vaccinated with a high dose of trivalent VLP vaccine had low titers of replicating virus and showed no signs of disease. The trivalent VLP vaccine elicited a broad immune response, thereby protecting the animals from homologous influenza challenge. Trivalent VLPs offer a potential public health benefit because they can easily be engineered and produced in a timely fashion, overcoming potential limitations of production of current egg-based influenza vaccines.

Figure 1

A



B

Vaccine Groups	Dose (based upon HA)
TVV	$3 \mu\text{g}$
TVV	$0.6 \mu\text{g}$
TVV	$0.12 \mu\text{g}$
H1N1 VLP	$3 \mu\text{g}$
H3N2 VLP	$3 \mu\text{g}$
B VLP	$3 \mu\text{g}$
TIV	$3 \mu\text{g}$
HIV-1 VLP	NA

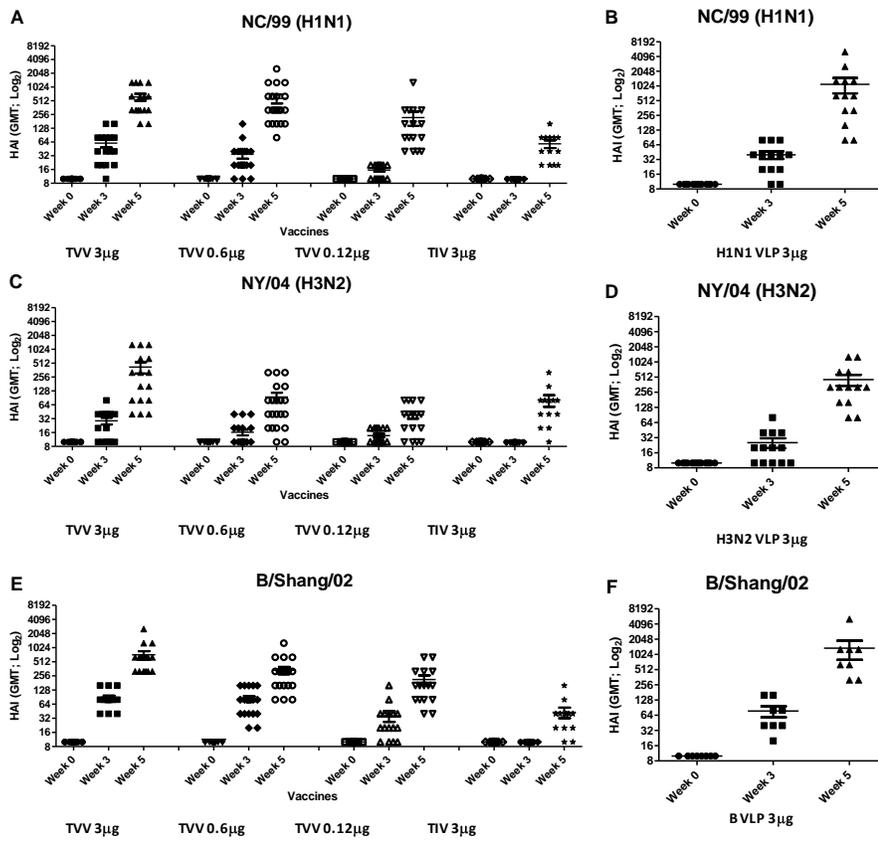


Figure 3

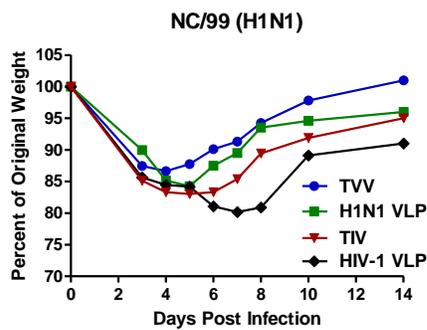


Figure 4

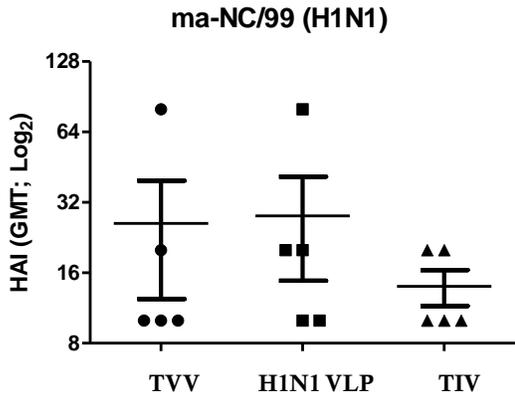
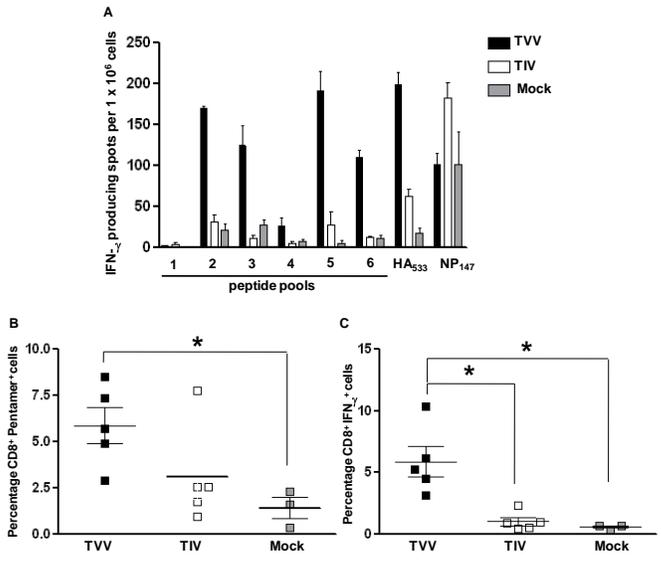


Figure 5



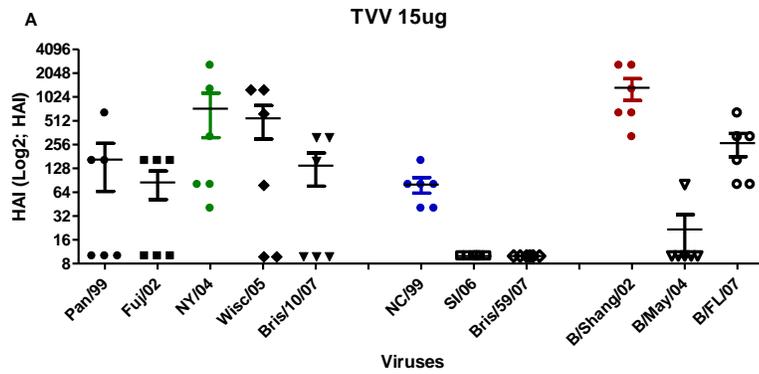


Figure 6

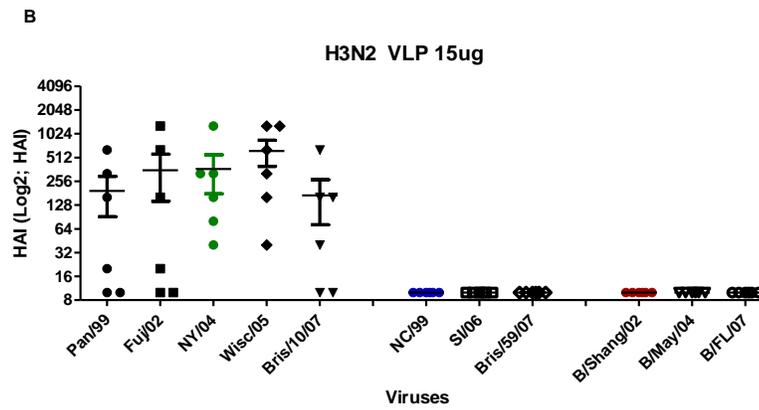
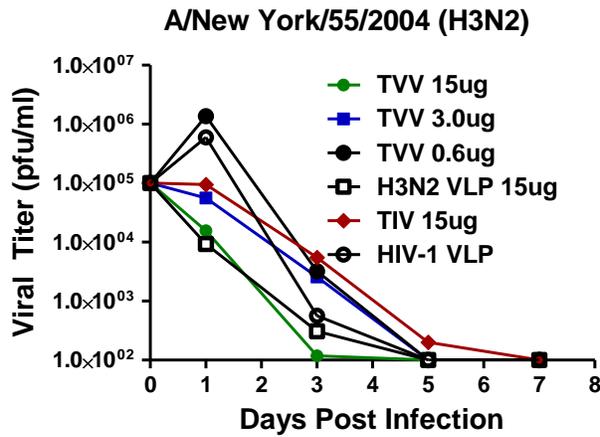


Figure 7



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

*DNA Repair Pathways and Their Influence on Tumorigenesis* - The purpose of this project is to explore novel features of three DNA repair pathways involved in mediating cellular response to DNA damage. Characterization of DNA repair has important implications for the development of cancer and for understanding certain kinds of resistance to anticancer chemotherapy. It is hoped that, through a better understanding of this fundamental process, more effective prevention and treatment strategies can be devised.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 3: Project Title and Purpose**

*Viruses and Cancer: Characterization of Host Cell-Virus Interactions* - Kaposi's sarcoma (KS), a lymphatic endothelial cell tumor, remains a critical public health problem world-wide. While rates of this disease have declined in the U.S. since their peak in the late 1980s, KS is now the most commonly reported tumor in sub-Saharan countries. In the U.S., specific populations remain at high risk for mortality from KS, including solid organ transplant patients who have a 40-60% mortality rate after contracting KS and the majority of survivors lose the transplanted allograft. Finally, while KS is currently well-controlled among AIDS patients, when these patients age it is expected that a resurgence of severe KS will occur. Therefore, developing vaccines to prevent or control this disease remains an important public health priority.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

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

*Cancer Inflammatory and Immune Response—Novel Insights and Targets for Therapy* - The experiments described in Aim 1 build upon the observation that the sera of patients with oral carcinoma contain tumor-derived membranous microvesicles (MV) that have been shown to induce death of activated immune cells and, thus, may contribute to immune dysfunction seen in these patients. The studies will characterize MV biology, importance for diagnosis, and prognosis of tobacco-related oral cancer. The experiments in Aim 2 extend observations related to how tumor cells die, examining the release of damage-associated molecular pattern molecules (DAMPs) and their effect on inflammatory cells. The effect of DAMPs, coupled with MV, on treatment response may enhance future therapeutic strategies and improve survival rates for cancer patients.

#### **Duration of Project**

1/1/2007 - 12/31/2007

#### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 5: Project Title and Purpose**

*Mechanisms for Antiestrogen Resistance in Breast Cancer* - The estrogenic signaling pathway is the target of proven and effective tamoxifen (a selective estrogen receptor modulator; SERM) chemoprevention and treatment for breast cancer. However, it is suspected that some breast cancers contain mutations in the estrogen receptor, and the Nichols lab has isolated one from a breast cancer that appears to be activated, not inhibited, by tamoxifen. This finding raises the question of whether expression of such a receptor converts normally sensitive breast cancer cells to antihormone resistance. By studying how cancer cells are inhibited by tamoxifen or similar SERMs and how they escape that inhibition, one can define alternative or additional therapy to minimize its effect. This new knowledge should lead to more successful combination strategies for chemoprevention, therapy, and disease-free survival for breast cancer patients.

#### **Duration of Project**

1/1/2007 - 12/31/2007

#### **Summary of Research Completed**

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

*Oral Cancer and Chromosomal Instability: Finding Novel Cancer Prevention Targets* - This project is designed around two major aims. The first aim seeks to determine how deregulation of cell cycle checkpoints leads to genomic instability in oral cancer cells. A significant subset of oral carcinomas is associated with high-risk human papillomaviruses (HPVs), and the HPV-16-encoded E7 oncoprotein will be used as a model system for further experiments. The second aim will focus on whether attenuation of the mitotic spindle checkpoint can be used to eradicate oral cancer cells. These projects will provide the framework for future translational studies to improve preventive approaches in patients at risk for HPV-induced oral cancer as well as nonvirus-induced oral carcinomas.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 7: Project Title and Purpose**

*Evaluating the Effects of Stress on Spontaneous Tumor Development* - The purpose of this project is to evaluate how chronic stress affects the development and progression of breast cancer in mice that spontaneously develop mammary tumors in a model that mimics the development of breast cancer in women. The investigators will also study the effects of chronic stress on the ability of these mice to mount immune responses that target a receptor on the breast tumors which is also expressed on human breast tumors and which is already known to be an antigen available for inducing immune responses in about 25 percent of women with breast cancer. These studies will provide new insights into how stress responses affect the induction of antitumor immunity to breast cancer.

### **Duration of Project**

1/1/2007 - 12/31/2007

### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 8: Project Title and Purpose**

*Hypermethylation of Genes in Secondary SCCHN in Relation to Smoking* - Most cases of squamous cell carcinoma of the head and neck (SCCHN) are strongly associated with chronic smoking. Almost half of the survivors of first tumors continue smoking after treatment, and many of these patients develop a second primary tumor (SPT) within two decades of initial diagnosis. The basic biological mechanisms that connect smoking and secondary SCCHN are not well understood. The Garte research team is interested in understanding these mechanisms by linking exposure to smoking with one of the more exciting recent fields of research into molecular carcinogenesis—changes in the function of genes due to hypermethylation, a specific way that cells control gene expression. The hypothesis to be addressed is that cigarette smoking is linked to hypermethylation of specific genes involved in secondary SCCHN tumorigenesis.

#### **Duration of Project**

1/1/2007 - 12/31/2007

#### **Summary of Research Completed**

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement 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 Infrastructure Project 9: Project Title and Purpose**

*Research Infrastructure: Vivarium Renovations for Barrier Facility* - Despite a vivarium staff's best efforts to run a pathogen-free facility, communicable disease outbreaks will still occur. The best method to minimize these outbreaks is to develop specialized standard operating procedures (SOPs) for moving animals, personnel, and materials into the facility; moving animals between facilities; and limiting facility access for outside individuals (public and researchers). The design of the existing vivarium facility in the Biomedical Science Tower South (BSTS) prohibits rigorous SOP implementation, leaving the animals vulnerable to adventitious disease outbreaks. The purpose of this project is to renovate the existing animal facility into a barrier facility to minimize communicable disease outbreaks.

#### **Duration of Project**

7/1/2007 – 11/30/2008

#### **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 10: Project Title and Purpose**

*Research Infrastructure: Caging Installation at Bridgeside Point II Vivarium Facility -* Bridgeside Point II (BPII) is a new research facility at the University of Pittsburgh. At this time, final fit-out of the core vivarium facility is being completed, and this project will provide necessary caging and related sanitary equipment. Because the facility is one mile from the heart of the main University campus, a fully equipped, on-site vivarium facility is essential for investigators working there.

### **Anticipated Duration of Project**

12/17/2009 – 12/31/2010

### **Project Overview**

To fully outfit the newest research building at the University of Pittsburgh, appropriate vivarium equipment must be purchased and installed. The first basic need for the facility is caging designed to accommodate the needs of the animals housed within according to the standards of the Association of Assessment and Accreditation of Laboratory Animal Care International (AAALAC). These standards include the air purification systems and cage washing facilities necessary to maintain a healthy vivarium environment.

### **Principal Investigator**

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### **Other Participating Researchers**

None

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

This project will enhance research infrastructure at the University of Pittsburgh by providing an on-site vivarium facility at the newest research building, BPII. Additional research space provides the infrastructure required to expand research programs, recruit new faculty members, and accommodate the changing needs of existing investigations, all of which are essential to advance biomedical progress and, in the long run, to translate findings from animal to human studies.

## **Summary of Research Completed**

All rodent and rabbit caging systems and related equipment have been delivered from the vendors. Ancillary equipment to support the caging is currently in storage at an outside warehouse while arrangements are made for the building opening and clinical cleaning. Large animal caging has been delivered and is installed in the large animal suites. Biosafety cabinets and NuAire animal transfer stations have been delivered and are on site.