



ANNUAL REPORT 2008

Tobacco-Related Disease Research Program
University of California



Annual Report 2008

from the University of California to the
California State Legislature on the progress of the
Tobacco-Related Disease Research Program,
established and administered by the University of California
pursuant to Proposition 99, The Tobacco Tax and Health Protection Act of 1988,
Senate Bill 1613 of 1989 and reauthorized pursuant to Assembly Bill 3487 of 1996

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EXECUTIVE SUMMARY

The Tobacco-Related Disease Research Program (TRDRP) is an integral component of California's internationally recognized effort to reduce the severe human and economic toll of tobacco use. TRDRP's mission is to mitigate the impact of tobacco-related illness by funding research on tobacco use and tobacco-related disease. This research has contributed to the success of the state's tobacco control efforts by identifying more effective policies and strategies for tobacco use prevention and cessation, particularly among our state's diverse communities. It has also identified promising new approaches to the treatment of tobacco-related diseases from which Californians suffer. TRDRP identifies the areas in which there is the greatest need for research, funds research that will address these needs, and disseminates the results of the research to the medical, scientific, and tobacco control communities. TRDRP is pleased to be a major contributor to prevention and treatment efforts within the state.

Tobacco consumption in California is at an all-time low due to an effective, comprehensive state tobacco control program and the price of tobacco products, including increases in state excise taxes on tobacco. The Department of Health Services reported that 14.0% of California adults were current smokers in 2005, a 38 percent decline since 1988 when California voters passed Proposition 99, which established the state's comprehensive tobacco research, education, and prevention programs.¹ Despite this decline, research on tobacco-related disease and tobacco use remains important because the state's taxpayers will be paying for decades to come for the treatment of tobacco-related diseases that are now developing in California's current smokers and in adolescents who are starting to smoke. According to a report by the Institute for Health & Aging at the University of California, San Francisco, the cost of smoking in California is nearly \$16 billion annually or \$3,331 per smoker every year, an avoidable cost borne by all California taxpayers.²

In 2008, 47 research grants were completed, representing cutting-edge science on tobacco-related disease and tobacco control policy and programs, particularly in those groups at highest risk for tobacco use and exposure to secondhand smoke. They include 10 on nicotine dependence, 5 on tobacco use prevention and cessation, 5 on tobacco control policy, 14 on cancer, 9 on heart and lung disease, and 4 on environmental tobacco smoke and effects of tobacco use on reproductive processes.

Brief summaries of the research findings, which appear at the end of this report, include:

- The prolonged facilitation of brain reward function induced by nicotine, as well as the conditioned negative affective states elicited by environmental stimuli, may contribute to relapse.
- Reliable measures of nicotine withdrawal in mice were developed that can be used to investigate the neurobiological and genetic factors contributing to the different aspects of nicotine withdrawal in future studies.
- The widely-used medication, bupropion, most likely improves smoking cessation by ameliorating the aversive effects of nicotine withdrawal rather than by making smoking less rewarding. Further, bupropion probably makes nicotine withdrawal less aversive by affecting either one or both of two chemical messengers found in the brain.
- Unexpectedly, 25% of Chinese- and Korean-American college students who had not smoked as college freshmen eventually did so during their remaining years in college.
- Qualitative interviews identified insights into why African Americans appear less likely than other groups to use nicotine replacement therapy.
- A unique, comprehensive, school-based, anti-tobacco program tailored to Deaf/HH youth was developed and implemented.
- Stores saturated with cigarette ads and promotions may increase smokers' daily consumption and reduce their resolve to quit.

¹ <http://www.dhs.ca.gov/tobacco/documents/press/PressRelease04-10-06.pdf>

² Max, W. et al. The Cost of Smoking in California, December 2002.

- Although exposure to secondhand smoke in indoor workplaces has decreased since the inception of the California Tobacco Control Program, a higher percentage of Hispanics/Latinas than other ethnic groups have reported exposure over time.
- The tobacco industry documents show that the industry was aware of the presence and the potential risk of radioactive polonium 210 in cigarette smoke for over 40 years but actively failed to reveal its presence.
- The tobacco industry is currently using the same advertising strategy to promote ostensibly “harm-reduced” tobacco products that they did decades ago with “low-tar” cigarettes.
- Although most African American churches have a health ministry, they neither address tobacco control nor have a written anti-tobacco policy.
- A novel device for early detection of laryngeal cancer, called optical coherence tomography, is being developed to produce extremely high quality images of the human larynx in an office-based setting.
- Demonstrated that endothelin, a vasoactive peptide, is produced in extremely high levels in the squamous cell carcinoma microenvironment and contributes to pain behavior in the cancer pain mouse model.
- Drugs called COX-2 inhibitors were shown to augment the immune response to cancer cells in laboratory lung cancer models and thus cause tumors to shrink.
- Lung damage caused by emphysema is considered irreversible. Believing that the molecule VEGF is essential for lung cell function, investigators demonstrated that general exercise is a possible way to reverse loss of function and increase endogenous VEGF in the lungs.
- Demonstrated that insulin-resistant smokers are most at risk of cardiovascular disease and found evidence for a potential pharmacological approach to decrease heart disease in these patients.
- The chemical processes leading to the formation of stable nicotine oxidation products under simulated indoor conditions were identified and characterized for the first time, which will help assess long-term exposure to secondhand smoke pollutants.
- Women exposed to SHS, based on a highly sensitive laboratory test, were 20%-37% less likely to conceive.
- A study of the effects of nicotine on uterine artery contractility found a major reason for the reduced uterine blood flow observed with smoking/nicotine exposure during pregnancy.

In 2008, TRDRP awarded \$13.9 million in 44 new grants (an additional 4 were declined) to scientists at 22 California non-profit research institutions. However, because of insufficient funds, TRDRP was unable to fund 51 research proposals that had been rated “outstanding” or “excellent” by expert peer reviewers, which was an increase over 2007.

The largest, multi-year grants addressed the following Primary Research Areas

- Cardiovascular and Cerebrovascular Disease
- Chronic Obstructive Pulmonary Disease
- Development of Nicotine Dependence Treatments
- Lung Cancer
- Prevention and Cessation of Tobacco Use and Tobacco-Related Health Disparities in California’s Diverse Populations
- Public Policy and Economics of Tobacco Use
- Secondhand Smoke and Outdoor Tobacco Smoke

INTRODUCTION

The Tobacco-Related Disease Research Program (TRDRP) is an integral component of California's internationally recognized effort to reduce the severe human and economic toll of tobacco use. TRDRP's mission is to mitigate the impact of tobacco-related illness by funding research on tobacco use and tobacco-related disease. This research has contributed to the success of the state's tobacco control efforts by identifying more effective policies and strategies for tobacco use prevention and cessation, particularly among our state's diverse communities. It has also identified promising new approaches to the treatment of tobacco-related diseases from which Californians suffer. TRDRP identifies the areas in which there is the greatest need for research, funds research that will address these needs, and disseminates the results of the research to the medical, scientific, and tobacco control communities. TRDRP is pleased to be a major contributor to prevention and treatment efforts within the state.

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OVERVIEW

Mission

TRDRP's mission is to mitigate the impact of tobacco-related illness by funding research on tobacco use and tobacco-related disease. The program's goals are consistent with the broader mission of Proposition 99, which is to reduce the human and economic costs of tobacco use by reducing the incidence, prevalence, morbidity, and mortality of tobacco-related disease in California.

Goals

TRDRP strives to meet the needs of the research community, the tobacco control community, the health care community, policy makers, and the public by:

- Funding high-quality and innovative research that contributes to the understanding of tobacco use and tobacco-related illnesses and serves California's diverse populations.
- Serving as an information resource for tobacco issues through dissemination of research findings and sponsorship of conferences and symposia.
- Funding research that will lead to more effective disease treatments for California's smokers and former smokers.
- Funding research that will lead to more effective strategies for tobacco use prevention and cessation.

¹ <http://www.dhs.ca.gov/tobacco/documents/press/PressRelease04-10-06.pdf>

² Max, W. et al. The Cost of Smoking in California, December 2002.

TRDRP strives to meet additional needs of the research community by:

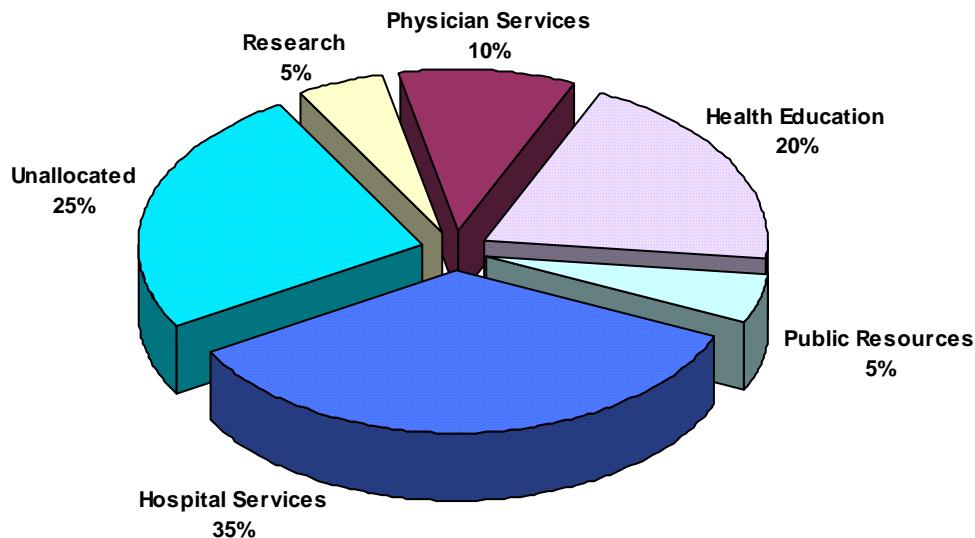
- Providing opportunities to researchers to conduct high quality and innovative research that advances tobacco-related science.
- Helping to build the research infrastructure in California that is critical for comprehensive tobacco-related disease research, in part by encouraging investigators to pursue careers in tobacco research through career development grant awards.

Program Administration

TRDRP was established as a result of the passage of Proposition 99 (“The Tobacco Tax and Health Protection Act of 1988”) in November 1988. The proposition increased the tax on cigarettes by 25 cents per pack and raised the tax on other tobacco products an equivalent amount. The initiative created the Cigarette and Tobacco Products Surtax Fund, consisting of six accounts in which specific percentages of the revenue are deposited annually (see Figure 1): the Research Account (5 percent), the Health Education Account (20 percent), the Hospital Services Account (35 percent), the Physician Services Account (10 percent), the Public Resources Account (5 percent), and the Unallocated (or General Purposes) Account (25 percent). Collection of the tax began on January 1, 1989.

Proposition 99 specified that the revenues from the Research Account be used to fund research on tobacco-related disease in California. The California Legislature subsequently asked the University of California to establish and administer a research program to facilitate the elimination of smoking in California, and to report annually to the Legislature about the activities of the Program. TRDRP manages all fiscal and programmatic aspects of the tobacco research funding from the Cigarette and Tobacco Products Surtax Fund. As stipulated by the legislation, funding for administrative expenses is limited to five percent of the Research Account. Within the Office of the President at the University of California, TRDRP is one of the Special Research Programs in the Office of Research and Graduate Studies.

Figure 1. Distribution of Tobacco Tax Revenue Specified by Proposition 99



REPORT ON 2008 ACTIVITIES

Completed Grants

In 2008, 47 research grants were completed, representing cutting-edge science on tobacco-related disease and tobacco control policy and programs, particularly in those groups at highest risk for tobacco use and exposure to secondhand smoke. They include 10 on nicotine dependence, 5 on tobacco use prevention and cessation, 5 on tobacco control policy, 14 on cancer, 9 on heart and lung disease, and 4 on environmental tobacco smoke and effects of tobacco use on reproductive processes.

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- A novel device for early detection of laryngeal cancer, called optical coherence tomography, is being developed to produce extremely high quality images of the human larynx in an office-based setting.
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- Women exposed to SHS, based on a highly sensitive laboratory test, were 20%-37% less likely to conceive.
- A study of the effects of nicotine on uterine artery contractility found a major reason for the reduced uterine blood flow observed with smoking/nicotine exposure during pregnancy.

Research Involving Women and Communities of Color

Of TRDRP's 190 active grants, 76 (40%) involve human subjects. Of the grants that involve human subjects, 73 (96.1%) involve both women and communities of color, 1 (1.3%) involves women but not communities of color, and 2 (2.6%) involve neither.

TRDRP Coordination with Tobacco Control and Education Programs Funded by the Proposition 99 Health Education Account

TRDRP coordinates its activities with the California Department of Health Services (DHS) and the California Department of Education (CDE) because the three agencies share the mission of reducing the harm and costs of tobacco use in the state. They receive funding from a common source, the Cigarette and Tobacco Products Surtax Fund created by Proposition 99. During 2008, TRDRP staff continued to work with their counterparts from the DHS Tobacco Control Program and the CDE Safe and Healthy Kids Program Office to keep abreast of developments in their respective programs, avoid duplication of effort, share expertise, and provide input into the development of each program's goals.

Dissemination of Research Findings

In accordance with state statutes, TRDRP regularly disseminates the findings of funded research in a number of ways. The knowledge gained from TRDRP-funded studies is helping to improve the effectiveness of the tobacco control programs supported by the Proposition 99 Health Education Account that are administered by the California Department of Public Health and the California Department of Education. Results of research on tobacco-related disease are also enhancing scientists' understanding of biological mechanisms underlying the cause of tobacco-

related disease and pointing the direction to technologies for the earlier detection and more effective treatment of lung disease, heart disease, and cancer.

- **Scientific Publications**

TRDRP-funded investigators have continued to actively disseminate findings from their research in scholarly publications and at scientific conferences. In 2008, funded investigators reported publishing 143 articles in refereed scientific journals, including 138 that had appeared in print and 5 that were accepted for publication and were awaiting appearance in print. Some of the peer-reviewed scientific journals in which the papers appeared include: *American Journal of Epidemiology; American Journal of Public Health; American Journal of Respiratory & Critical Care Medicine; Atherosclerosis, Thrombosis, and Vascular Biology; Biology of Reproduction; Birth Defects Research; Cancer Research; Circulation; Clinical Cancer Research; Environmental Science and Technology; FASEB Journal; International Society for Biological Therapy of Cancer; Journal of Biological Chemistry; Journal of Epidemiology and Community Health; Journal of Immunology; Lung Cancer; Nature Medicine; Neuroscience; Nicotine and Tobacco Research; Plos Medicine; Proceedings of the American Association for Cancer Research; Psychopharmacology; Science; Stroke; Tobacco Control; Vascular Medicine.*

- **Biennial Scientific Conference**

Scientific conferences are one of the most effective ways to disseminate recent research findings in a timely manner. TRDRP has hosted conferences at which its funded investigators report their latest findings. The program has expanded the traditional scientific conference model by including tobacco control professionals to give them the opportunity to learn about the latest findings directly from the scientists who are conducting the research.

Approximately 300 researchers and tobacco control professionals attended TRDRP's Biennial Conference in Sacramento on October 8-9, 2007. The conference theme was "Future Research Opportunities." The keynote speaker in the opening session was David Kessler, M.D., Dean of the School of Medicine and Vice Chancellor for Medical Affairs at the University of California, San Francisco and former FDA Commissioner. The luncheon speaker was Bill Lockyer, California State Treasurer and former California Attorney General. The plenary session included talks on future research opportunities in developing nicotine vaccines, reducing health disparities, and pharmacogenomics.

- **Newsletter**

In 2008, TRDRP published two issues of its newsletter, *Burning Issues*, which contained articles on critical research topics in tobacco-related disease and tobacco use, and information about the program and notices of upcoming events. They included articles on the Altria Group, Inc. and Phillip Morris International split; possible FDA regulation of tobacco products; and the University of California Regents action on accepting research funds from the tobacco industry. The newsletters are available on TRDRP's Web site, <http://www.trdrp.org/publications/Newsletters.asp>.

- **Web site**

Visitors to TRDRP's Web sit (www.trdrp.org) can search research grants, as well as view all program publications and announcements.

2007-2008 FUNDING CYCLE

• Research Grants Awarded

In 2007-2008, TRDRP awarded \$13.9 million in 44 new grants (an additional 4 were declined) to scientists at 22 California non-profit research institutions. However, because of insufficient funds, TRDRP was unable to fund 51 research proposals that had been rated “outstanding” or “excellent” by expert peer reviewers, which was an increase over 2006-2007. Details of 2007-2008 awards, including abstracts, can be found in TRDRP’s Compendium of Awards, which can be accessed at: <http://www.trdrp.org/publications/compendiums/comp07.pdf>.

The largest, multi-year grants addressed the following Primary Research Areas

- Cardiovascular and Cerebrovascular Disease
- Chronic Obstructive Pulmonary Disease
- Development of Nicotine Dependence Treatments
- Lung Cancer
- Prevention and Cessation of Tobacco Use and Tobacco-Related Health Disparities in California’s Diverse Populations
- Public Policy and Economics of Tobacco Use
- Secondhand Smoke and Outdoor Tobacco Smoke

• Award Types

- **Research Project Awards** fund investigator-initiated research projects on all aspects of tobacco-related disease and tobacco use. These awards support research that is judged likely to yield valuable outcomes. The projects are judged to be feasible and likely to succeed because they employ sound scientific approaches and offer promising supporting data from preliminary studies.
- **Innovative Developmental and Exploratory Awards (IDEAs)** fund developmental or exploratory research that is not yet sufficiently mature to compete successfully for an individual research award. Although the proposed research might lack adequate pilot data or proven methods, it is creative, intellectually exciting, and shows clear promise to yield findings that could lead to breakthroughs in the field.
- **Research career development awards.** TRDRP offers three award types that are aimed at enhancing the scientific infrastructure for tobacco-related research in California by supporting the development of careers in research. **New Investigator Awards** are aimed at encouraging newly independent investigators to conduct research on tobacco-related issues. **Postdoctoral Fellowship Awards** allow researchers early in their careers to receive training in tobacco-relevant disciplines. **Dissertation Research Awards** provide support for the dissertation research of doctoral candidates who wish to pursue tobacco-related research.
- **Collaborative research awards.** **Community-Academic Research Awards (CARA)** are intended to stimulate and support collaborations between community-based organizations and university-based investigators to perform scientifically rigorous research into tobacco control issues important to California’s diverse communities. **School-Academic Research Awards (SARA)** are intended to stimulate and support collaborations between schools and university-based investigators to perform scientifically rigorous research into tobacco control issues that: 1) are identified as important to schools in the state; 2) are likely to produce results that are meaningful to school-based prevention and intervention efforts; and 3) use methods that are relevant, culturally appropriate, and appropriate in terms defined and accepted by the schools. SARAs are jointly funded by the California Department of Education (CDE) and TRDRP.

• Cornelius Hopper Diversity Award Supplements

The Cornelius Hopper Diversity Award Supplements (CHDAS) are designed to encourage TRDRP-funded principal investigators to mentor individuals who want to pursue careers in research on tobacco use and tobacco-related disease. Qualified applicants for the CHDAS are from groups that are underrepresented among researchers who investigate tobacco use or tobacco-related disease, and/or individuals who will work directly with

underrepresented groups that are disproportionately impacted by tobacco use. Nine funded investigators received supplements to their TRDRP grants for support of additional project personnel (see Table 2).

Table 2. CHDAS awarded in 2008

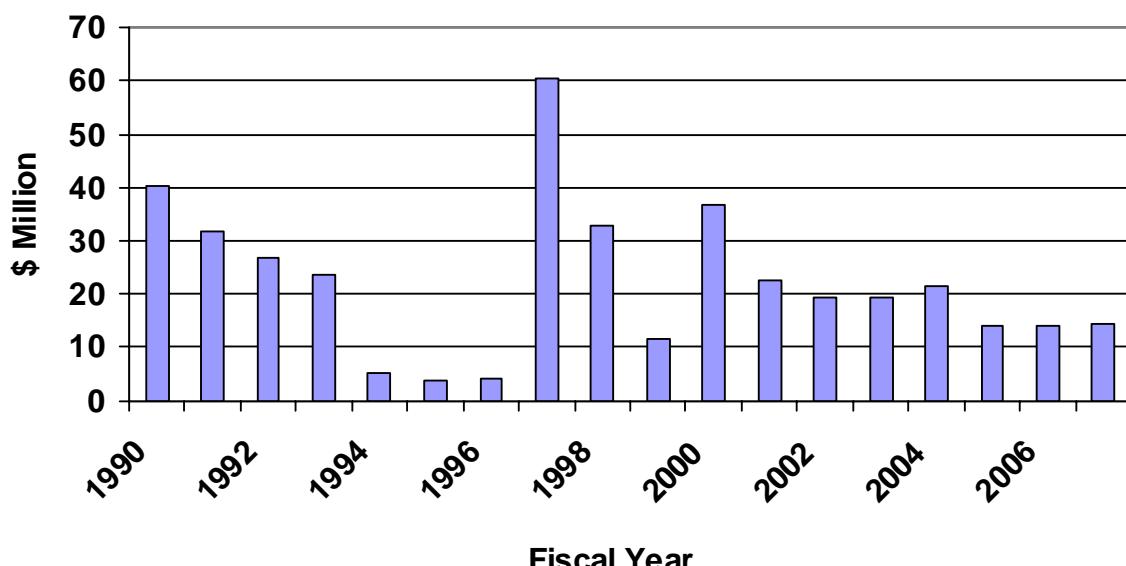
Trainee	Mentor	Institution	Grant title
Munoz, Ricardo	Jimenez-Camargo, Luis A.	University of California, San Francisco	Internet health research center: Smoking, Latinos and the Web
Munoz, Ricardo	Liang, Jennifer	University of California, San Francisco	Internet health research center: Smoking, Latinos and the Web
Lopez, Ivan	Shahram, Yalda	University of California, Los Angeles	Mild carbon monoxide exposure impairs the inner ear
Tsoh, Janice Y.	Wang, Vivian W.	University of California, San Francisco	A stage based schedule smoking intervention for Chinese
Choudhry, Shweta	De Giacomo, Anthony	University of California, San Francisco	Tobacco gene-environment interactions and minority asthmatics
Richardson, Russell	Nishiyama, Steven K.	University of California, San Diego	Oxidative stress and skeletal muscle dysfunction in COPD

HISTORY

Appropriations

The sole source of TRDRP funds is the revenue from the tobacco surtax that was established when California voters passed Proposition 99 in 1988. Proposition 99 specified that five percent of this tax revenue be deposited in the Research Account and that it be used exclusively for research on tobacco-related disease. Tobacco sales in California have steadily declined since the Proposition 99 tobacco excise surtax went into effect in 1989. Between 1990-91 and 2004-05, TRDRP resources declined from \$26.9 million to \$14.3 million annually. Appropriations from the Research Account to the University of California have shown large fluctuations – from \$40.3 million in 1990 to \$3.65 million in 1995 to \$60.4 million in 1997 (see Figure 2).

Figure 2: Appropriations to TRDRP from Proposition 99 Research Account, 1990-2008



Starting in 2000-2001, the amount appropriated from the Research Account to the California Department of Health Services was increased from approximately \$1.7 million to approximately \$5 million annually. During the first ten years of Prop. 99-funded programs, the annual appropriation to DHS remained at approximately 6 percent of available funds (i.e., revenue, interest, and Proposition 10 backfill), regardless of the amount appropriated to UC. For example, in 1999-2000 it was 7.5 percent. Starting in 2000-2001, however, the DHS appropriation was increased to more than \$5 million which is now 24 percent of the total available.

Grants Awarded

Since its inception in 1989 through 2008, TRDRP awarded \$387 million in 1,208 grants to approximately 800 scientists at 80 California institutions. The grants awarded constituted 24 percent of the applications received. The dollar amounts and number of grants awarded by subject area are displayed in Table 1.

Table 1. Award Totals by Subject Area, 1989-2008

Subject Area	Number of Awards	Amount (\$)
Cancer	231	\$62,928,303
Cardiovascular Disease	147	\$46,155,794
Epidemiology (through 2006)	149	\$58,341,303
General Biomedical Science	141	\$35,554,586
Nicotine Dependence	135	\$37,815,895
Public Health/Policy	121	\$33,252,196
Pulmonary Disease	146	\$40,984,404
Social/Participatory	138	\$57,685,350
Total	1,208	387,154,112

Evaluation of Research Grant Applications

Research grant proposals submitted in response to TRDRP's Call for Applications are first screened for relevance to the program's mission. Relevant proposals are assigned to a committee of peer reviewers who are experts in the scientific discipline and subject matter of the proposed research (these committees are known as "study sections"). Peer reviewers are drawn from outside California to minimize actual and apparent conflicts of interest with the applicants. Each study section evaluates applications for their scientific merit. Following state statutes, the evaluation procedure is modeled on the one used by the National Institutes of Health. The study sections' merit ratings are transmitted to TRDRP's Scientific Advisory Committee (see below). The committee uses the scientific merit ratings together with the degree to which a proposal is responsive to funding priorities to make funding recommendations. The awards recommended for funding by the Scientific Advisory Committee represent important and innovative research that promises to advance knowledge needed to improve tobacco control; tobacco use prevention and cessation; protection from secondhand smoke; and prevention, treatment, and diagnosis of tobacco-related disease.

SCIENTIFIC ADVISORY COMMITTEE

In accordance with enabling legislation, a Scientific Advisory Committee advises the University on the administration of TRDRP. Members, who represent major California organizations involved in health research, are appointed to three-year terms, are not compensated, and may not receive TRDRP funding while serving on the committee (see Table 3). The committee is charged with recommending the strategic objectives and priorities of TRDRP and with making final recommendations on grants to be funded based on the established priorities and the scientific merit of the proposals as determined by peer review.

Table 3. Scientific Advisory Committee Roster, 2008

CHAIR	REPRESENTING	TERM
Geraldine V. Padilla, Ph.D. Professor & Associate Dean for Research UCSF School of Nursing 2 Koret Way, Room N339 San Francisco, CA 94143-0604	<i>Professional medical or health organization</i>	2005-2008
MEMBERS		
Sara Courtneidge, Ph.D. Professor The Burnham Institute for Medical research 10901 North Torrey Pines Road La Jolla, CA 92037	<i>Biomedical Research</i>	2008-2011
Lawrence W. Green, Dr.P.H. Adjunct Professor Department of Epidemiology and Biostatistics University of California, San Francisco Box 0981, 185 Berry Street 6611 San Francisco, CA 94143-0981	<i>Social/Behavioral Science</i>	2006-2009
Carlene E. Henriques, CHES Program Coordinator Sacramento County DHHS Tobacco Education Project 9719 Lincoln Village Drive, Suite 300A Sacramento, CA 95827	<i>Community-based provider of health education and prevention services</i>	2005-2008
Fredric B. Kraemer, M.D. Professor of Medicine Division of Endocrinology Stanford University Medical Center Stanford, CA 94305-5103	<i>American Heart Association, Western States Affiliate</i>	2005-2008
Paul Murata, MD, MSPH Medical Institute of Little Company of Mary 20911 Earl Street, Suite 400 Torrance, CA 90503	<i>American Cancer Society, California Division</i>	2005-2008

Marilyn Newhoff, Ph.D. Dean, College of Health and Human Services San Diego State University 5500 Campanile Drive, M/C 4124, ED-154B San Diego, CA 92182-4124	<i>Tobacco-Related Disease Research Institute</i>	2007-2010
Gerd P. Pfeifer, Ph.D. Professor of Biology Beckman Research Institute, City of Hope 1500 E. Duarte Road Duarte, CA 91010	<i>Biomedical Research</i>	2004-2007
Kim D. Reynolds, Ph.D. Associate Professor Institute for Health Promotion & Disease Prevention Research Keck School of Medicine University of Southern California 1000 South Fremont Avenue, Unit 8 Alhambra, CA 91803	<i>Independent Research University</i>	2005-2008
Thomas Scott, Ph.D. Vice President for Research San Diego State University 5500 Campanile Drive San Diego, CA 92182	<i>Tobacco-Related Disease Research institution</i>	2004-2007
Randall S. Stafford, M.D., Ph.D. Associate Professor of Medicine Stanford Prevention Research Center Stanford University Medical School Hoover pavilion, Room N229 211 Quarry Road Stanford California 94305-5705	<i>Independent research university</i>	2005-2008

RESULTS OF COMPLETED RESEARCH GRANTS, 2008

This section summarizes research findings from grants that ended in 2008.

Nicotine Dependence

Neurobiological Substrates of Nicotine Addiction (12RT-0231)

Athina Markou, Ph.D.

The Scripps Research Institute

Because the risk for developing diseases associated with tobacco smoking is reduced when smoking is stopped, there is incentive to invest research resources in elucidating the neurobiological substrates of nicotine dependence and the factors that lead to relapse.

Elevations in brain reward thresholds for nicotine are seen during spontaneous nicotine withdrawal. These elevations can be precipitated in nicotine-treated rats with a variety of nAchR antagonists injected systemically or into specific brain sites. In Specific Aim 1, the investigators studied the effects of the nAchR antagonist dihydro- β -erythroidine (DH β E) injected into limbic brain sites, such as the ventral tegmental area, the nucleus accumbens, the central nucleus of the amygdala and the bed nucleus of the stria terminalis, on reward thresholds in nicotine- and saline-treated rats. Administration of the nAchR antagonist into the ventral tegmental area, but not into the shell of the nucleus accumbens, central nucleus of the amygdala, or the bed nucleus of the stria terminalis, was sufficient to induce the reward threshold elevations. Further, there was increased sensitivity of dopamine D1-like receptors in the ventral tegmental area, but not into the stria terminalis, in nicotine-dependent subjects to a D1-like receptor antagonist, but no differential effects to a dopamine D2-like receptor antagonist. Thus, there is decreased nAchR number/function with the development of nicotine dependence in the ventral tegmental area, but not in the nucleus accumbens, central nucleus of the amygdala, or the stria terminalis that may contribute to withdrawal symptomatology and relapse. Elevations in reward thresholds reflect “diminished interest or pleasure” in the rewarding electrical stimuli and are a measure of the depressive symptom of anhedonia that characterizes tobacco withdrawal.

Studies under Specific Aim 2 developed and used new animal models of nicotine dependence to explore whether the adaptations that occur in the function of the cholinergic system with the development of nicotine dependence in specific brain sites (explored in Specific Aim 1) are long-lasting and persistent. Findings indicated that nicotine differs from other psychomotor stimulant drugs in two respects. First, there does not appear to escalation in nicotine intake when subjects are allowed extended access to nicotine self-administration. Secondly, nicotine appears to have prolonged effects in facilitating brain reward function, an effect opposite to those seen during withdrawal from other psychomotor stimulants, such as cocaine and amphetamine. Finally, additional novel findings indicated that environmental stimuli can be associated with the negative affective state of nicotine withdrawal and precipitate a conditioned negative affective withdrawal-like state. Such prolonged facilitation of brain reward function induced by nicotine, as well as the conditioned negative affective states elicited by environmental stimuli may contribute to relapse.

MicroRNA and Dopamine-induced Dendritic Protein Synthesis (14DT-0040)

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Addiction has been called a disease of learning and memory. Synaptic plasticity (the strength of the connection between synapses as measured by the postsynaptic potential) is believed to play an important role in addiction since it is one of the neurochemical underpinnings of learning and memory. The addictive property of nicotine is believed to arise from its ability to induce dopamine release in regions of the brain that respond to rewards. This project investigated the importance of dendritic protein synthesis in response to dopamine, especially the synthesis of GluR1 protein, a subunit of AMPA-type glutamate receptor, which is important for synaptic plasticity.

This laboratory previously discovered that dopamine induces the synthesis of new proteins in neuronal dendrites. To disrupt the synthesis of proteins in dendrites, the investigators successfully delivered small-interfering RNAs (siRNAs) into neurons using penetratin, a small peptide that facilitates the entry of siRNAs into cells. siRNAs are mimics of endogenous microRNAs, which are potent inhibitors of protein synthesis. New protocols were developed to conveniently conjugate siRNAs to penetratin peptides. Once inside the cell, the disulfide linker allows the automatic release of the siRNA from the peptide. To monitor the entry of siRNA-penetratin into neurons, the researchers tagged the siRNA with a fluorescent marker. Under laser-scanning microscopy, they observed that most neurons had taken up the siRNA, and the fluorescent signal was clearly present in dendrites.

Next, they synthesized siRNAs against GluR1. In dissociated hippocampal neuron cultures, application of GluR1-siRNA-penetratin resulted in decreased levels of GluR1. When the siRNA conjugate was applied to hippocampal

slices from the rat brain, they observed changes in electrophysiological properties consistent with the reduction in GluR1 levels. Having developed the novel reagent that allows the rapid blocking of GluR1 synthesis in dendrites, they are now poised to examine its physiological role when neurons receive dopamine signaling.

Development of Nicotine Withdrawal Measures in Mice (14IT-0053A)

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The aversive aspects of nicotine withdrawal including affective and ‘physical’ or somatic components are powerful motivational factors contributing to the maintenance of tobacco smoking. This project developed measures of nicotine withdrawal in C57BL/6 and BALB/c mouse strains. Measures of the affective aspects of nicotine withdrawal included brain reward deficits (e.g., anhedonia), anxiety-like behavior and prepulse inhibition. Nicotine withdrawal was: a) spontaneously induced by termination of chronic nicotine delivery through 14- or 28-day subcutaneous osmotic minipumps; or b) precipitated by administration of the nicotinic acetylcholine receptor antagonists mecamylamine or dihydro- β -erythroidine to mice chronically treated with nicotine.

Reward deficits of nicotine withdrawal were characterized using the intracranial self-stimulation procedure in C57BL/6 mice. Results showed that only 28-day, but not 14-day, exposure to nicotine induced threshold elevations during days 1-3 of spontaneous withdrawal. Precipitated nicotine withdrawal also induced marked deficits in brain reward function in mice that were exposed to nicotine for shorter periods than those required to induce spontaneous nicotine withdrawal. Anxiety-like behavior was assessed in the light-dark box and the startle reactivity tests during nicotine withdrawal after 14-day exposure to a wide range of nicotine doses with small increments between doses. Additionally, the duration of nicotine withdrawal observation period was extended to days 1, 3 and 5 and there were no marked changes in anxiety-like behavior in both C57BL/6 and BALB/c mice. By contrast, extended 28-day exposure to the highest nicotine dose significantly increased anxiety-like behavior in the light-dark box in C57BL/6 mice. Finally, withdrawal from a wide range of nicotine doses and 14-day exposure did not alter significantly prepulse inhibition response in either C57BL/6 or BALB/c mice indicating that nicotine withdrawal had no effect on pre-attentional cognitive deficits at the nicotine doses tested.

The results for somatic signs of nicotine withdrawal indicated that there was no effect of spontaneous nicotine withdrawal after 14-day nicotine exposure in either C57BL/6 or BALB/c mice. When exposure to nicotine was extended to 28 days, there was a significant increase in the number of somatic signs in C57BL/6 mice indicating that longer nicotine exposure was required to induce spontaneous withdrawal. Finally, they investigated the role of 5-HT₇ receptors in prepulse inhibition (PPI) of the startle response using 5-HT₇ knockout mice. The results indicated that the 5-HT₇ receptor knockout mice may provide a useful tool to study the role of 5-HT₇ receptor function in the action of atypical antipsychotic drugs, schizophrenia and the substrates mediating the high smoking rates among schizophrenia patients.

The results demonstrated reward deficits (anhedonia) during both spontaneous and nicotinic receptor antagonist precipitated withdrawal in the mouse. They also identified experimental conditions, such as nicotine dose and exposure time, necessary to induce detectable affective and somatic aspects of spontaneous nicotine withdrawal in the mouse. In sum, the present studies developed reliable measures of nicotine withdrawal in mice that can be used to investigate the neurobiological and genetic factors contributing to the different aspects of nicotine withdrawal in future studies.

Effects of Bupropion in Animal Models of Nicotine Dependence (14FT-0056A)

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Although bupropion is one of the most effective anti-smoking medications, neither the behavioral nor neurochemical mechanisms by which works are well-understood. The current project explored the effects of

chronic bupropion administration on the components of nicotine dependence and provided data on which pharmacological effect of bupropion is important in improving smoking cessation rates.

Chronic bupropion use had no effect on the direct rewarding effects of nicotine, but partially decreased the indirect rewarding effects of nicotine. Environmental cues associated with nicotine delivery are hypothesized to play an important role in maintaining smoking behavior in humans. In the present studies, chronic bupropion enhanced the motivational properties of nicotine-associated environmental cues. Nicotine withdrawal is associated with decreased brain reward function ('anhedonia'). Chronic bupropion administration diminished anhedonia during nicotine withdrawal, and also decreased the somatic signs of nicotine withdrawal.

Although the tricyclic antidepressant desipramine shares one pharmacological effect with bupropion, unlike bupropion, chronic desipramine administration ameliorated the anhedonic and somatic components of nicotine withdrawal. A second pharmacological effect of bupropion was identified as important in ameliorating the anhedonic component of withdrawal. Finally, although rats allowed to self-administer nicotine for extended periods of time exhibited the somatic component of nicotine withdrawal, no evidence of anhedonia was obtained. It is likely that nicotine intake is insufficient to induce both components of nicotine withdrawal. In summary, bupropion most likely improves smoking cessation by ameliorating the aversive effects of nicotine withdrawal rather than by making smoking less rewarding. Further, bupropion probably makes nicotine withdrawal less aversive by affecting either one or both of two chemical messengers found in the brain.

Cell Adhesion at Nicotinic Synapses (14DT-0092)

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It is widely known that long-term tobacco use leads to a strong addiction due primarily to the chemical nicotine acting at nicotinic synapses. Degeneration or inappropriate formation of nicotinic synapse are believed to underlie many neurological disorders including schizophrenia, Parkinson's, and Alzheimer's disease. Despite the obvious importance of nicotinic synapses very little is known about how these connections are formed and modulated.

This project studied L1, an adhesive protein that bridges the gap between neurons at nicotinic synapses. Preliminary work showed that L1 molecules located on both sides of the synapse work together to induce and stabilize components needed for acetylcholine release at the right places. It was shown that adding L1 to several cell types enables them to acquire more acetylcholine release machinery from the upstream cell. Experiments on neuron-muscle synapses showed that organization of release machinery also requires L1 in the upstream cell. L1 in both the upstream and downstream cell cooperate to achieve this synaptic organization. These findings were confirmed *in vivo* at the neuron-muscle synapse and the neuron-neuron nicotinic synapse.

In conclusion, the adhesive protein L1 was found on the upstream and downstream side of nicotinic synapses and acts from both locations to organize acetylcholine release machinery in the upstream cell. This novel function for L1 occurs at the neuron-muscle synapse and the neuron-neuron nicotinic synapse in the culture dish and in the animal.

Effects of Nicotine on Hematopoietic Stem Cell Migration (14FT-0126)

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Stem cells, both embryonic and somatic, possess a great potential for tissue regeneration and the restoration of organ function in human diseases. Transplantation of human hematopoietic stem cells (HSC) is currently a required procedure for patients who have undergone high-dose chemotherapy and irradiation. The regenerative function of transplanted stem cells depends on their ability to home to the sites of injury. Although investigators previously demonstrated that nicotine negatively affects migration of HSC, the mechanisms of this phenomenon have not been investigated.

Gene expression profiling demonstrated that components of the cholinergic system, including choline acetyltransferase, acetylcholinesterase and nicotinic acetylcholine receptors (nAChRs), are expressed in embryonic stem cells (ESC) and differentiating embryoid bodies. These receptors are physiologically active as measured by Ca²⁺ influx. Triggering of nAChRs expressed in embryoid bodies by nicotine resulted in activation of signaling cascade and shifts of spontaneous differentiation toward hemangioblast. Similarly, in vivo exposure of the developing embryo to nicotine resulted in higher numbers of hematopoietic progenitors in fetal liver. However postpartum, the number of hematopoietic stem/progenitor cells (HSPC) was decreased, suggesting an impaired colonization of the fetal bone marrow with HSPCs. This correlated with increased number of circulating HSPC and decreased expression of CXCR4 that mediates migration of circulating cells into the bone marrow regulatory niche. In addition, protein microarrays demonstrated that nicotine changed the profile of cytokines produced in the bone marrow regulatory niche. This correlated with the decreased repopulating ability of HSPC in vivo and diminished hematopoietic activity in bone marrow cultures treated with nicotine.

The data provided evidence that the nicotine-induced imbalance of the cholinergic system during gestation interferes with normal development and provides the basis for negative health outcomes postpartum. These results potentially provide a basis for the development of new strategies for stem cell transplantation in smoking patients.

Nicotine Receptors: Role in Nicotine Addiction (15FT-0030)

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This project studied nicotinic ACh receptors in the brain, which are the receptors for nicotine found in cigarette smoke, specifically, the alpha6 receptor subunit. The overall goal was to construct a genetically engineered mouse that would allow isolation and amplification of the alpha6-specific responses to develop a compound for smoking cessation therapy and to better understand the basic neurobiology of nicotine dependence. The project succeeded in generating a knock-in mouse model that expresses a hypersensitive alpha 6 allele for studying nicotine addiction. However, it did not succeed in expressing alpha6 receptors *in vitro* to design a suitable hypersensitive mutation, as planned.

Genes Affecting Nicotine Response in C. elegans (15RT-0216)

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The addictive properties of nicotine are a major factor that prevents smokers from quitting. Although the consequences of nicotine addiction are well-known, much remains to be learned about the genes and molecules that affect the brain's responses to nicotine.

This project used a simple animal model, the roundworm *Caenorhabditis elegans*, to identify such genes and determine how they affect the nervous system's response to nicotine. The investigators developed behavioral tests for nicotine response in *C. elegans* and identified several lines of mutant nematodes with increased or decreased nicotine sensitivity. One of these lines carried a mutation in the gene *npr-2*, which encodes a molecule related to human hormone receptors. They showed that *npr-2* affects the sensitivity of two behaviors to nicotine, locomotion and egg-laying. In particular, mutants that lack *npr-2* function are almost completely resistant to the effects of nicotine on egg-laying.

Tobacco-Use Prevention and Cessation

Smoking Prevention for Asian American College Students (12RT-0004)

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The present study continued the evaluation of college students begun in a previous TRDRP-funded project. It continued evaluation of these students during their third and fourth years of college to examine changes in their smoking behavior over time and further study factors influencing tobacco use during the college years.

Overall the project was successful in identifying risk and protective factors related to smoking initiation and examining ethnic and gender differences in smoking patterns. However, because there were relatively few smokers in the sample, only partial success was achieved in identifying risk and protective factors related to progression of smoking through the college years as well as characterizing patterns of smoking over this period.

Major accomplishments were: 1) Evaluated 85% of the original participants over the duration of the study. 2) Identified gender and ethnic differences in smoking rates. Specifically, Koreans smoked more than Chinese American students, and males more than females. 3) A novel and unanticipated finding was that significant initiation to smoking occurred during the college years. 25% of those who had not smoked as college freshmen eventually smoked a cigarette during their 4 years in college. This suggests that programs for primary prevention of smoking may be useful on college campuses. 4) Contrary to predictions and to previous research, students' level of acculturation did not predict any aspect of smoking. This finding contrasts with previous studies, and suggests that acculturation may have less effect on smoking in college students. 5) Despite the observed differences in smoking rates between groups, there was no evidence for ethnic differences in the factors that influence smoking. Notably, smoking-related risk and protective factors identified for Asian American students are similar to those for youth of other ethnicities. This finding suggests that ethnicity specific prevention programs may not be needed for Chinese or Korean-American students. 6) Alcohol use during freshman year was the most consistent predictor of future smoking initiation and progression. This finding suggests that efforts to prevent alcohol use on college campuses may also help prevent smoking.

Bupropion for Hospital-Based Smoking Cessation (12RT-0148)

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Millions of adult smokers are hospitalized yearly often for smoking-related illnesses. These hospital admissions provide a window of opportunity to help smokers quit. The Agency for Health Care Policy and Research guidelines currently recommend that all hospitalized smokers be offered smoking cessation treatment during hospital admissions. To date, however, relatively few controlled clinical trials examined whether hospital-based smoking cessation interventions are effective, and only one other study among smokers admitted for possible heart attacks has examined whether bupropion, a unique antidepressant medication as an aid in smoking cessation, is useful when begun in a hospital setting. The objective of this project was to determine whether bupropion when combined with standard smoking cessation counseling during and immediately after hospital admission increases smoking cessation rates.

This clinical trial recruited 85 participants who were assigned randomly to receive bupropion plus standard cognitive-behavioral therapy or placebo plus standard cognitive behavioral therapy. Participants received study medication for a total of 7 weeks and were followed for 6 months to assess whether they had quit smoking and remained abstinent. Although data analysis is not completed, the addition of bupropion did not increase the long-term probability of quitting, similar to the results of a recently published study of smokers hospitalized for chest pain.

Forty-two subjects were randomized to the experimental group and 43 were randomized to the placebo group; two experimental subjects withdrew and five placebo subjects withdrew. One participant randomized to receive bupropion died during the follow-up period versus two participants randomized to receive placebo. One participant in each group was lost to follow-up. By self-report, 39% of the bupropion group reported not smoking at the end of treatment (8 weeks) versus 36% of the placebo group ($P=0.82$). At the end of drug treatment (8 weeks), quit rates validated by spousal proxy or saliva cotinine were 28% in the bupropion group versus 31% in the placebo group ($P=0.80$). Of the 77 participants who were followed through 6 months, the self-reported quit rates were 30% for the bupropion group and 46% for the comparison group ($P=0.23$). Six month validated quit rates were 15% for the bupropion group and 32% for the comparison group, respectively ($P=0.11$). This study, along with the other bupropion study for smokers hospitalized with chest pain, suggests that bupropion has no special advantage in this setting over other drugs or interventions used to aid in smoking cessation.

The study did encounter significant barriers. The main obstacle was the inability to achieve recruitment goals despite screening almost 15,000 patients at the San Francisco VA Medical Center. The investigators concluded that the feasibility of any hospital-based smoking cessation study is questionable because the pool of potential participants is limited by the current very short duration of hospital stays.

Protecting the “Hood” against Tobacco: Cessation Project (12AT-1701)

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The goals of this project were to sustain and expand a productive community-academic partnership infrastructure, continue the community work initiated in the pilot, and contribute to smoking cessation among adult African Americans in the Bayview-Hunter's Point (B-HP) community of San Francisco. The project, which evolved through a community process in which input was sought from individuals and groups as to what was needed to more effectively address tobacco in the community, was designed as a community participatory project that included a randomized clinical trial of an innovative, tailored smoking cessation based on industry denormalization and social justice messages. This study also included a qualitative interview study of participants and a process evaluation of the project.

The collaborating investigators succeeded in developing an innovative intervention to be tested, recruiting for and mounting a clinical trial including developing and running both the intervention and control group programs. They also conducted qualitative interviews with participants and provided community capacity building in the form of training and engaging with numerous individuals and organizations and becoming a respected presence within the community.

The primary barrier was the collapse of the control program, which required significant additional time, personnel and resources. A second obstacle was challenges in recruiting sufficient participants given the multitude of social and personal issues faced by people in this disadvantaged community. Despite aggressive recruitment efforts, the investigators were unable to recruit enough people to achieve the necessary sample size. Finally, the tensions between the flexibility required to honor the tenets of community participatory research, the recruitment difficulties and the exactness needed to conduct a valid randomized clinical trial created challenges.

Although the study did not achieve sufficient statistical power to make causal inferences, the data suggest that the intervention program might have an incremental benefit over a standard approach in helping African American smokers quit. Qualitative interviews identified insights into why African Americans appear less likely than other groups to use nicotine replacement therapy. The investigators contributed to the literature on ethical issues in conducting community participatory research with a highly-read paper describing the inability to gain IRB approval for one pilot project. They also completed work from the pilot on tobacco industry targeting of inner city

communities. In addition, community research partners noted enhanced skills, willingness to advocate for tobacco control measures and to sustain smoking cessation, and academic partners noted enhanced skills in working with community members and improved understanding of the need for flexibility in research design in community participatory approaches.

School-Based Tobacco Control Programming for Deaf/HH Youth (12HT-3201)

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Deaf and hard-of-hearing (Deaf/HH) youth are at risk for tobacco use and many of those who smoke want to quit. However, anti-tobacco programming, including school-based curriculum, although badly needed, have never been developed for this population or rigorously evaluated. This study extended a collaboration, established through a TRDRP pilot School-Academic Research Award (SARA), of educators with state and nationally recognized expertise in Deaf youth culture, behavior, and education [California School for the Deaf, Fremont (CSDF)] and researchers with specific experience relevant to tobacco use in this population (UCLA Division of Cancer Prevention and Control Research, School of Public Health and Jonsson Comprehensive Cancer Center). Building on the cumulative findings from a nine-year program of TRDRP-funded research, the only one of its kind in the nation, the investigators developed, implemented, and are rigorously evaluating a comprehensive school-based anti-tobacco program tailored to the unique social, cultural, and communication needs of Deaf/HH youth.

They designed, developed and produced a tobacco-prevention program for Deaf/HH students, grades 7-12, "Hands Off Tobacco! An Anti-Tobacco Program for Deaf Youth." The curriculum was implemented during the 2004-2005, 2005-2006, and 2006-2007 school years at the two intervention sites. A video and teacher's guide were completed. Appropriate tobacco prevention brochures were distributed at the two control sites. The student survey was drafted; pilot tested for content and administration strategies; reviewed by two faculty focus groups; finalized; and administered at baseline and follow up at all intervention and control sites. The faculty/staff/administrator survey was finalized and administered at all sites. The curriculum was extended through the design and development of tobacco-prevention programming for Deaf/HH students in grades 5 and 6. The curriculum (hard copy and DVD), with a revised introduction and additional (grades 5 and 6) materials, is available for dissemination, as is the companion DVD and teacher's guide.

Nonsmokers Helping Smokers and the Role of Culture (13RT-0023)

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Smokers who quit often report getting informal help from nonsmokers, but few studies have examined the phenomenon of nonsmokers seeking formal help on behalf of smokers. This study recruited nonsmokers who called the California Smokers Helpline asking for materials and advice to help a smoker quit. The specific aims were to measure the characteristics of the nonsmokers who call, to assess natural support behaviors, and to develop and pilot test a telephone-based training protocol for nonsmokers to better help smokers quit smoking.

From July 2004 to June 2008, we assessed the characteristics of nonsmokers who called the English, Spanish, Cantonese, Mandarin, Korean, and Vietnamese language lines of the Helpline. We collected data on 7,020 nonsmokers: 6.4% of total calls to the Helpline.

To examine specific helping behaviors we developed and pilot tested a questionnaire for general and specific support. These questions are being used to assess baseline helping behavior and the effect of the training intervention on helping. We also developed a way to examine clients' "mental model" of quitting smoking-- basically their beliefs about what it takes to quit. This tool was incorporated into the training protocol with the belief that shifting the nonsmokers' mental models might lead to a more sustainable behavior change in their

support of their smokers' efforts to quit. The final aspect of this study was to develop a training protocol for working with nonsmokers. We developed and pilot tested the protocol and created a 9-page self-help booklet for nonsmokers who want to help smokers quit. The planned sample is 200 subjects and the present report summarizes data from the 136 subjects currently in the study.

This research project has achieved a number of objectives. First, it provides a description of the phenomenon of nonsmokers seeking help for smokers. These nonsmokers ("proxies") call the statewide telephone quitline to seek help for smokers they care about. The study found a large difference across ethnicities in the proportion of Helpline callers who are proxies, from about 2% among American Indians to about 37% among Chinese-speaking Chinese. However, despite the variations across ethnic and language groups in the proportions of proxies, the characteristics of those who call are quite consistent — most are nonsmoking women who live with a smoker. Moreover, most of these callers have either explicit or implicit understandings with the smokers that it is all right to call a cessation program on their behalf. Identifying these consistent characteristics has made development of an intervention for this group easier.

Second, this project has led to the development of a proxy-specific booklet and a telephone based counseling protocol that focuses on empowering proxies to press for change at home. Nonsmoking women, especially those who take the initiative to call for a smoker they live with, represent an untapped resource for helping smokers to quit, especially for ethnic groups such as Asian Americans and Latinos, known to have lower proportions of smokers who seek help in quitting.

Third, this project has tested the feasibility of intervening with nonsmoking proxies. Not only do proxies call for help, they agree to work with a trainer to find ways to change their own helping behavior. The training includes identifying a target behavior (e.g., having a discussion with the smoker about quitting) and developing a plan for carrying it out. Proxies engage in the process through a comprehensive planning call and through follow up calls with the trainer.

Fourth, this project found that nonsmokers' self report of how much support they give to their smoking husband or significant other is correlated with the likelihood of the smoker quitting later. It also found that a single question on the nonsmoker's supportiveness was an efficient measure of social support.

The nonsmoking proxies are very involved in the quitting process. Smokers report high rates of support from the proxies, even higher than the proxies themselves report providing. At the same time, smokers report that proxies generally give them a "hard time" about quitting. Proxies use the materials sent by the Helpline and provide them to the smoker. They talk to the smoker about quitting and they encourage calling the Helpline. According to the proxies, about 10% of the smokers quit smoking in the two months after proxies call the Helpline, a rate which leaves plenty of room for improvement. However, there appears to be no difference in any of the outcomes between the group involved in the training and the group that simply received materials in the mail, so the intervention will need further refinement.

Effects of Pro- and Anti-Smoking Cues in Stores on Craving (13RT-0123)

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Two experiments examined the impact of retail cigarette marketing and graphic warning labels on urge to smoke. Study 1 tested whether minimally deprived smokers pay more attention to cigarette ads and pack displays than to ads and displays for non-tobacco products, and whether retail cigarette ads stimulate craving. Smokers and nonsmokers (ages 18-24) (n=63) were paid \$50 to abstain from smoking overnight and to complete a lab experiment. Smokers were categorized as light (2-10 cigarettes per day) (n=19) or moderate (11 or more per day) (n=25) because previous research suggests that light smokers may be more sensitive to environmental cues to smoke. After providing a breath sample to confirm overnight abstinence, all participants were exposed to the same set of

visual cues, 20 retail signs or displays for cigarettes and 20 signs or displays for non-tobacco products, such as toothpaste, soap, and batteries. Random assignment determined whether participants saw the block of tobacco or non-tobacco cues first. SuperLab Pro measured secondary task reaction time to a visual probe. Immediately following each block of cues, participants completed two measures of self-reported craving, the QSU-brief and a 100-mm visual analog scale. Regardless of presentation order, smokers took longer to respond to the visual probes during tobacco than non-tobacco cues, suggesting an attentional bias that was not observed for nonsmokers. However, the between-group differences were not significant. As predicted, smokers reported higher levels of craving and greater urge to smoke after exposure to tobacco cues than to non-tobacco cues regardless of presentation order ($p=.03$), but light and moderate daily smokers did not differ.

Study 2 examined whether exposure to cigarette packs with graphic warning labels would reduce craving. Ten graphic warning labels were selected from Australia, Canada, and the EU based on young adult smokers' ratings of negative emotion (fear, disgust), potency (accuracy of message), and perceived effectiveness (ability to reduce urge to smoke, encourage cutting down, quitting). Using Adobe Photoshop, two cigarette packs for five top-selling brands were created with a different warning label for each. As in Study 1, young adult smokers ($n=69$) were paid \$50 to abstain from smoking overnight. Participants were randomly assigned to see either 10 pictures of cigarette packs with graphic warning labels (treatment group) or to see 10 pictures of nontobacco items sold in convenience stores (control group). Paper-and-pencil measures of craving and quit intentions were completed before and after exposure. Participants were escorted to a break area approximately 70 feet from the building, where a confederate measured latency to smoke (i.e. how long until a cigarette was lit). As predicted, the group exposed to graphic warning labels reported a greater reduction in craving ($p=.03$), waited longer to smoke ($p=.11$), and expressed greater intentions to quit ($p=.01$) than the control group.

Results from these experiments suggest that stores saturated with cigarette ads and promotions may increase smokers' daily consumption and reduce their resolve to quit. These effects may be reduced by packaging cigarettes with graphic warning labels that are visible at the point of sale. Thus, tobacco control efforts would benefit from policies to decrease the quantity of pro-smoking cues and increase the visibility of anti-smoking cues in stores.

Tobacco Control Policy

Influences that Promote Ethnic Disparities in Smoking (12KT-0158)

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The goals of this project were to estimate how the California Tobacco Control Program (CTCP) and the 1998 Master Settlement Agreement between the State Attorneys General and the tobacco industry affected smoking, especially initiation and cessation, and smoking restrictions among ethnic minority groups.

Findings have suggested significant ethnic disparities in the age of smoking initiation, with the majority of Asian Americans/Pacific Islanders (AA/PI) and African Americans (AA) initiating as young adults. Reductions in AA adolescent smoking in the 1980s were offset by increased initiation among young adults during this time period and suggest that the window for uptake of regular smoking shifted to older ages for AAs more so than non-Hispanic whites (WH). Continued overall higher smoking prevalence among AA adults in California is due to higher smoking prevalence and less successful quitting in older age groups. Since 1996, there has been a significant decline in current non-daily smoking prevalence among Hispanic/Latina (HL) women who speak mostly English at home, but no significant reduction in current daily smoking regardless of language spoken at home. HL smokers use nicotine replacement therapy (NRT) less than WHs and are less likely to receive physician advice to quit compared to WHs. Although exposure to secondhand smoke in indoor workplaces has decreased since the inception of the CTCP, the percentage of HLs reporting exposure has been greater than other ethnic groups over time. Wide disparities in smoking behaviors exist by income and education among WHs but no such disparity was present

among HLs. Finally, our most recent findings suggest that AA and AA/PI adolescents are less receptive to tobacco marketing practices relative to WHs, even among susceptible never smokers.

The above findings suggest that smoking prevention strategies should begin at a young age and continue throughout young adulthood, especially for ethnic minority populations as evidenced by delayed initiation among AAs and AA/PIs. Smoking prevention and cessation efforts among HLs remain a priority because of greater exposure to secondhand smoke in indoor workplaces, and less likelihood of receiving physician advice to quit and use NRT. Increased efforts to reduce smoking among low-income WHs warranted. Efforts in these regards would contribute to reducing the burden of tobacco related disease among racial/ethnic minorities in California.

Exploration of Tobacco Use among Asian American and Pacific Islander Youth (13AT-3000 & 13AT-3001

Rod Lew MPH

Association of Asian Pacific Community Health Organizations (AAPCHO)

Sora Park Tanjasiri DrPH, MPH

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Tobacco use among California's multiethnic youth is growing, yet there is little research indicating what environmental factors impact use and prevention. Asian Americans and Pacific Islanders (AAPIs) are a very diverse population, consisting of over 100 ethnic groups and in addition, make up 13% of the residents. However, there is still a paucity of information and understanding concerning the pro- and anti-tobacco factors affecting these tremendously varied and disparate communities. The specific aims of this study were: 1) To identify, inventory and explore the pro-tobacco and anti-tobacco environmental characteristics in two low-income AAPI populations (Cambodian and Chamorro) compared with non-AAPI communities using: 1a) Geocoding/asset mapping and 1b) Photovoice; and 2) To study the relationship between environmental characteristics (both pro- and anti-tobacco) and tobacco initiation and use among youth in one AAPI population (Cambodian).

In Richmond, the youth used GPS devices to log in waypoints of positive tobacco control and negative tobacco influences in their communities. They identified 27 positive influences compared to 99 negative influences that were classified into the following categories: food, shops, health, ads, general community, stores, and schools. In addition, there were 27 locations that the youth perceived as both positive and negative influences. These waypoints were then plotted on a map of each community with the AAPI population densities color-coded. The youth also took photographs several of these waypoints and a selection of these photographs was analyzed using the SHOWeD method of Photovoice and captions were created. Significant progress towards Specific Aim #2 were also made, with all 298 surveys completed and all data entered and cleaned. Additionally, findings from the interviews, surveys, and GIS mapping are helpful to assist in advocating for and developing effective tobacco control interventions for the AAPI community.

Tobacco Radioactivity & Public Health Policy (14IT-0001)

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The investigators retrieved and analyzed declassified "secret" tobacco industry and published academic findings to address the issue of cigarette smoke polonium 210 (^{210}Po) radioactivity and lung cancer risk in active smokers. The findings uniformly showed that the inhaled cigarette smoke from all domestic and foreign brands contains considerably higher of alpha particle emitting ^{210}Po and its parent lead 210 (^{210}Pb) than smoke-free air. The average cigarette content of alpha particle was 16 mBq/cigarette. Post-mortem human lungs had higher levels of radioactivity compared to non-smokers. Both industry executives and independent investigators recognized since 1964 the possibility of a causal link between ^{210}Po and increased lung cancer risk based on colocalization of lung tissue malignancies and sites with relatively high alpha particle activity. A case for alpha particle carcinogenicity is made by epidemiological evidence in humans inhaling, ingesting or receiving alpha particle injections from sources other than cigarette smoke. Calculations using reported cigarette ^{210}Po content, fraction of cigarette smoke inhaled,

lung distribution, lung retention time and half-life show that smoking of two-packs of cigarettes a day is 10 times higher than the approved EPA level of 4 pCi/L resulting in 120-380 lung cancer deaths in 1,000 smokers over 25 years.

The tobacco industry documents show that the industry was aware of the presence and the potential risk of polonium in cigarette smoke for over 40 years but actively failed to reveal its presence in cigarette smoke thus keeping the smokers unaware of the presence of tobacco-specific alpha particles in cigarette smoke. Industry documents show that the motivation of not taking action to remove 210Po from cigarette was directly linked to the fear that the procedures designed to remove 210Po would reduce the rate of nicotine delivery to the brain of smokers and thus eliminate the much sought after feeling of rapid “nicotine kick.” Legislative action is warranted to warn smokers of cigarette smoke radioactivity and the potential of lung cancer death that claims the lives of 160,000 American smokers every year.

Marketing Low-Tar Cigarettes and New Harm-Reduced Products (14FT-0013)

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Between the 1950s and the 1970s, the tobacco industry realized that the public was becoming concerned about health risks of smoking. Manufacturers introduced filtered cigarettes and low-tar cigarettes and used advertising to convince the market not only that smoking high-filtration and low-tar cigarettes was preferable to quitting but also that socially desirable people smoked low-tar products. The tobacco industry is currently using the same advertising strategy to promote ostensibly “harm-reduced” tobacco products. A number of “potential reduced exposure products” (PREPs) have been developed and advertised as safer alternatives to conventional cigarettes for smokers who are concerned about smoking-related diseases. These products are also marketed on a social acceptability platform, wherein appeals are made to smokers who do not like the cosmetic drawbacks of smoking (stale smoke odor, stained teeth or household fabrics) or the negative social aspects (pressure from nonsmokers exposed to secondhand smoke).

Iterative analysis of tobacco industry documents and print advertising collections were used to analyze advertising messages in low-tar advertising campaigns. Important psychosocial needs unrelated to smoking and salient to low-tar smokers were identified, and the tobacco industry’s identification and targeting of these needs with carefully orchestrated advertising campaigns to keep health-concerned smokers from quitting were traced. The study compared and contrasted low-tar cigarette advertising and advertising for PREPs marketed to similar segments of the market. Although many of the concerns are similar between the first low-tar consumers and the current potential consumers of PREPs, the “mainstreaming” of low-tar (roughly 90% of American smokers use some form of reduced-tar product) has been accompanied by a mainstreaming of low-tar advertising as well. On the other hand, PREPs marketing retains at least an implicit focus on health; however, tobacco companies have availed themselves of a wide array of newer and stealthier marketing techniques.

Major accomplishments:

- 1) Identification of the salient psychosocial needs of low-tar smokers according to the tobacco industry’s own market research records.
- 2) Description of tobacco industry strategies for designing advertising images and marketing messages that incorporate salient psychosocial needs into campaigns targeting low-tar smokers to discourage them from quitting.
- 3) Evaluation of the focus on non smoking-related psychosocial needs in low-tar marketing and the importance to the industry of shifting attention away from health and onto more intangible concerns.
- 4) Identification of the “mainstreaming” of low-tar products and the consequent abandonment of health messages in favor of promotion of brand personalities and brand images once restricted to the “full-flavor” or “regular” brands.

- 5) Assessment of the manner in which PREPs have taken the place of traditional low-tar products as the healthy choice for concerned smokers.

Effects of Social Capital and Smoking: Elasticity and Pathways (14IT-0175)

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This project examined whether higher levels of community-level social capital (CSC) increase levels of smoking cessation in California using the California Tobacco Survey for 1999 and 2002. CSC can be theoretically defined as the density of social connections in a given geographical location. Higher levels of CSC are theorized to affect smoking cessation behavior by making it more likely for the following things to occur: the spread of information on how to stop smoking, social pressure to stop smoking, psychological support to help smokers quit, and the availability of medical care. In addition to determining the overall effect of CSC on smoking cessation, we also examined whether CSC is related to the spread of information on how to stop smoking and social pressure to stop smoking.

Our aims included (1) an analysis of the how CSC influences the demand for cigarettes in comparison with tax policies and media campaigns, (2) whether CSC affects cigarette consumption through its impact on general attitudes towards smoking, and (3) whether the above relationships vary by race/ethnicity. We were not able to directly examine media campaigns due to statistical issues in the data that could not be overcome. We examined the effect of tax policies by examining whether prices (which include taxes) affect smoking cessation independently from CSC. We also examined the influence of CCS on general attitudes towards smoking. Finally we were not able to adequately examine whether differences in the effect of CSC on smoking cessation differ by race/ethnicity due to statistical issues that could not be overcome.

The major accomplishments of this research project are as follows. First, we were able for the first time to make a causal connection between CSC and smoking cessation. Second, we determined for the first time that neither the spread of information on how to quit smoking, nor negative social pressure is the reason that CSC works in California. This suggests that CSC may possibly work in this case through positive psychological support and/or access to medical care. Third, we were able to show for the first time that CSC has much larger effects on smoking cessation in California than price differences (which are equivalent to tax changes).

One barrier to overcome in our study was the issue of making a causal claim. In order to make the claim that higher CSC causes higher levels of smoking cessation; we are required to find some mechanism, to sort people into groups of low CSC and high CSC, which was not related to a person's smoking behavior. This allows us to, in effect, have treatment and control groups sorted in a way similar to randomization without actually performing randomization. We used weather conditions in different geographic areas as our sorting mechanism, as smoking behavior and the decision of what kind of weather to live in are not related. Yet, weather and CSC are related, as worse weather results in less frequent in-person social contacts. We were unable to find such an instrument to sort people by the extent to which they were exposed to media campaigns.

A second barrier was having an adequate sample size of racial/ethnic groups in order to do a subsample analysis of whether the relationship between CSC and smoking cessation differs by race/ethnicity. The sample sizes for African-American and Hispanics were simply too small to perform this analysis properly.

African American Church Role in Tobacco Norm Change (14IT-0188)

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Health Education Council

Using the African American church as a venue for tobacco control/prevention initiatives provides a community competent opportunity to decrease tobacco-related disease disparities within this population. This project examined

the influence of church-based tobacco policy on tobacco norms among congregation members and the greater community in Sacramento.

A total of 45 adult church leader representatives completed a phone survey that queried their knowledge of the church, congregation, pastor and church policies. In the second phase, 963 adult congregants of those churches were solicited and surveyed in the church setting on their tobacco use and perspectives about the church anti-tobacco policy. Each survey instrument was designed to address the following project aims: 1) To determine the existence and nature of any written tobacco-free policy statements for church members, on church facilities and church sponsored events; 2) To determine the number of congregation members that are smokers and 3) To determine the number of congregation members who are motivated to quit.

Findings reveal that most churches have a health ministry addressing chronic disease but not tobacco control. While most pastors preach about smoking they do not have written anti-tobacco policy. Most congregants responded that they were non-smokers, but when the question was asked about the type of cigarettes that they smoke, most gave a brand preference. This suggests that 26% of congregants are actual smokers and that contradictory responses might reflect the tendency of African Americans to provide socially-desirable responses by denying tobacco and drug use, perhaps particularly in the context of a survey distributed in churches. Most congregants did not know if their pastor preached about tobacco use which suggest the need for more prominent tobacco prevention programming. Current cigarette smoking among congregants was not related to pastor preaching. However, most smokers indicated an intention to quit smoking within the next 30 days.

Implications of this study include the readiness of the Black church to integrate tobacco prevention programming as well as to establish institutional tobacco use policy given the resources. Congregants are smokers, but are not currently aware of their pastor's commitment to tobacco prevention through integration of tobacco messages in sermons and cessation through the health ministry. An expanded study measuring the impact on smoking norm change after a culturally tailored, faith-based tobacco prevention intervention would provide much needed data on the efficacy of tobacco prevention in the African American church.

Cancer

Office-Based OCT Laryngoscopy of Vocal Cord Cancer (12RT-0113)

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University of California Irvine

Head and neck cancer is inexorably linked to the use of and exposure to tobacco and tobacco byproducts. Laryngeal (voicebox) cancer is the most common cancer in the head and neck. Although early diagnosis of this disease can lead to higher cure rates, most laryngeal cancers are unfortunately diagnosed and detected at an advanced stage. This study focused on developing a device to produce extremely high quality images of the human larynx in an office-based setting using light. The technology is called optical coherence tomography (OCT) and is a cutting edge medical imaging method.

Early laryngeal cancer is very difficult to diagnose because the symptoms of hoarseness, throat pain, and change in the quality of voice are also seen in many benign disorders. At present, the only way early laryngeal cancers can be diagnosed is by a biopsy, which must be performed with the patient asleep in the operating room. Vocal cord biopsies can cause irreparable damage to the quality of the voice, and hence physicians are quite reluctant to perform them.

To date, over 200 patients with cancers or other disorders of their larynx and related regions have been examined using OCT. A considerable database and catalog of OCT images of both normal tissues and cancers have been developed. Images can be obtained *in vivo* without the need for sedation and anesthesia.

Peripheral Mechanisms of Oral Cancer Pain in a Mouse Model (12KT-0166)

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Although pain is the primary determinant for a negative quality of life for oral cancer patients, traditional medications including narcotics and non-steroidal anti-inflammatory drugs are not effective and the underlying cause of oral cancer pain is not known. The hypothesis of this project is that pain-producing mediators are expressed by the oral cancer and sensitize nerve fibers in the tumor microenvironment. The primary aim was to identify mediator(s) that could be contributing to cancer pain. A mouse model for oral cancer and a behavioral assay to assess pain secondary to mechanical stimulation were developed. A nociceptive mediator responsible for oral cancer pain was identified.

The project demonstrated that endothelin, a vasoactive peptide, is produced in extremely high levels in the squamous cell carcinoma microenvironment and contributes to pain behavior in the cancer pain mouse model. The research also confirmed that the peripheral concentration of endothelin is a primary determinant of the magnitude of cancer pain and demonstrated a complete reversal of the pain with a peripherally administered endothelin antagonist. They demonstrated increased expression of endothelin in both human oral cancers as well as the mouse cancer model. Finally, they demonstrated that the level of pain produced by the cancer depends on the peripheral concentration of endothelin. These findings represent a significant step forward in understanding the underlying causes of oral cancer pain and will clearly impact therapeutic approaches to controlling it.

Total Synthesis of the Clinical Anticancer Drug Etoposide (13DT-0012)

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California Institute of Technology

Etoposide is a potent clinical anticancer drug that provides the first line of defense for lung cancer. Unfortunately, previous attempts at making Etoposide have generally proceeded through long, inefficient processes, so lung cancer patients must currently rely upon a diminishing supply of plant extracts to provide them with this important remedy. These restrictions have also prevented scientists from making many compounds related to Etoposide, which could have promising anticancer properties. This research therefore investigated a novel and efficient synthesis of this drug, which could lead to the discovery of new, more effective, lung cancer treatments and would represent contributions to both organic chemistry as well as the treatment of tobacco-related diseases.

The studies resulted in two distinct approaches toward the total synthesis of Etoposide. The investigators performed an extensive investigation of the oxidative kinetic resolution reaction, which serves a crucial role in the completion of this project. The results were extremely promising and led to the discovery of a number of substrates that perform well in this transformation. Although the project faced a number of challenges in advancing these substrates further, some exciting leads for future development and advancement in the synthesis were discovered. The investigation explored an alternative strategy using an aryne cascade reaction, which also yielded some extremely promising results.

COX-2 Regulation of Vaccination Responses in Lung Cancer (13RT-0031)

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Lung cancer cells induce immune suppression. During the current funding period we have discovered that a substance produced by the lung cancer cells, PGE2, can profoundly contribute to this immune suppression by inducing the activity of T regulatory cells (T reg cells). T reg cells are immunosuppressive lymphocytes that function to suppress the activity of other lymphocytes. Thus the tumor can induce the activity of the T reg cells and increase their numbers, thereby blocking the patient's immune response against the cancer cells. The investigators found that drugs which block the enzyme responsible for the production of PGE2 can limit the capacity of T reg

cell activity. They also found that these drugs, called COX-2 inhibitors, can augment the immune response to cancer cells in laboratory lung cancer models and thus cause tumors to shrink.

IAP Antagonists for Lung Cancer Treatment (13RT-0130)

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Lung cancer is caused in part by a failure of lung cells to die in accordance with the natural lifespan regulation of cells. Normally, in the lung and other self-renewing tissues, billions of cells are produced daily, offset by a commensurate amount of cell death. This cell suicide mechanism becomes defective in cancers, contributing the malignant cell accumulation and making tumor cells difficult to kill by chemotherapy and radiation. Among the endogenous proteins that block cell death in cancers is XIAP, which is over-produced in many lung cancers. The goal of this grant was to devise drugs that inhibit XIAP, thus restoring the normal cell suicide mechanism in advanced lung cancers.

The investigators produced synthetic chemicals that bind XIAP and that negate its ability to block tumor cell death. Multiple variants of the chemicals were produced to explore the structural features optimal for suppression of XIAP. Several equally effective, optimized chemicals were generated and shown to kill cancer cells in laboratory experiments. In cell culture experiments, the XIAP inhibitory compounds demonstrated cytotoxic activity against human lung cancer cells and displayed synergistic activity with some conventional anticancer drugs. XIAP antagonists also showed anti-tumor activity against human lung cancer cells grown in mice. Initial pharmacokinetics and biodistribution studies were performed in rodents, providing insights into development of improved dosing regimens.

Using the optimized chemical inhibitors of XIAP, the investigators plan to complete analysis of the safety and pharmacological properties in animals. A new pharmaceutical company was formed to undertake further preclinical development of the XIAP antagonists, including large-scale solution synthesis, formulation and large animal pharmacokinetics and toxicology studies. These efforts will lay a foundation for eventual clinical trials.

Progress toward Chemical Synthesis of Communesin B, a Novel Antineoplastic (14FT-0002)

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The discovery of drugs possessing novel modes of action could significantly impact our ability to treat lung cancer. Many currently employed drugs act by hampering the uncontrolled cell division exhibited by tumor cells, and typically target the cellular protein tubulin. Communesin B is a natural product that has shown promising toxicity against cancer cells, and may act by binding the functionally critical cellular protein actin, which has been targeted to a much less extent in efforts to treat lung cancer. The aim was to develop a laboratory synthesis of communesin B to address its scarce availability from natural sources and to explore its potential in lung cancer chemotherapy.

In progress toward the synthesis of Communesin B, the researchers completed a synthesis of a key intermediate, the alkaloid aurantioclavine, in the spatial arrangement of atoms that exists in nature and is that required for Communesin B. They developed a method allowing access to the congested array of atoms present in Communesin B and also further explored the scope of 3-halo-oxindoles as reactants to provide such crowded atomic arrays.

Lung Cancer Treatment: The Total Synthesis of Ineleganolide (14DT-0004)

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California Institute of Technology

The chemical structure of the molecule ineleganolide is unlike that of other anticancer drugs. This unique structure may open a new cancer treatment class if greater quantities can be produced. This project has attempted to build ineleganolide from inexpensive, readily available starting materials by uniting two chemical fragments and further developing the combined structure. Each fragment was formed and joined together efforts have continued to

advance this structure toward ineleganolide. The investigators were forced to develop two new methods for forming one of the fragments, advancing science further than anticipated by the original proposal.

They have secured additional support from the National Institutes of Health to complete the structure of ineleganolide. These efforts should provide enough of this anti-tumor natural product to explore its mode of action and range of bioactivities.

Role of *GLI2* in Oral Mucosa Maintenance and Carcinogenesis (14FT-0011)

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The 5-year survival for oral squamous cell carcinoma (SCC) is only 40% and has not improved over the past 40 years. Smoking tobacco is the primary etiological agent for oral cancer and the risk cancer increases with the number of cigarettes smoked and is similar for pipe and cigar smoking.

The investigators found a number of previously unappreciated genes as candidates for participation in the process of oral cancer disease initiation or progression, and focused on particular a gene named *GLI2* (Gli-Kruppel Family Member 2). Instead of the standard model of growing cells on a plastic surface in a two-dimensional monolayer, the investigators used an organotypic cell culture approach which integrates the different components found in the oral cavity into a three-dimensional, multi-cell layer reconstruct.

Since previous reports showed that induction of *GLI2* in the skin-derived cells grown on plastic results in down-regulation of differentiation markers, the investigators first asked if overexpression of *GLI2* has a similar effect when cells are grown in three-dimensional organotypic reconstructs. Reconstructs of HaCaT cells in which *GLI2* was induced on either oral or skin derived fibroblasts displayed an undifferentiated phenotype manifested by a decrease in nuclear size and an increase in nuclear density. Immunohistochemical staining specific for proliferation and differentiation markers confirmed that in reconstructs in which *GLI2* was induced most cells remained in cycle throughout the entire epithelial layer and did not express classic differentiation markers, suggestive of cells undergoing cancerous transformation. Interestingly, even though a large number of cells remained in cell cycle in the presence of *GLI2*, the rate of proliferation remained unaffected. In control reconstructs, on the other hand, most cells withdrew from the cell cycle and terminally differentiated. These data strongly suggested that *GLI2* opposes differentiation in organotypic cultures and may play a role in cancer pathogenesis.

Role of p53-regulated sestrin genes in lung cancer (14FT-0054)

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Reactive Oxygen Species (ROS), which are produced by tobacco smoking, might play an important role in lung carcinogenesis by inducing genetic changes and accelerating tumor growth and metastasis. Two related genes, Sesn1 and Sesn2, which are members of the Sestrin gene family, are important regulators of ROS accumulation and cell viability. These genes are often dysregulated in lung cancers and their inactivation accelerates the growth of model tumors in mice. Deficiency of either or both these genes might increase the mutational burden and genetic instability in lung tissue and lead to deregulation of signaling pathways involved in carcinogenesis. The two specific aims of this project were 1) to study the role of the p53-regulated Sestrin genes in initiation and progression of lung cancer and 2) to characterize signaling pathways regulated by p53-dependent sestrins.

Sesn2-deficient mice were generated but did not show any abnormalities. Lung-specific knock-down mice were generated by applying high-titer lentiviral vectors expressing two different Sesn1 shRNA delivered through intratracheal injection to downregulate a Sesn1 expression in lung parenchyma cells. Sesn2 wildtype and knock-out mice as well as lung-specific Sesn1 knock-down mice were treated with NNK and tobacco smoke and the combination of both. Only a small number of mice BL6/129 mixed background produced benign adenomas in a 1-year period and tobacco smoke did not significantly increased tumor incidence. Sesn2 knock-out mice or Sesn1

lung-specific knock-down mice did not show any difference in tumor incidence and size. NNK or NNK/TS slightly increased expression of pro-inflammatory cytokines and chemokines such as TNF, MAP1 and MCP1 in a Sesn1- and 2-independent manner, but no significant effect of NNK on activation of p53 and p53-target genes was observed.

To study the role of Sesn1 and 2 in regulation of intracellular signaling the investigators examined the activity of different signaling pathways in cells with ectopic expression of Sesn1/2 as well as Sesn1/2 knocked-deficient cells. Despite the inability to determine any role of Sestrins in regulation of MAPK pathways, they found out that Sesn1 and 2 downregulated activity of mTOR kinase, a critical regulator of translation, cell growth, proliferation and metabolism. They showed that the lack of significant effect of Sesn1 and 2 on lung tumor development can be explained by high resistance of mice of BL6/129 mixed background to NNK and tobacco smoke, and the possible role of Sestrins in late but not the initial stages of carcinogenesis.

Novel, 3-Dimensional Approach to Non-Invasive Diagnostics (14IT-0097)

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Tobacco use is the main cause of oral cancer and early detection of cancer and its curable precursors remains the best way to ensure patient survival and quality of life. The long-term goal of this research is to develop a non-invasive clinical modality for (1) diagnosing and monitoring oral lesions that have the potential for malignant change (leukoplakias, erythroplakias), and (2) rapid screening of high-risk patient groups (which are clearly defined for oral malignancy). This project investigated, *in vivo*, ultra-fast, high-resolution, 3-dimensional Fourier Domain Optical Coherence and Optical Doppler Tomography (3D-FD-OCT/ODT) for these purposes. They researchers:

1. Performed studies in 120 animals using their prototype cutting-edge 3D-FD-OCT/ODT technology;
2. Imaged 10 patients: 5 with oral squamous cell carcinoma and 5 with oral dysplasia;
3. Demonstrated that *in vivo* 3-D imaging of relatively large tissue areas is possible, covering larger tissue areas at greater speeds than previously possible. Three-D imaging permitted rapid visual identification of regions of interest, which could then be examined more closely using 2-D slices extracted from the 3-D images.

A Novel Total Synthesis of Brazilin and Brazilide A (14DT-0130)

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Recently, it has been discovered that unlike normal lung tissues, upregulation of telomerase, an enzyme that restores the length of the telomere (a region of DNA at the end of the chromosome involved in chromosomal replication and stability), occurs in 98% of small cell lung cancer. As for non-small cell lung cancer, telomerase activity is regarded as an important prognostic factor after surgical resection. Such discoveries suggest that the telomerase expression is an adverse prognostic factor for both lung cancers. There are very few small molecule inhibitors of telomerase known. Brazilin is reported to inhibit telomerase at concentration of less than 100 micromolar and to kill cancer cells *in vitro*. Moreover, it is reported to nick DNA strands. This “double whammy” activity was deduced after the surprising observation that cancer cells doped with brazilin tinctures for visualization died unexpectedly. And Brazilin is not toxic up to 5 minimolar concentration based on the mammalian cells test. Through synthesis, refinement, and testing, the investigators proposed to discover the mechanism by which this class of compounds interacts with telomerase.

The investigators successfully finished a short total synthesis of (\pm)-brazilin. They also devised a new strategy for producing optically-enriched brazilin. In cooperation with Professor Norbert O. Reich’s group at UCSB, the investigators’ synthetic brazilin and its analogs are being analyzed for their ability to inhibit telomerase in comparison with BIBR-1532, a well-known telomerase inhibitor they prepared and used as a standard.

Defining Mutagenesis Pathways in Tobacco-Related Cancer (14DT-0137)

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The Scripps Research Institute

The greatest difficulty in fighting cancer is that all cancers require different treatments because they are distinct from one another. However, all forms of cancer, including those that are tobacco-related, do begin the same way, with the mutation of DNA. This project sought to understand the process of mutation through the use of the model organism budding yeast and mutagens that mimic the effect of many of the carcinogens found in tobacco. In addition, the proteins identified during the course of this work represent exciting new drug-targets to prevent and treat tobacco-related cancer.

The main objectives were to identify all of the genes involved in the process of mutagenesis and organize them into groups based on experimentally determined properties. The investigators screened 4,848 *Saccharomyces cerevisiae* gene deletion strains to identify genes involved in damage induced mutation of the *CAN1* gene. Extensive quantitative validation of the strains identified by the screen in different genetic backgrounds and with different mutation assays led to the identification of ten genes. Among the identified genes were those functioning in error-prone post-replication repair as well as two additional genes, *FYV6* and *RNR4*. Genetic characterization of *FYV6* and *RNR4* demonstrated that they are epistatic with respect to induced mutation, and that they function independently of post-replication repair, although *FYV6* plays a smaller role. This novel pathway of induced mutation appears to be mediated by an increase in deoxynucleotide levels that facilitates lesion bypass by the replicative polymerase Polδ, and is as important as error-prone post-replication repair in the case of UV- and MMS-induced mutation. We propose that Rnr4/Polδ and mutagenic post-replication repair constitute the two dominant pathways by which *S. cerevisiae* induce mutation in response to DNA damage.

Effect of PDGF Inhibition on Angiogenesis in Lung Cancer (14FT-0152)

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Like normal organs, tumors depend on a blood supply for survival. The formation of new blood vessels (angiogenesis) is regulated by substances such as vascular endothelial growth factor (VEGF) and platelet derived growth factor-B (PDGF-B). Studies have shown that VEGF can inhibit angiogenesis and cause regression of tumors. Relatively little is known, however, about the action of PDGF-B inhibition. Recent studies indicate that inhibition of PDGF potentiates the action of VEGF inhibitors or chemotherapy on tumors, but the mechanism of this potentiation is unclear.

The researchers found that PDGF-B inhibition reduced pericytes on tumor blood vessels after 2 days of treatment. Tumor vessels lacking pericytes regressed after 4 days of treatment. Longer treatment resulted in a progressive reduction in tumor vessels. By 28 days, the only vessels remaining had a "normalized" phenotype. Despite the large reduction in tumor vessels, tumor growth increased. PDGF-B inhibition improved tumor vessel efficiency and increased tumor cell proliferation and tumor cell death. A chemotherapeutic, cyclophosphamide, reduced tumor cell proliferation which warrants its combination with PDGF-B inhibition. Thus, PDGF-B inhibition appears to result in two populations of tumor vessels. In one population, tumor vessels lack pericytes and may be vulnerable to other angiogenic inhibitors. The other population of "normalized" tumor vessels, may provide a more efficient route for drug delivery of chemotherapeutics to tumor cells.

Synthesis and Evaluation of Anticancer Agent Celogentin C (15DT-0015)

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Stanford University

Natural products which inhibit microtubule polymerization have been used as anticancer treatments. The original goal was the synthesis and study of one such antimitotic agent, celogentin C. However, when it became evident that the project would not approach completion within a reasonable timeframe, the focus was switched to a related peptide-derived antimitotic natural product, phomopsin B. The total synthesis of phomopsin B was recently

completed. This work has been summarized and accepted for publication in *Angewandte Chemie International Edition*. After completing the synthesis, and verifying its success by comparison to an authentic sample, the presence of a previously unreported equilibrium species was observed. Full characterization of this equilibrium species and evaluation of phomopsin B in cancer cell lines, including non-small cell lung cancer, are the logical directions for future study.

Heart and Lung Disease

Protective Function of VEGF on the Development of Emphysema (12RT-0062)

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Emphysema is characterized by abnormal enlargement of the air spaces in the lungs which results from progressive degradation of the alveolar walls. Many diseases can cause emphysema, especially chronic obstructive pulmonary disease (COPD), which is estimated to affect over 16 million people and is the fourth leading cause of death in the U.S. Since emphysema involves the chronic and persistent damage to the alveolar cells and extracellular matrix, emphysematous lung injury is usually thought to be irreversible. Vascular endothelial growth factor (VEGF) stimulates the growth of endothelial and epithelial cells necessary to form new capillaries. The investigators hypothesized that VEGF is an essential molecule for cell functions involved in the maintenance and remodeling of the normal lung architecture. On the one hand, low VEGF activity impairs lung remolding ability and emphysema is more likely to progress. On the other hand, overexpression of VEGF will enhance the remodeling ability of the lung and delay the development of emphysema caused by smoking.

A transient decrease in pulmonary VEGF was found to lead to increased alveolar and bronchial cell apoptosis (cell death), air space enlargement, and changes in lung elastic recoil (processes that are characteristic of emphysema) which persisted for at least eight weeks. General exercise was shown to be a possible way to reverse this loss and increase endogenous VEGF in the lungs. Decreased VEGF led to increased alveolar septal cell and bronchial epithelial cell apoptosis. Other results suggested that an active remodeling or repair response in VEGF-deficient lungs is mediated by the protein, matrix metallopeptidase 3 (MMP-3). Surfactant production was altered by targeted ablation of the VEGF gene. One transgenic mouse line (mouse VEGF promoter expressing firefly-luciferase) was established and licensed by UCSD for analyzing VEGF promoter activity in the lungs and other organs.

Tobacco Smoke and Airway Remodeling in Asthma (12RT-0071)

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The goals of the research project were to determine whether passive smoking (i.e. exposure to low levels of environmental tobacco smoke or ETS) is likely to increase airway remodeling and airway hyperreactivity (AHR) in a mouse model of asthma. These studies are relevant to whether allergic children with mothers who smoke develop increased reactivity and remodeling of their airways.

Different groups of mice were sensitized to ovalbumin (OVA) allergen and then chronically exposed by inhalation to either ETS (low levels of side-stream smoke), OVA, or the combination of ETS and OVA for up to three months. Mice exposed to the combination of ETS and OVA had significantly increased levels of peribronchial fibrosis and smooth muscle thickening compared to mice exposed to OVA or ETS alone. Mice exposed to the combination of ETS and OVA had significantly increased numbers of peribronchial cells expressing TGF- β suggesting that the increased levels of TGF- β could account for the enhanced airway remodeling when mice are exposed to the combination of ETS and OVA.

Studies were also done to determine whether corticosteroids and CpG DNA can inhibit remodeling induced by exposure to allergen and ETS. These studies demonstrated that CpG DNA and corticosteroids are both effective at preventing airway remodeling induced by exposure of mice to ETS and allergen. The mechanism by which corticosteroids inhibit airway remodeling in mice exposed to ETS and allergen is similar to that mediated by CpG. Corticosteroids and CpG DNA both reduce eosinophilic inflammation as well as the number of peribronchial cells expressing TGF- β in the remodeled airway. As TGF- β is an important mediator of airway remodeling, the ability of both corticosteroids and CpG DNA to inhibit expression of TGF- β in the remodeled airway of mice exposed to ETS and allergen, may be an important mechanism by which both corticosteroids and CpG DNA inhibit airway remodeling.

Smoking, Insulin Resistance, and Endothelial Dysfunction (12RT-0159)

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Smoking is a well-recognized cardiovascular disease risk factor and this risk is markedly attenuated with smoking cessation. However, the pathophysiological link between smoking and cardiovascular disease risk is not clear, and many smokers are unwilling or unable to stop smoking. The results of this project addressed both of these issues. The investigators showed that, despite being less heavy, approximately 50 % of smokers can be classified as being insulin resistant; a prevalence greater than that seen in the population at large. Results of previous large population-based epidemiological studies have identified an atherogenic lipoprotein phenotype that is characteristic of smokers; a high triglyceride and a low high-density lipoprotein cholesterol concentration. The investigators have shown that these changes do not occur in all smokers, but are essentially confined to those smokers that are also insulin resistant. Even when some cardiovascular disease risk factors were seen in both insulin resistant and insulin sensitive smokers, there was significant interaction between smoking and insulin resistance. For example, adiponectin is secreted by the adipose tissue, and low circulating concentrations are currently considered to both decrease insulin sensitivity and increase cardiovascular disease risk. The results of these studies have shown that plasma adiponectin concentrations are lower in smokers, as compared to nonsmokers. Although this observation was true of both insulin resistant and insulin sensitive smokers, the concentrations were lowest in the insulin resistant subgroup. Finally, they have demonstrated that despite continued tobacco use, administration of pioglitazone, a pharmacological agent known to improve insulin sensitivity in nonsmokers, was able to accomplish the same goal in insulin resistant smokers who were unable or unwilling to stop smoking, associated with decreased evidence of inflammation and improved lipid metabolism. These findings are of particular relevance in view of studies showing that pharmacological treatment of the dyslipidemic characteristic of smokers has been shown to reduce cardiovascular disease, and this effect was most prominent in insulin resistant individuals.

In summary, the findings have provided: 1) evidence that it is the insulin resistant smokers who are most at risk of cardiovascular disease; 2) insights into the metabolic abnormalities that exist in these individuals; and 3) a potential pharmacological approach to decrease heart disease in individuals determined to continue smoking.

Smoking, Atherosclerosis and MCSF Gene Variations (13DT-0006)

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Cigarette smoking is a widely recognized risk factor for cardiovascular disease (CVD). Atherosclerosis, the formation of lesions in blood vessel walls, is an underlying process in the development of CVD. One important component in the development of atherosclerosis is Macrophage Colony Stimulating Factor (MCSF), a growth factor that activates specific immune cells called monocytes. Activated monocytes enter blood vessel walls and cause an inflammatory response that leads to thickening and remodeling of vessel walls and may ultimately result in atherosclerosis. In fact, MCSF is so important to the process of atherosclerosis, that in mice unable to produce MCSF as a result of a genetic mutation, little or no atherosclerosis occurs.

A genetic variation of the MCSF gene exists in humans and appears to substantially increase atherosclerosis risk among smokers. The investigators hypothesized that in response to vessel damage caused by smoking, the variant gene produces excessive amounts of MCSF which increase the numbers of activated monocytes migrating to vessel walls. This causes increased remodeling and thickening of the blood vessel walls, ultimately leading to more atherosclerosis than observed among people with the normal MCSF gene.

Preliminary results included increased atherosclerotic progression among smokers and regression among former smokers. The investigators completed additional genotyping of single nucleotide polymorphisms of the MCSF gene. This appears to be the first study to: 1) link a variant MCSF gene to smoking-related atherosclerosis, and 2) investigate a mechanism by which smoking increases atherosclerosis.

The Role of CCL18 in Pulmonary Inflammation (13RT-0083)

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CCL18 is a chemokine which is up-regulated in the lung in various chronic inflammatory diseases (chronic asthma and fibrotic lung diseases), and recent reports indicate that it may be a promising pharmacological target in these diseases. The investigators chose to determine its effects in *in vitro* models by 1) determining whether cigarette smoke extract (CSE) causes increased CCL18 expression, 2) asking what effect CCL18 had on macrophage (Mf) behavior, and 3) asking what effect it had on other cells in the lung.

While CSE did not increase CCL18 production on its own, it greatly increased CCL18 production in the presence of the chronic inflammatory cytokines IL-4 and IL-13, which are increased in asthmatics and to a lesser degree in smokers. After realizing that one of the initial hypotheses - that CCL18 is involved in Mf recruitment to the lung - was wrong, the investigators concluded that other chemokines are responsible for this and that CCL18 instead has a major, long-lasting effect on Mfs that have already arrived in the lung. Therefore, these cells were characterized. The effects of CCL18 included prolonged Mf survival, the production of other chemokines, which can recruit various types of inflammatory cells, and the production of factors that can cause airway remodeling. CCL18-stimulated monocytes matured into a chronic inflammatory Mf type. This is a novel observation for any chemokine, which will be further pursued. In the last aim they switched their emphasis from endothelial cells and fibroblasts to cell types that are less well known, but which appear to play a role in chronic lung inflammation, hematopoietic progenitors (HSPCs) and fibrocytes. Both of these cells respond to CCL18, but they have not yet determined whether CCL18 recruits these cells to the lung.

The following milestones were achieved: 1) A synergistic effect between cigarette smoke and IL-4/IL-13 was observed in the up-regulation of CCL18 by Mfs. Thus it would appear that CCL18 will be up-regulated primarily in smokers with co-existing up-regulation of IL-4 or IL-13, which is prominent in chronic asthma. 2) CCL18 causes Mf maturation to a cell-type which plays a major role in converting an acute inflammatory response into one of chronic inflammation, which is much more resistant to current therapies. These cells could play a major role in smoking associated lung remodeling. 3) HSPCs, which are increasingly being recognized as playing a role in chronic lung inflammation, respond to CCL18. 4) Fibrocytes, which similarly play a role in airway remodeling, respond to CCL18.

CCL18-stimulated Mfs appear to contribute to airway remodeling seen in the lungs of smokers, in particular those with co-existing asthma by producing chemokines/cytokines and other proteins, which cause airway remodeling.

Tobacco Use and Atherosclerosis Protection by Paraoxonase (13RT-0087)

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When blood flow to arteries in the heart is stopped, it is often caused by a disease called atherosclerosis. Although this abrupt cessation of blood flow to the heart is an acute event, many other events that occur very early in life lead

up to the acute event, and this is called chronic atherosclerosis. Early atherosclerotic disease changes are seen even in young adults and infants. The rate at which atherosclerosis progresses in individuals can differ. Tobacco use or exposure to environmental tobacco smoke will accelerate the rate at which this disease gets worse. Why cigarette smoke enhances or exacerbates atherosclerosis is currently unknown. We know that cigarette smoke travels to the lungs and from there to the blood stream. We also know that there are components in this tobacco smoke that can damage cells within the artery wall that the blood flows through. Some of these components within tobacco smoke can initiate chemical reactions that damage molecules as well as cells. Lipoproteins are components of blood that are particularly susceptible to the damage by tobacco smoke. These are lipid-rich proteins that contain phospholipids as well as neutral lipids including triglycerides and cholesterol. When lipoproteins become oxidized by tobacco smoke, they are believed to become proatherogenic, i.e., they are bad for the cells lining the blood wall and contribute to their death or cell changes. Normally, when lipoproteins become oxidized in small amounts, this lipoprotein oxidation can be reversed *in vivo* by an enzyme called paraoxonase 1. This enzyme, which also travels in the blood, is made by the liver and can repair oxidative damage to the plasma lipoprotein. Tobacco smoke is a double whammy in the sense that it can also reduce the activity of this protective oxidation sparing enzyme. The main site for paraoxonase production is in the liver.

The investigators hypothesized that increasing the amount of circulating paraoxonase by generating additional cells within the body that make this enzyme (such as macrophages which are found in atherosclerotic lesions in the artery wall) would be protective. To test this, they made mice that have macrophages that produce large amounts of paraoxonase. The tobacco smoke exposed recipient mice were given bone marrow transplantation from the macrophage expressing paraoxonase donor mice. Control animals or animals that were exposed to a comparable amount of tobacco smoke but did not receive extra paraoxonase via bone marrow-derived macrophages were also studied. When the extent of atherosclerosis in the recipient mice was measured, the amount of paraoxonase that was provided by the bone marrow transplanted macrophages did not provide enough protection against tobacco smoke-induced increases in atherosclerosis.

Although they were able to document successful bone marrow transplantation and that tobacco smoke exposure was accomplished, the studies did not show the expected efficacy for two reasons: (1) either the amount of paraoxonase expressed by the bone marrow-derived cells was insufficient or (2) other anti-oxidant enzymes are needed to completely neutralize the proatherogenic lipoproteins that become oxidized upon exposure to tobacco smoke.

Airway Transcriptome Changes in Smokers (131T-0097)

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The lungs comprise the airways that conduct air to the gas-exchanging region of the lungs, the alveoli. The topic addressed by this grant is the effect of cigarette smoking on the expression of genes in the lining of the airways of the lung. The investigators analyzed the expression of genes in samples of airway cells collected by bronchoscopy. They used gene chips to measure the expression patterns of thousands of genes in samples of airway biopsy tissue from smokers and a healthy control group. The first aim was to establish the gene expression profile of airway mucosal cells in smokers with and without airflow obstruction compared to healthy subjects and disease controls (asthmatic subjects). They examined two sample types – brushings of the lining of the airway and biopsies of the airway wall. In both samples types they used very conservative statistics to figure out which genes are abnormally expressed – this conservative approach allowed them to be quite confident that observed differences were real and not statistical artifacts. In epithelial brushings they found differential expression for 40 genes (27 genes displayed increased expression and 13 were decreased). In epithelial brushings they found differential expression for 23 genes (21 genes increased and 2 decreased). Ten genes appear on both lists of differentially expressed genes. Many of the differentially expressed genes are oxidoreductases or scavenger molecules which serve to limit smoke induced activation of epithelial cells. One of the genes called CABYR is an interesting protein that gains calcium-binding capacity when phosphorylated and which has recently been shown to be upregulated in lung cancer tissues. This

gene is worthy of further investigation for a possible role in COPD. The second aim was to validate the leads generated by the gene chips by confirming them using a different technology for measuring gene expression. This work is ongoing.

Tobacco Oxidants, EGF Receptor Stability, and Lung Disease (13FT-0126)

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Cigarette smoke contains an abundance of free radicals/oxidants per puff and these reactive oxidants have been linked to the occurrence of lung disease. However, the molecular and cellular mechanisms responsible for oxidant-induced lung disease are not well understood. This study was undertaken to examine the effects of hydrogen peroxide (H_2O_2), one of the detected oxidants in cigarette smoke, on the epidermal growth factor receptor (EGFR) and its role in the development of lung cancer.

The EGFR is involved in the regulation of cell growth and proliferation but when this receptor is activated and is not deactivated through its normal degradation pathway, uncontrolled cell growth and tumor promotion may occur. The hypothesis is that H_2O_2 from cigarette smoke can aberrantly activate the EGFR in a way that allows the active receptor to circumvent degradation and to continue signaling, leading to uncontrolled cell growth and tumor promotion.

They exposed human airway epithelial cells to either H_2O_2 or cigarette smoke (CS) and found that the EGFR is indeed activated under both conditions and the H_2O_2 measured from CS-exposed cell culture medium is comparable to the levels used in H_2O_2 exposures. Furthermore, the H_2O_2 - and CS-activated EGFR is not degraded due to the aberrant phosphorylation of the receptor and subsequent lack of ubiquitination and lysosomal degradation.

Concomitantly, H_2O_2 and CS exposures result in the activation of downstream Akt and ERK1/2 survival and proliferation pathways.

Removal of the EGFR from the cell surface by sorting for degradation is important in inhibiting this receptor's oncogenic potential since the oncogenic action of the EGFR has been shown to depend on its localization at the plasma membrane. This study demonstrates that the H_2O_2 component of CS can aberrantly activate the EGFR, causing it to remain active at the plasma membrane and continue to propagate growth signaling, thereby providing some insight into one mechanism by which cigarette smoke may promote lung cell hyperplasia.

Integrin Receptors and Heart Disease (14FT-0033)

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At the root of blood clotting are platelets, cells that circulate in the blood and stick to each other and the insides of vessel walls once stimulated. At the molecular level, this process is controlled by a protein that sits on the platelet surface called the alphaIIb/beta3 integrin. A special characteristic of this protein is that it exists in two interchangeable shapes called the inactive and active conformations. Normally, the integrin maintains the inactive conformation which prevents the cells from clumping together. However, in certain instances, such as cut to the skin, platelets are stimulated causing the integrin to change its shape to the active conformation. At this point the integrin proteins become 'sticky' causing platelets to clump together and adhere to the blood vessel wall forming a clot that stops the bleeding.

Unfortunately, integrin activation can be initiated inappropriately, for example, alphaIIb/beta3 is activated by the rupture of a coronary plaque. Such aberrant activation has the potential to block intact blood vessels, something that is very dangerous if it occurs within the heart since it can result in a heart attack. This research investigated the molecular details of how the integrin switches from the inactive shape to the active one. It is currently known that integrins consist of two non-identical halves called subunits. If one imagines each subunit to look like a dumbbell, it is thought that these two subunits come together in an 'X'-like fashion with the crossing point at the 'bar' region

of the dumbbells. Based on the literature and findings from this laboratory and other leading groups, the central hypothesis is that the conversion of the inactive integrin to the active conformation involves a change in the length of the ‘bar’ and the angle at which the two halves of the protein cross. To gain further support of this idea, the investigators approached this problem using both biophysical techniques and cell biological assays. By approaching this concept from different angles they hope to definitively show how the integrin activation process works. The biophysics projects have provided various isotopically enriched integrin proteins to a collaborator who is progressing rapidly towards a three-dimensional structure of the transmembrane subunit of the beta3 transmembrane helix. The cell biology assay which measures the effect of point mutations on integrin activation has provided exciting new insights.

The project was not completed because the investigator terminated the grant one year early.

Environmental Tobacco Smoke & Effects of Tobacco Use on Reproductive Processes

Reactivity of Surface Nicotine Towards Indoor Ozone (12KT-0178)

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Lawrence Berkeley National Laboratory

Secondhand tobacco smoke (SHS) is a complex chemical mixture. While many volatile SHS constituents can be removed in short times from indoor environments by ventilation, other components tend to sorb (“stick”) to the surface of indoor materials, and can be slowly desorbed over periods of weeks and months following their original release. Nicotine is an important semivolatile SHS constituent, used often as an indoor tracer for tobacco smoke. It has a particularly high affinity for surfaces, which leads to an extended indoor residence time due to sorption. Surface nicotine can be slowly desorbed into indoor air over extended periods of time, but it also is subject to reaction with ozone and other atmospheric oxidants.

This project investigated the reactivity of surface nicotine towards indoor ozone. The investigators designed, built and evaluated an environmental chamber and its ancillary experimental setup. They developed sampling and analytical procedures for the quantitative determination of gas-phase chamber nicotine and its oxidation products using gas chromatography/mass spectrometry (GC/MS) and gas chromatography/nitrogen-phosphorus detection (GC/NPD). Experiments were performed by initially depositing a known amount of nicotine over various model surfaces: Teflon, cotton and wallboard. These materials were characterized by determining their effective surface area exposed to the gas phase, as well as their water uptake capacity. The interaction of ozone with surface nicotine was investigated by monitoring its desorption over a week following equilibration in dry or humid air (65-70 % RH). In dry air, gas phase nicotine concentrations decreased with respect to baseline (no ozone) levels by 2 orders of magnitude for Teflon after 50 h under 20–45 ppb O₃, and by a factor of 10 for cotton after 100 h with 13–15 ppb O₃. For wallboard, in both dry and humid air, gas phase nicotine concentrations decreased with respect to baseline (no ozone) levels by 2 orders of magnitude after 100 h and 3 orders of magnitude after 200 h, at 40-60 ppb O₃. The ratios of pseudo first-order rate constants for surface reaction (*r*) to long-term desorption (*k*) were *r/k* = 3.5 and 2.0 for Teflon and cotton surfaces, respectively. In the case of wallboard, the ratios of pseudo first-order rate constants for surface reaction (*r*) to long-term desorption (*k*) were *r/k* = 2.5 and 3.7 for dry and humid air, respectively. These results clearly indicate that under moderate ozone levels, the rate of nicotine oxidation is competitive with its desorption rate, and surface oxidation can reduce significantly the gas phase concentrations of re-emitted nicotine. Interactions of nicotine and pyridine with model surface materials were further evaluated at the molecular level using FTIR spectroscopy, observing that acid-base association with surface sites reduced desorption rates. In chamber experiments, formaldehyde, N-methylformamide, nicotinaldehyde, cotinine and myosmine were identified as oxidation products, indicating that the pyrrolidinic N was the site of electrophilic attack by O₃. Considering the effect of relative humidity, co-adsorbed water present in the cotton samples completely inhibited nicotine oxidation. However, the same effect was not observed for materials such as Teflon and wallboard, with much lower water uptake capacity.

This study has, for the first time, identified and characterized chemical processes leading to the formation of stable nicotine oxidation products under simulated indoor conditions. Those oxidation products are expected to be present in a variety of indoor settings as a consequence of ozone-driven chemical aging processes, and should be taken into account in order to assess long-term exposure to SHS pollutants. Better understanding of indoor chemical processes taking place on surfaces is needed to evaluate the impact of SHS aging processes on human exposure to toxic pollutants.

Does Tobacco Exposure Delay Conception? (12RT-0202)

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Sequoia Foundation

Several studies have suggested that smoking by women or their partners can affect fertility and delay conception, but it is not known whether women exposed to second-hand smoke have reduced fertility or conception delay. Previous studies may have underestimated smoking effects by not taking passive exposure into account among non-smokers and relying on women's self-reports of smoking. This study examined the relationship between tobacco exposure and time-to-pregnancy using a biochemical measure of tobacco exposure capable of detecting passive exposure levels.

Women obtaining pregnancy tests were surveyed to determine use of birth control and duration of unprotected intercourse. A total of 2,083 specimens were selected for analysis for cotinine, a metabolite of nicotine, using a highly sensitive laboratory test to quantify exposure to second-hand smoke, as well as active smoking. The probability of conception at each menstrual cycle (fecundability) was statistically modeled within a sample of 995 women selected at random. The probability of conception at each cycle was reduced by 32% among women with exposure to second-hand smoke (urinary cotinine concentrations ≥ 0.165 ng/ml), relative to those with minimal exposure (<0.165 ng/ml), adjusting for the age of the woman. Second-hand smoke exposure effects were observed at low doses for the random, pregnant and live-birth groups. However, among those surveyed at delivery, only those with the heaviest second-hand smoke exposure (urinary cotinine concentrations between 7.4 and 99.5 ng/ml) had significantly lower fecundability. Across the study groups, the results consistently support a role for second-hand smoke in delayed conception, with estimated reductions in fecundability ranging from 20-37%. Active smoking (urinary cotinine ≥ 100 ng/ml) was associated with a 25% reduction in fecundability relative to non-smokers, and a 46% reduction in fecundability relative to those with minimal second-hand smoke exposure.

Oral Clefts, Smoking & Variation of Toxin Biotransformation (13RT-0109)

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The purposes of this research were to investigate susceptibility to orofacial clefts caused by smoking during pregnancy or exposure to environmental tobacco smoke. The investigators assessed the association between maternal smoking and environmental tobacco smoking and the occurrence of orofacial clefts using two large population-based case-control studies: 1) The National Birth Defects Prevention Study (NBDPS), an ongoing, 9-state case-control study of environmental and genetic risk factors for major birth defects; and 2) the California Study of Birth Defects Causes (CII), based in three California counties (LA, Santa Clara, SF). Among the 3,390 control mothers in NBDPS, 20% smoked during the month before conception and two months following conception, compared to 24% of mothers of infants with cleft lip ± cleft palate and 23% for mothers of infants with cleft palate alone. By contrast, of mothers in the CII who delivered from 1999-2003, 10% of mothers of cleft palate infants and 13% of mothers of cleft lip+palate were classified as smokers, as compared to 11% of mothers of control infants. In the NBDPS results but not in the CII population, maternal smoking was associated with an approximate 20% increase in the occurrence of cleft lip ± cleft palate, and a higher risk for bilateral cleft lip. Among the heaviest smokers, risk for bilateral cleft lip ± cleft palate was nearly 4-fold higher than among nonsmoking mothers. In NBDPS data, environmental tobacco exposure among nonsmoking mothers was not associated with higher risk for cleft lip. However, environmental tobacco exposure was associated with increased

risk for infants with cleft palate with associated major malformations and females infants with isolated cleft palate. These are two of the largest population-based, case-control studies to investigate associations between tobacco exposures during pregnancy and risk for orofacial clefts.

For the candidate genes which were investigated, there was little evidence that their variants interacted with maternal smoking to influence risk for clefts. One exception was the observation of higher risk of cleft lip among smoking mothers whose infants had a specific alleles of nitric oxide synthase (NOS3 -922G or 894T allele) (for homozygotes, OR= 2.5; 95% CI; 1.2-5.6).

This project showed that smoking during pregnancy nearly doubles the risk to have an infant with an orofacial cleft. It also showed that environmental tobacco exposures contribute in a small additive manner to risk, at least in a 9-state study with a higher (20%) percentage of pregnant women who smoke. However, in a population limited to California counties where only 11% of control women smoked during the periconceptional period, the increased risk of cleaving was not confirmed.

Nicotine's Effects on Uterine Artery Contractility (14FT-0075)

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Recent studies indicate that cigarette smoking/nicotine increases maternal blood pressure and decreases uterine blood flow in pregnancy. This in turn can decrease the supply of nutrients and oxygen delivered to the growing fetus, thereby leading to decreased birth weight and increased perinatal mortality rate. However, the mechanisms are not fully understood.

During pregnancy, uterine artery blood flow significantly increases to ensure normal fetal development. One of the major mechanisms accounting for this increase is a significant decrease in uterine artery vascular tone. The investigators previously demonstrated that alpha 1-adrenoceptor-mediated signaling pathway and endothelial function in the uterine artery play key roles in precise regulation of uterine artery contractility during pregnancy. In the current studies, they tested the hypothesis that chronic nicotine exposure directly decreases endothelium-dependent relaxation and increases vascular contractility of the uterine artery in pregnancy. They found that chronic nicotine exposure significantly enhanced alpha1-adrenoceptor agonist phenylephrine-induced contractions. Furthermore, chronic nicotine treatment increased protein kinase C (PKC) activity and potentiated PKC-mediated uterine artery contraction in pregnancy. In contrast, chronic nicotine treatment decreased endothelial nitric oxide synthase activity and gene expression, leading to a decrease in uterine artery relaxation.

These findings suggest that nicotine directly impairs uterine vascular function in pregnancy. Although it is not clear at present whether the increased vasoconstriction and the inhibition of endothelial nitric oxide synthase activity could be a major reason for the reduced uterine blood flow observed with smoking/nicotine exposure during pregnancy, these findings provide a potential mechanism.

Effects of Side Stream Tobacco Smoke on DNA Deletions (14DT-0121)

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It is widely accepted that secondhand cigarette smoke causes cancer, but the mechanisms of secondhand-smoke-induced cancer are not understood nor are the reasons why some people but not others develop cancer after exposure to smoke. Many factors may contribute to carcinogenesis caused by smoking including free radicals, inflammation, and DNA damage. Free radicals can lead to DNA damage. To understand how secondhand smoke may cause cancer, the study used mice with a reduced capacity to repair DNA damage caused by free radicals. The mice lacked the gene *Ogg1* or *Myh*, or both which are involved in base excision DNA repair removing oxidatively damaged DNA bases. The investigators exposed mice lacking *Myh*, *Ogg1*, or both to side-stream tobacco smoke (SSTS) during embryonic development. These mice have a specific repeat in their genome which, if deleted, leads

to a black-colored cell in their eye. Therefore, if the DNA is damaged by SSTS, and the damage leads to that deletion, it is detected as a spot in the eye. DNA deletions or other genome rearrangements are one cause of cancer and the investigators have previously shown that this DNA deletion assay highly correlates with genetic and environmental cancer-causing factors including cigarette smoke. Myh-deficient mice were found to be more sensitive to SSTS than their wildtype littermates when exposed to 1 mg/m³ total particulate matter for 10 days. Thus, humans with polymorphisms in the *Myh* gene may be more susceptible to SSTS-induced DNA damage. Other polymorphisms such as Ogg1 polymorphisms, may also lead to increased susceptibility to SSTS-induced DNA damage, which will be tested in animals in future experiments.

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