7TH ANNUAL INVESTIGATOR MEETING

Tobacco-Related Disease Research Program

AIM 2002

TRDRP
Tobacco-Related Disease Research Program

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7th Annual Investigator Meeting

AIM 2002

Women and Smoking

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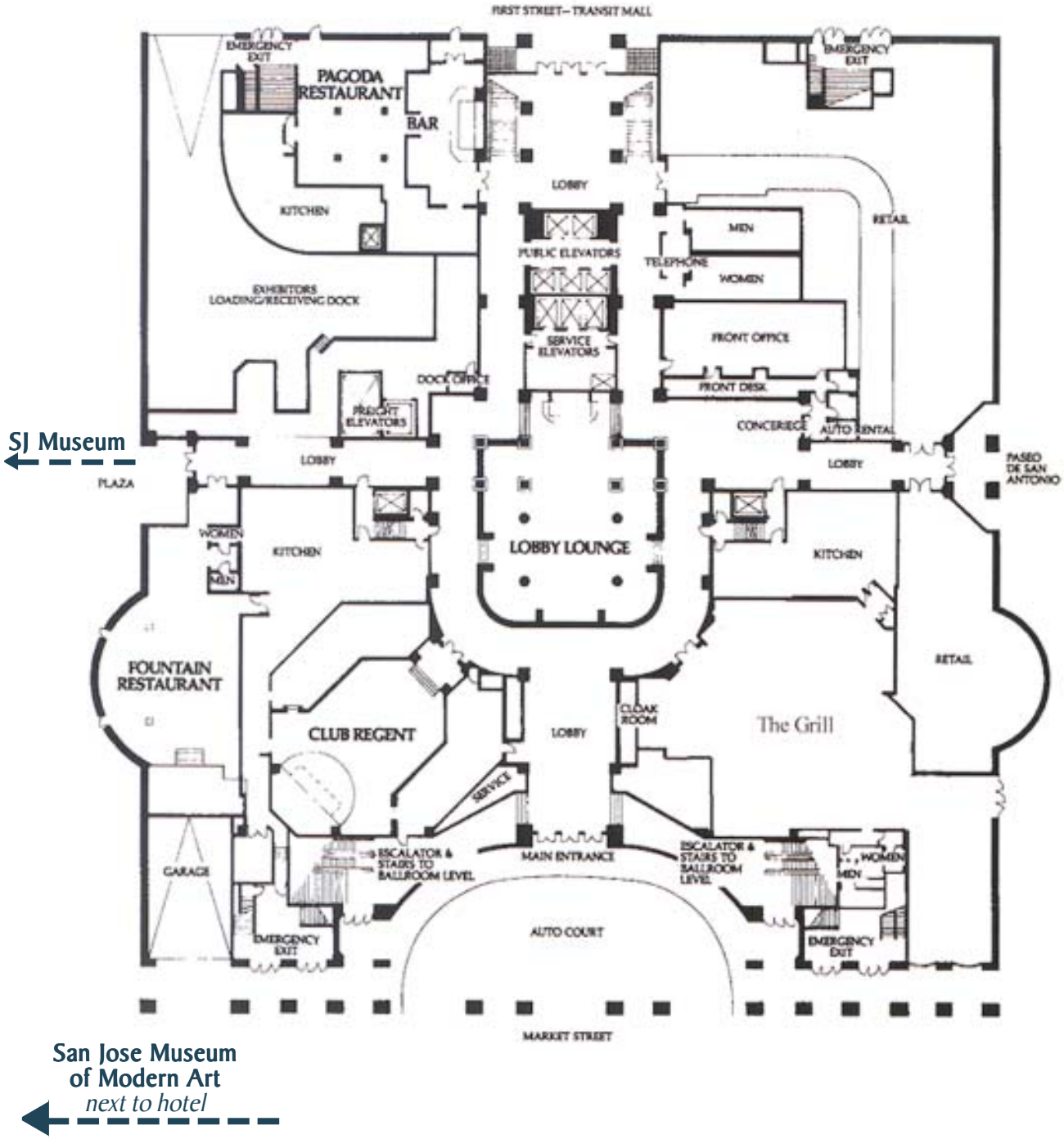
On behalf of the Tobacco-Related Disease Research Program and the University of California, I would like to extend a warm welcome to TRDRP's 7th Annual Investigator Meeting. The conference theme is *Women and Smoking: Smoking Has No Glass Ceiling.* Smoking by women and their exposure to environmental tobacco smoke present real challenges to researchers, clinicians, and tobacco control professionals on many levels, from tobacco use prevention to treatment of tobacco-related disease. In the U.S., women started smoking in very large numbers several decades after men and, as a result, are experiencing the health consequences later. We have learned that the tobacco companies market directly to women and adolescent girls, and target specific ethnic groups, which has likely contributed to the incidence and prevalence of smoking. We expect to learn about recent advances in knowledge of women's tobacco use patterns, their susceptibility to tobacco-related disease, and approaches to tobacco control among girls and young women.

I encourage you to talk to the poster presenters, especially those outside your own area of expertise, to learn about advances being made in other fields to combat the detrimental effects of tobacco use.

I am confident you will leave the conference with an increased understanding of the recent advances made in tobacco research in California. In my view, the meeting will be a rousing success if it stimulates discussions about emerging research issues that lead at least some of you to new research collaborations with colleagues from other disciplines.

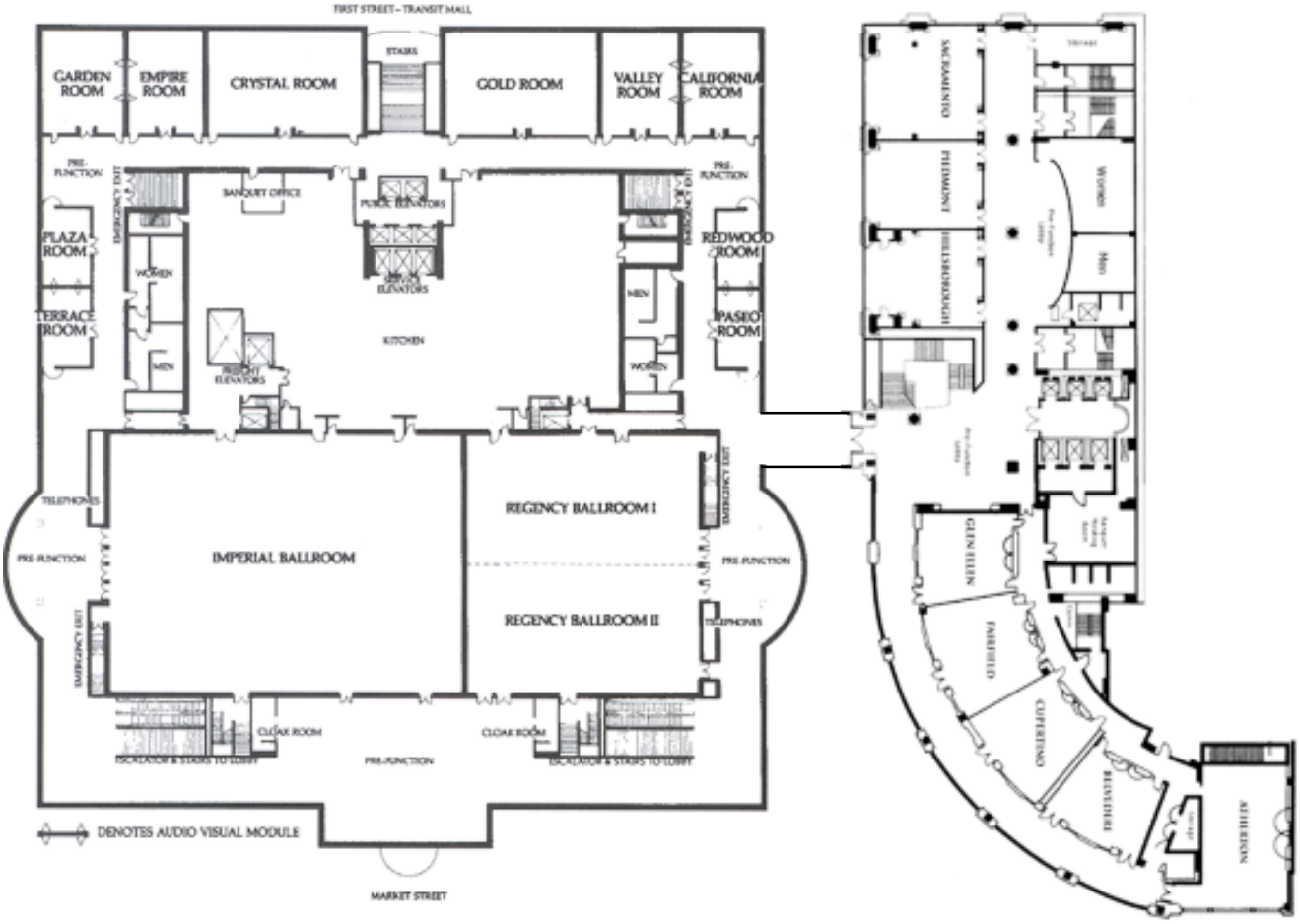
Charles L. Gruder, Ph.D.
Executive Director, Special Research Programs
Acting Director, TRDRP

LOBBY LEVEL


FIRST STREET– TRANSIT MALL
EMERGENCY EXIT
PAGODA RESTAURANT
BAR
KITCHEN
LOBBY
PUBLIC ELEVATORS
MEN
WOMEN
TELEPHONE
RETAIL
EXHIBITORS LOADING/RECEIVING DOCK
SERVICE ELEVATORS
FRONT OFFICE
FRONT DESK
DOCK OFFICE
FREIGHT ELEVATORS
CONCIERGE
AUTO RENTAL
SJ Museum
PLAZA
LOBBY
PASEO DE SAN ANTONIO
WOMEN
MEN
KITCHEN
LOBBY LOUNGE
KITCHEN
FOUNTAIN RESTAURANT
CLUB REGENT
LOBBY
CLOAK ROOM
The Grill
RETAIL
SERVICE
GARAGE
ESCALATOR & STAIRS TO BALLROOM LEVEL
MAIN ENTRANCE
ESCALATOR & STAIRS TO BALLROOM LEVEL
WOMEN
MEN
EMERGENCY EXIT
AUTO COURT
EMERGENCY EXIT
MARKET STREET
San Jose Museum of Modern Art
next to hotel

BALLROOM LEVEL

TOWER MEETING ROOMS



FAIRMONT - FLOOR PLAN

December 4th–WEDNESDAY

2ND FLOOR LOBBY

7:30am–6:30pm **Registration & ContinentaL Breakfast**
Imperial Ballroom Lobby

8:00am–12:00pm **Poster SETUP** — *Imperial Ballroom*

8:00am–12:00pm **Exhibitor Setup** — *Imperial Ballroom (east side of lobby)*

9:00am–12:00pm **Workshops**

A. Smoking and Breast Cancer — *Atherton*

B. Cardiovascular Disease in Women — *Cupertino*

C. TCS Data on Women and Smoking in California — *Belvedere*

12:00pm–1:30pm **Poster Viewing (W1-46)** — *Imperial Ballroom*
PIs will be at their posters from 12:00 – 12:45pm
(Posters will be on display for the duration of the meeting)

12:00pm–5:00pm **Exhibits Open** — *Imperial Ballroom (east side of lobby)*

12:30pm–1:30pm **Lunch** — *Imperial Ballroom*
What 's New at TRDRP

1:30pm–4:30pm **Workshops**

D. Tobacco Use Research Centers Panel Discussion — *Atherton*

E. Cutting Edge Issues in COPD — *Cupertino*

F. ETS and Adverse Pregnancy Outcomes — *Belvedere*

5:00pm–6:30pm **Town Hall – Harm Reduction** — *Crystal Ballroom*

6:30pm–8:00pm **Reception** – *San Jose Museum of Modern Art*
(Next to Hotel)

December 5th–THURSDAY

2ND FLOOR LOBBY

7:30am–2:30pm — **Registration and Continental Breakfast**
Imperial Ballroom Lobby

8:00am–4:30pm — **Exhibits Open** — *Imperial Ballroom (eastside of lobby)*

8:00am–11:00am — **Plenary Session: *Women & Smoking***
Imperial Ballroom

Moderator

Rosemarie Henson, M.S.S.W., M.P.H. — *Director*
CDC's Office on Smoking and Health

Panelists

Cheryl Healton, Ph.D. — *President and CEO*
American Legacy Foundation

Virginia Ernster, Ph.D. — *Professor Emerita*
Department of Epidemiology & Biostatistics
University of California, San Francisco

Jill Siegfried, Ph.D — *Professor of Pharmacology & Co-Director*
The Lung Cancer Program, University of Pittsburgh

Sherri Watson Hyde — *Executive Director*
National African American Tobacco Prevention Network

11:00am–12:30pm — **Poster Viewing (T47-97)** — *Imperial Ballroom*
PIs will be at their posters from 11:00 – 11:45am
(Posters will be on display for the duration of the meeting)

12:30pm–2:00pm — **Lunch with Keynote Speaker** — *Imperial Ballroom*
Diana M. Bontá, R.N., Dr. P.H.—*Director*
California Department of Health Services

2:30pm–4:30pm — **TRDRP Listens** — *Gold Room*
Open Forum

WORKSHOP A

AMERICAN CANCER ASSOCIATION
CALIFORNIA BREAST CANCER RESEARCH PROGRAM
TOBACCO-RELATED DISEASE RESEARCH PROGRAM

Smoking and Breast Cancer: Is There a Connection?

Moderator

Phillip Gardiner, Dr. P.H.

Research Administrator, Social & Behavioral Sciences/Nicotine Dependence

Is there a relationship between smoking and breast cancer?
How does secondhand smoke affect breast cancer incidence?

Epidemiology of Smoking & Breast Cancer

Susan Murin, M.D.— University of California, Davis

Secondhand Smoke & Breast Cancer

Marilie Gammon, Ph.D.— University of North Carolina Chapel Hill

Tobacco Carcinogens and Breast Cancer

Steven Hecht, Ph.D.— University of Minnesota

Closing Comments

John Baron, M.D., M.Sc.— Dartmouth Medical School

WORKSHOP B

AMERICAN HEART ASSOCIATION

Cardiovascular Disease in Women

Moderator
Ronald Krauss, M.D.
Children's Hospital Oakland Research Institute

9:00 am **Postmenopausal Hormones and Coronary Heart Disease**
Dr. Marcia Stefanick, Ph.D. — Stanford University

9:40 am **Smoking and Coronary Heart Disease**
Dr. Rita Redberg, M.D. — University of California, San Francisco

Break

10:40 am **Diabetes, Obesity, and Coronary Heart Disease**
Dr. Alka Kanaya, M.D. — University of California, San Francisco

11:20 am **Estrogens and Atherosclerosis**
Dr. Carole Banka, Ph.D. — La Jolla Institute for Molecular Medicine

WORKSHOP C

CALIFORNIA DEPARTMENT OF HEALTH SERVICES TOBACCO CONTROL SECTION

CDHS and TCS Data on Women and Smoking in California

Moderator

Jessica R. Schumacher, M.S

California Department of Health Services, Tobacco Control Section

Impact of Ethnicity on Girl-Boy Differences in California Adolescent Tobacco Use

William J. McCarthy, PhD.—Senior Researcher, WestEd; Adj. Associate Professor of Psychology, University of California, Los Angeles Division of Cancer Prevention and Control Research

Gender Differences in Smoking Behavior

Elizabeth A. Gilpin, M.S.—Director, Biostatistics Shared Resource, University of California, San Diego Cancer Center; Clinical Professor of Biostatistics, Department of Family and Preventive Medicine, University of California, San Diego

Is it Harder for Women to Quit Smoking?

Shu-Hong Zhu, Ph.D.—Associate Professor; Principal Investigator, California's Smokers' Helpline, Department of Family and Preventive Medicine, University of California, San Diego

Factors Associated with smoking Among Women in California and the U.S.

Jessica R. Schumacher, M.S.—Research Scientist, California Department of Health Services, Tobacco Control Section

Countering Tobacco Industry Influence on Women through Media

Schaelene Rollins, B.A.—Media Specialist, California Department of Health Services, Tobacco Control Section

WORKSHOP D

NATIONAL CANCER INSTITUTE
TRANSDISCIPLINARY TOBACCO USE RESEARCH CENTERS

Moderator

Glen Morgan, Ph.D.
Program Director—TTURCs, National Cancer Institute

C. Anderson Johnson, Ph.D.
Director—TTURC, University of Southern California

Frances Leslie, Ph.D.
Director—TTURC, University of California, Irvine

Dan Stokals, Ph.D.
Principle Investigator—Transdisciplinary Core, TTURC, University of California, Irvine

Juliana Fuqua, Ph.D.
Postdoctoral Fellow—TTURC, University of Southern California

WORKSHOP E

AMERICAN LUNG ASSOCIATION

Cutting Edge Issues in COPD

Moderator & Organizer

Richard Casaburi, Ph.D., M.D.

Harbor-UCLA Research and Education Institute

1:30pm **Are Women More Susceptible than Men?**
Carolyn Rochester, M.D.—Yale University

2:05pm **New Pharmacologic Agents**
Richard Casaburi, Ph.D., M.D. —Harbor-UCLA Research & Education Institute

2:40pm **Perfecting Pulmonary Rehabilitation**
Andrew Ries, M.D.—University of California, San Diego

3:15pm **Long-Term Oxygen Therapy –Technology and Economics**
Brian Tiep, M.D.—Pulmonary Care Continuum

3:50pm **Update on Volume Reduction Surgery**
Matthew Brenner, M.D. —University of California, Irvine

WORKSHOP F

CALIFORNIA DEPARTMENT OF HEALTH SERVICES
TOBACCO-RELATED DISEASE RESEARCH PROGRAM

Environmental Tobacco Smoke and Adverse Pregnancy Outcomes: Views from Multiple Disciplines

This workshop consists of a panel of prominent investigators from multiple disciplines who will be asked to respond to the following four papers on ETS and birth weight:

1. Haddow JE, Knight GJ, Palomaki GE, McCarthy JE. Second-trimester serum cotinine levels in nonsmokers in relation to birth weight. *Am J Obstet Gynecol* 159(2):481-4 (1988).

2. Rebagliato M, Florey Cdu V, Bolumar F. Exposure to environmental tobacco smoke in nonsmoking pregnant women in relation to birth weight. *Am J Epidemiol.* 1995 Sep 1; 142(5):531-7.

3. Eskenazi B, Prehn AW, Christianson RE. Passive and active maternal smoking as measured by serum cotinine: the effect on birthweight. *Am J Public Health.* 1995 Mar; 85(3):395-8.

4. Kharrazi M, DeLorenze GN, Kaufman FL, Eskenazi B, Bernert JT, Graham S, Pearl M, Pirkle J. *Influence of low-level environmental tobacco smoke on pregnancy outcomes.* Manuscript.

Each panelist will be asked to provide a critique of the papers from his/her own discipline. For example, what evidence from his/her discipline supports or does not support a causal relationship between ETS and slowed fetal growth. What research needs to be done to more solidly evaluate this relationship?

1:30 PM **Moderator Introductions**—Francisco O. Butching, Ph.D., TRDRP

1:40 PM **Epidemiologist**—Gayle Windham, Ph.D.,Yale University

2:00 PM **Animal Scientist**—Laura Van Winkle, Ph.D., University of California, Davis

2:20 PM **Pharmacokinetics**—Neal Benowitz, M.D., University of California, San Francisco

2:40 PM **Placental Pathologist**—Olga Genbacev, Ph.D., University of California, San Francisco

3:00 PM **Male-Mediated Effects**—Andrew Wyrobek, Ph.D., Lawrence Livermore National Laboratory

3:20 PM **Genetics**—Xiaobin Wang, M.D., M.P.H., Sc.D., Boston University

3:40 PM **Obstetrician**—Aaron Caughey, M.D., M.P.P., M.P.H., University of California, San Francisco

4:00 PM **Question and Answer Session**

TOWN HALL MEETING: HARM REDUCTION

Moderator

Dr. Kathy Sanders-Phillips
Center for Drug Abuse Research, Howard University
and TRDRP Scientific Advisory Committee Member

Featured Participants

David Burns, M.D.—Professor of Family and Preventive Medicine, University of California, San Diego

Neal Benowitz, M.D.—Chief-Division of Clinical Pharmacology, University of California, San Francisco

Richard Hurt, M.D.—Professor of Medicine, Mayo Medical School

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The goal of this Town Hall Meeting is to generate discussion among AIM participants about the concept and application of

Harm Reduction in Tobacco Control and Mitigation of Tobacco-Related Diseases

In the past five years, harm reduction has emerged as a critical issue in tobacco control. A harm reducing product is one that "lowers total tobacco-related mortality and morbidity even though use of that product may involve continued exposure to tobacco-related toxicants" according to the Institute of Medicine's comprehensive report on this topic in 2001. The recognition that many people cannot or will not stop using tobacco leads to the premise that a harm reduction approach—encouraging tobacco users to switch to a less dangerous method of nicotine consumption – would benefit these individuals and potentially benefit the overall public health.

The theoretical feasibility of tobacco harm reduction may have broader support than the much more debatable issue of whether any existing tobacco products qualify as "harm reducing". The increasing number of untested and unregulated new tobacco products entering the U.S. market that claim to contain reduced levels of certain toxins present additional challenges. Are modified cigarettes – like Advance Lights—and new smokeless nicotine products – like Ariva – more or less dangerous than existing brands? Should researchers, public health professionals and doctors recommend any tobacco products as harm reducing? What assays and studies are needed to demonstrate that a product is harm reducing? What is the effect of harm reduction on cessation efforts? Can harm reduction work without stricter regulation of new tobacco products, and even traditional tobacco products, and their safety claims?

PLENARY SESSION
Women and Smoking

Moderator
Rosemarie Henson, M.S.S.W., M.P.H.
Director—CDC Office on Smoking and Health

Speakers
Cheryl Healton, Ph.D.
President and CEO—American Legacy Foundation
Counter-Marketing Against Tobacco
A Critical Factor in Reducing Smoking in Women and Girls

Virginia Ernster, Ph.D.
Professor Emerita—Department of Epidemiology & Biostatistics
University of California, San Francisco
Health Effects of Smoking and Trends in Smoking Prevalence Among Women

Jill Siegfried, Ph.D.
Professor of Pharmacology & Co-Director of The Lung Cancer Program, University of Pittsburgh
Potential Sex Differences in Pathways of Lung Carcinogenesis

Sherri Watson Hyde
Executive Director, National African American Tobacco Prevention Network
Role of Research in Activating Communities of Color

KEYNOTE SPEAKER

Diana M. Bontá, R.N., Dr.P.H.

Director—California Department of Health Services

Diana M. Bontá, R.N., Dr.P.H., is the Director of the California Department of Health Services [DHS]. DHS is one of the largest departments in California State Government, with a budget over $30 billion and 6,000 employees. DHS administers a wide array of programs and services to help meet the needs of the State's diverse population and oversees such programs as California's tobacco control, Medi-Cal, HIV/AIDS, family planning, domestic violence, communicable disease control, environmental health, and chronic disease. The Department has numerous regulatory roles and is responsible for licensure of nursing homes, hospitals, and primary care clinics.

Dr. Bontá has 30 years of experience in the health care field, including her prior position as Director of the Department of Health and Human Services for the City of Long Beach. In addition, Dr. Bontá has held the positions of Deputy Executive Director of the Los Angeles Regional Family Planning Council, Regional Administrator of Rural Health Programs for the State of California, and various other positions with hospitals as a clinical instructor and head nurse of medical and pediatric units in Los Angeles, Buffalo, and New York City. Her doctorate and master's degrees in public health were earned at the University of California, Los Angeles, and Dr. Bontá's B.S. in nursing was earned at State University of New York in Buffalo.

Dr. Bontá has been an instrumental community leader in California and the national. Dr. Bontá was awarded the Milton and Ruth Roemer Prize for Creative Local Public Health Work by the American Public Health Association; the California Women's Law Center Annual Pursuit of Justice Award; the Latino Journal, Latino Perspectives, National Hispanic Employee Association 2001 Excellence in Public Service Award; the Hispanic Business Magazine "Elite Women" profile of distinguished women in the field of government April 2002; and the University of California, Los Angeles School of Public Health Inaugural induction into the Alumni Hall of Fame. She was elected to serve as Chair of the Executive Board of the American Public Health Association. Dr. Bontá is a member of the Centers for Disease Control and Prevention Advisory Committee to the Director, the Association of State and Territorial Health Officials, and the Anti-Terrorism Preparedness Task Force.

VIRGINIA L. ERNSTER, PH.D.

University of California, San Francisco

Dr. Ernster is Professor emerita in the Department of Epidemiology and Biostatistics, School of Medicine, University of California , San Francisco (UCSF). She has served as Chair of her department at UCSF, as well as Associate Director of the UCSF Cancer Center, and leader of its Tobacco Control Program. She has been a member of the National Cancer Institute's Board of Scientific Advisors and many other national committees, has served on the Board of the American College of Epidemiology, and has been on the editorial board of several medical journals. Dr. Ernster has a long-standing interest in the effects of smoking on women's health and was Senior Scientific Editor of the 2001 Surgeon General's Report Women and Smoking. She chaired the Policy Advisory Committee of the NCI-funded COMMIT community intervention smoking cessation trial and was a member in 1998 of the NCI's Tobacco Research Implementation Group. Dr. Ernster also has been actively involved in breast cancer research and was principal investigator of a project funded by the NCI to establish a population-based mammography registry for San Francisco. Her publications include work on the epidemiology of breast cancer and female lung cancer, cigarette advertising targeted to women, the health effects of smokeless tobacco use, mammography screening, and ductal carcinoma in situ (DCIS).

For over two decades, Dr. Ernster co-directed the core course in Epidemiology and Biostatistics for UCSF medical students, designed to instill skills for critical evaluation of the medical literature and the practice of evidence-based medicine. She received the UCSF Distinguished Teaching Award as well as other awards for excellence in teaching and in 1996 was the recipient of the Career Achievement Award of the UCSF Women's Faculty Association.

CHERYL HEALTON, PH.D.

American Legacy Foundation

Dr. Cheryl Healton is the American Legacy Foundation's first president and chief executive officer. Dr. Healton is a researcher, professor, and public health administrator with more than 20 years experience in public health. She has served on an array of national, state, and local committees and task forces for public health and policy issues including HIV/AIDS, violence, substance abuse and promotion of women's health. Dr. Healton has extensive experience in tobacco control issues. She developed a program to study the effects of tobacco marketing and counter-marketing on youth tobacco use for the U.S. Centers for Disease Control and Prevention. Dr. Healton also developed a series of prevention partnerships linking public health researchers with New York State tobacco health policymakers, and she has evaluated intervention programs for the state's largest youth tobacco prevention program.

Dr. Healton joined Legacy from Columbia University's Joseph L. Mailman School of Public Health in New York where she served as Head of the Division of Sociomedical Sciences and Associate Dean for Program Development. She founded and directed the school's Center for Applied Public Health, conceptualizing and implementing applied research in emerging issues in public health. Prior to serving as Associate Dean at the School of Public Health, Dr. Healton was Associate Dean of Columbia's College of Physicians and Surgeons. In this capacity she was responsible for a wide range of health related programs. Dr. Healton holds a doctorate from Columbia University's School of Public Health and a master's degree in Public Administration at New York University for health policy and planning.

ROSEMARIE HENSON, M.S.S.W., M.P.H.

*Director—Office on Smoking and Health, Centers for Disease C*ontrol and Prevention

Rosemarie Henson is the Director of CDC's Office on Smoking and Health, the federal government's leading organization for tobacco control. As Director, she is responsible for leading and coordinating strategic efforts and programs aimed at eliminating tobacco use as a major public health problem. Ms. Henson has played a leadership role in developing policies and programs to implement cancer control initiatives, promote women's health, and prevent HIV/AIDS. Prior to being named the Director of the Office on Smoking and Health, she served as the Deputy Director of the National Center for Environmental Health. While serving in this position, Ms. Henson dealt with a broad range of public health issues, including asthma, tobacco research, environmental health tracking, genetics, and biomonitoring. From 1992 to 2000, she was the Branch Chief for Program Services in CDC's Division of Cancer Prevention and Control. Ms. Henson was responsible for the significant growth of the National Breast and Cervical Cancer Early Detection Program that supports screening for millions of underserved women nationwide. Ms. Henson came to CDC from the Massachusetts Department of Health where she was instrumental in developing strategic partnerships with community-based organizations and implementing innovative programs in the areas of child health, chronic disease prevention, environmental health, and HIV/AIDS.

Ms. Henson received the Secretary of Health and Human Services Senior Management award, as well as several awards from national organizations, recognizing her leadership and work in breast and cervical cancer control. She has served on several agency committees and boards, including the American Cancer Society's National Task Force for Cancer in the Poor and Underserved, the World Education Board, and the Cambridge City Health Policy Board. Ms. Henson holds a Masters Degree in Public Health and a Masters of Science in Social Work from Columbia University.

JILL M. SIEGFRIED, PH.D.

University of Pittsburgh

Jill M. Siegfried, Ph.D. is Professor and Vice-Chairman of Pharmacology at the University of Pittsburgh and Co-Director of the Lung Cancer Program at the University of Pittsburgh Cancer Institute. Her research interests include growth factors and their receptors, risk factors for lung cancer, and novel therapeutic approaches to lung cancer. She is the recipient of several grants from the National Cancer Institute in the area of biology and therapy of lung cancer, including a Specialized Program of Research Excellence (SPORE) in Lung Cancer. She was the recipient of the 15th Annual Alton Ochsner Award Relating Smoking and Health in 2000 in recognition of her efforts to identify gender-specific pathways in lung carcinogenesis.

Dr. Siegried graduated with a doctorate in Pharmacology in 1981 from Yale University and received postdoctoral training at the Lineberger Cancer Program at the University of North Carolina. She joined the University of Pittsburgh in 1988, after a six-year tenure at the U.S. Environmental Protection Agency's Environmental Research Center in Research Triangle Park, N.C. Dr. Siegfried has been the vice-chair of the Department of Pharmacology at the University of Pittsburgh since 1994. Dr. Siegfried has served as a grant reviewer for the NIH, the ACS and the Department of Defense, as well as contributing to the TRDRP research program by serving as a member of the Cancer study section in 2000 and 2001.

SHERI WATSON HYDE

National African American Tobacco Prevention Network

Sherri Watson Hyde currently serves as the Executive Director of the National African American Tobacco Prevention Network (NAATPN). She is also one of the founding members of the Network which has existed for the past 3 years. NAATPN provides technical support to African Americans interested in reducing tobacco use within the African American Community and is dedicated to facilitating the development and implementation of comprehensive and community competent tobacco prevention and control initiatives to benefit people of African descent.

Ms. Watson Hyde came to NAATPN from the American Medical Association's SmokeLess States National Tobacco Control Policy Initiative, where she was a Regional Grant Liaison Officer in the southeast. She has also served as National Tobacco Control Field Manager and Consultant for the American Heart Association, Office of Public Advocacy in Washington, DC; Director of Cultural Diversity Programs for the American Lung Association National Office; Federal Advocacy Staff for the American Lung Association Government Relations Division; and Health and Judiciary Legislative Aide to Former U.S. House of Representatives' Majority Whip, David E. Bonior, and U.S. Representative David E. Price of North Carolina (who is still serving in the US House of Representatives). Ms. Watson Hyde is also a member of the American Legacy Foundation's African American Steering Committee for Priority Populations, the American Public Health Association and numerous other organizations. Ms. Watson Hyde is a graduate of the University of North Carolina at Chapel Hill, and has done graduate work at the University of Massachusetts in Amherst.

EXHIBITORS

American Cancer Society

American Indian Tobacco Education Network

American Legacy Foundation

American Lung Association

California Black Health Network

California Breast Cancer Research Program

Community-Campus Partnership For Health

Hispanic-Latino Tobacco Education Network

Next Generation California Tobacco Control Alliance

San Francisco Tobacco Free Coalition

The Ride Project-Public Health Institute

Tobacco-Related Disease Research Program

University Of California, San Francisco

Universitywide AIDS Research Program

POSTER PRESENTATIONS

WI-46
T47-97

W1

Effect of nicotine on bone marrow cells

Khaldoyanidi, Sophia
La Jolla Institute for Molecular Medicine

Proliferation, differentiation and self-renewal of hematopoietic stem/progenitor cells (HSPC) are complex and highly regulated process, which are controlled by a number of soluble factors, adhesion molecules, as well as by the extracellular matrix (ECM). Some pathophysiological factors, including cigarette smoke and its byproducts, could interfere with normal hematopoiesis leading to an imbalance in the production of mature blood cells. Most of the adverse effects of cigarette smoke have been associated with nicotine. We have previously demonstrated that nicotine affects the production of non-adherent cells and formation of hematopoietic foci in LTBMC. We further investigated whether nicotine can influence the ability of stromal cells of the hematopoietic microenvironment to maintain HSPC and to support their proliferation. Our results indicated that exposure of the bone marrow-derived hematopoiesis-supportive stromal cell line S17 to physiological concentrations of nicotine (10^{-5} M to 10^{-8} M) during co-culture with freshly isolated bone marrow cells results in a significantly decreased number of recovered HSPCs. To examine the molecular mechanisms mediating the inhibitory effect of nicotine on the hematopoiesis-supportive function of S17 stromal cells we performed a gene expression array. We found that nicotine induces differential expression of at least 12 genes that can be potentially involved in regulation of HSPC proliferation and self-renewal. We also observed that trafficking of HSPC from the bone marrow to the periphery and back was significantly affected in mice exposed to nicotine. This further suggests that nicotine influences HSPC-endothelial cell interactions. Therefore, we next examined the effect of nicotine on HSPC rolling and adhesion under conditions of physiological shear force. While the number of rolling HSPC was not affected, nicotine significantly facilitated the adhesion of HSPC to the bone marrow-derived endothelial cells. This in turn may lead to arrest of circulating HSPC on the vasculature and inhibition of HSPC mobilization and homing. Overall, our studies provide us with a better understanding of cellular and molecular mechanisms involved in nicotine–induced hematopoietic stem cell dysfunction and progression of tobacco associated diseases.

W2

The effect of nicotine on the protease-induced apoptosis in *Porphyromonas gingivalis* W83.

Fletcher, Hansel M.
Loma Linda University

Porphyromonas gingivalis is an important etiological agent in adult human periodontitis and is associated with cardiovascular diseases. Cigarette smoking has been found to significantly increase the risk for both periodontitis and cardiovascular diseases. Our overall objective is to elucidate the mechanism(s) by which smoking contributes to the pathogenesis of *P. gingivalis* associated diseases by characterizing the potential impact of smoking on the virulence of *P. gingivalis*.

Proteases produced by *P. gingivalis* are widely accepted as an important virulence factor. To explore the effects of these proteases on endothelial cells and assess a role for smoking metabolites in this process, we exposed bovine coronary artery endothelial cells (BCAEC) to protease active extracellular protein preparations from isogenic mutants of *P. gingivalis* in the pres`ence and absence of nicotine. BCAEC treated with protease active extracellular protein preparations exhibited a rapid loss of cell adhesion properties that was followed by high levels (>50%) of apoptotic cell death. This effect was correlated with the different levels of cysteine-dependent proteolytic activity of the isogenic mutants tested. Furthermore, gingipain RgpB appears to be the most significant in this process. In the presence of a non-cytotoxic concentration of nicotine, there was a significant increase of protease-induced apoptosis of BCAEC indicating that nicotine enhanced cell death. We tested the possibility that proteases from *P. gingivalis* induced loss of cell adhesion properties via proteolysis of BCAEC proteins involved in cell adhesion. Cleavage of N-cadherin, a cell surface protein involved in cell adhesion and signal transduction, was observed in immunoblots of lysates from detached cells. There was a direct correlation between the kinetics of N-cadherin cleavage and cell detachment. Immunoprecipitated N-cadherin molecules from cell lysates were also degraded by protease active extracellular protein preparations from isogenic mutants of *P. gingivalis*, suggesting a bacterial protease(s) capable of cleaving these endothelial cell-cell adhesion proteins. Loss of cell adhesion and N-cadherin cleavage could be inhibited by preincubation of *P. gingivalis* extracellular protease preparations with the cysteine protease inhibitor TLCK.

Taken together, these results indicate that nicotine can modulate *P. gingivalis* protease-induced apoptosis. Further studies to understand the mechanism(s) by which smoking metabolites modulate *P. gingivalis*-induced cell death, will facilitate the design of an appropriate intervention strategy to aid in the prevention and/or reversal of the negative impact of smoking on periodontitis or cardiovascular diseases.

W3

Effects of tobacco smoke on airway bactericidal activity

Di, Yuan-Pu P.
University of California, Davis

Tobacco smoke (TS) is known to induce pulmonary diseases such as emphysema and lung cancer and has effects on the host defense mechanism against pathogens, but the molecular mechanisms by which this occurs is not completely understood. In this research project, we are trying to elucidate how TS affects on the airway antibacterial activity and the related lung diseases. We have recently identified and characterized a novel secretory protein in upper respiratory tract, *spurt,* which displayed antibacterial activity and airway tissue specificity in our preliminary studies. The secretion of *spurt* was elevated in sputum samples obtained from patients with COPD compared with normal subjects. In addition, *spurt* homologue (*spurta*, alternatively spliced form of *spurt*) has also been shown to be secreted at higher level in smokers' nasal lavage fluids (NLF) than non-smokers.' To elucidate the regulatory mechanism of *spurt* by TS exposure, we performed nuclear run-on studies and results suggested TS induced *spurt* gene regulation is regulated at the transcriptional level. We then used transient transfection studies using *spurt* promoter construct in luciferase vector and to examine promoter activities changes before and after TS exposure. Our results indicated that promoter activities of *spurt* gene are higher after TS exposures than controls. Furthermore, we also examined the signaling events after TS exposure and confirmed the involvement of epidermal growth factor receptor (EGFR) and mitogen-activated protein kinases (MAPKs) signaling pathways in smoke induced *spurt* promoter activities. Our research is relevant to lung infectious diseases. Results from the first year's TRDRP funding support provide a possible regulatory mechanism of how TS exposure affects on airway antibacterial function by regulating the gene expression and the protein secretion of bactericidal proteins.

W4

Mild carbon monoxide exposure impairs the developing auditory system of the rat.

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Carbon monoxide (CO) is one of the most common of the active compounds in tobacco smoke. It is produced and accumulates as the result of incomplete combustion, from burning tobacco, vehicle exhaust, and other heat producing systems. Inhaled CO binds to hemoglobin in red blood cells and decreases their oxygen carrying capacity. CO is also known to block respiration within cells by inhibiting cytochrome oxidase. A small concentration of CO is produced naturally by mammals and is potentially involved in signal events in nerve cells. The important period for the brain growth spurt and onset of myelin formation takes place after birth and over the milk-feeding period of the rat pup. Within this period of growth the brain undergoes profound cell division and the development of an intricate multi-cellular organization. The consequences of mild exposure to CO during this critical period are not known.

The objective of our study was to determine if chronic exposure to mild concentrations of CO caused changes in auditory function during development. We exposed rat pups chronically to CO concentrations, 12 to 50 ppm in air starting at day 8, through 22 days of age. By using gastrostomy-reared rat pups we were able to compare mother-reared pups with the gastrostomy-reared pups, with or without CO exposure. The CO exposed animals had reduced amplitudes of the eighth cranial nerve's action potential upon examination of the auditory brain-stem response. We examined the central auditory regions for basal neuronal activity, using c-Fos immunoreactivity as a marker. In the central nucleus of the inferior colliculus (CIC), the basal c-Fos immunoreactive cells were significantly decreased in the CO exposed animals when compared to controls. There was, however, no difference in the number of c-Fos expressing cells in the external nucleus of the inferior colliculus in exposed and control animals. Analysis of the cochlea from CO exposed animals at 25 ppm revealed neurofilament expression was decreased in the neurons of the spiral ganglion. In addition, histochemical analysis revealed reductions of NADPH diaphorase, cytochrome oxidase and calcium ATPase. No changes were observed in the morphology of the neurons in the spiral ganglion and in the inner and outer hair cells. Synaptophysin immunoreactivity was also normal in the hair cells. We conclude that several critical components of the auditory pathway (e.g. cochlea and CIC) are selectively affected by mild CO exposure during development. The deficits in the CIC appear to be permanent as they persist into adulthood.

Our studies may be related to the auditory development of humans where mild CO concentrations occur in air. Carbon monoxide can be transported across the placental barrier, and exposure *in utero* constitutes a special risk to the fetus. Infants and young children are generally believed to be more susceptible to carbon monoxide than adults. Several areas in California have increased levels of carbon monoxide in ambient air over the 9 ppm established as the EPA's National Ambient Air Quality Standard (1997).

W5

Smoking-induced oxidant stress and platelet Integrin $\alpha_{IIb}\beta_3$

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Integrin $\alpha_{IIb}\beta_3$ is the platelet fibrinogen receptor that mediates platelet adhesion and aggregation. This receptor has a key role in hemostasis and thrombosis. Because cigarette smokers have a dramatically increased risk of vascular disease, this study tests the hypothesis that cigarette smoke directly alters the function of platelet integrin $\alpha_{IIb}\beta_3$.

The principle mode of regulation of $\alpha_{IIb}\beta_3$ is an on/off switch, the switch from "off" to "on" being commonly referred to as activation. The precise mechanism by which $\alpha_{IIb}\beta_3$, or integrins in general, are activated and deactivated is still not completely understood. The on/off switch is known to have physiologic importance because $\alpha_{IIb}\beta_3$ is maintained in the "off" state on circulating platelets, but is turned on when platelets encounter agonists such as ADP or thrombin. This activation enables $\alpha_{IIb}\beta_3$ to bind fibrinogen, leading to platelet aggregation that halts the loss of blood.

Our work with $\alpha_{IIb}\beta_3$, suggests that the activation of integrin $\alpha_{IIb}\beta_3$ is controlled by a "redox site" within the integrin. We believe that this site is comprised of several unpaired cysteine residues which rearrange during integrin activation. These observations led us to the hypothesis that the redox site within αIIbβ_3 is directly modified by constituents of cigarette smoke, and that this perturbation leads to widespread dysregulation of platelet adhesion and aggregation.

The initial objective of our study is to localize the redox site in $\alpha_{IIb}\beta_3$. Based on disulfide mapping experiments, it has generally been assumed that all fifty-six highly conserved cysteine residues of the integrin β subunits are paired. Yet, here we show that $\alpha_{IIb}\beta_3$ contains multiple free cysteine residues. We show that modification of the free cysteine residues of the redox site by a sulfhydryl-reactive reagent prevents the interconversion between resting and active integrin. We demonstrate that $\alpha_{IIb}\beta_3$ can be activated by the reducing agent DTT, and that the active form of the integrin can be de-activated by an oxidizing environment. Using mass spectrometry, we have localized the majority of free sulfhydryl residues to the cysteine-rich domain of the β subunit. Future work will reveal the precise location of all of the cysteines that comprise the redox site and will determine which of these are altered by the constituents of cigarette smoke. Such information will lead to a better understanding of how cigarette smoke precipitates vascular disease, and may point to methods of protecting the integrin redox site from cigarette smoke.

W6

Isoform-specific effects of apolipoprotein E on fatty acid metabolism

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Atherosclerosis, stroke, and heart disease are a primary cause of mortality and morbidity in western countries. Smoking is one of the most important risk factors. Even though the etiology of these diseases is multifactorial, it is known that the mechanism involves changes both in the blood vessel walls and in the metabolism of the lipoproteins that carry lipids in the blood. Since apolipoprotein (apo) E assists in the processing of lipoproteins and in clearing of lipids from the blood, it is in a prominent position to affect the development of these diseases. In humans, apoE exists in three isoforms (apoE2, apoE3 and apoE4), which produce different effects on lipid metabolism. Compared with the most common apoE form (apoE3), apoE2 is associated with a decrease in the risk of atherosclerosis, whereas apoE4 increases the risk. The exact mechanisms for these effects are not known. We have found that apoE may play a role in lipid metabolism independent of its well-characterized effects on the metabolism of lipoproteins. ApoE appears to interact with free fatty acids (FFA) and to alter the uptake of FFA by cells in an isoform-specific manner. The goals of this project are (1) to characterize the physical interaction of apoE with FFAs, (2) to determine the biological mechanism for the isoform-specific effects of apoE on the uptake of FFAs by cells *in vitro*, and (3) to determine if apoE exerts isoform-specific effects on the metabolism of FFAs *in vivo*. First, using a turbidimetric assay, we found that apoE interacts with FFA emulsions *in vitro*. ApoE added to FFA emulsions rapidly reduces the turbidity of the solution. Interestingly apoE4 does so more effectively than apoE2 or apoE3. Second, we discovered, using radiolabeled FFA, that the apoE isoforms have different effects on cellular FFA uptake. The uptake was greatest with apoE2, intermediate with apoE3, and lowest with apoE4. This finding was confirmed by measuring the effect of the apoE isoforms on the uptake of linoleic acid (an essential FFA), when added to the cellular medium. In addition, when the linoleic acid content of the cells was measured by gas-liquid chromatography, we found that the uptake is increased with apoE2, significantly decreased with apoE4, and unchanged with apoE3. Unexpectedly, the apoE isoforms also have the same effect on the total cellular lipid mass, suggesting that apoE alters the overall lipid profile rather than eliciting an effect on a particular FFA. Third, we are currently optimizing methods for separating and quantitating the different lipid classes in animal tissues (particularly in the brain), in preparation for examining the effects of apoE on the uptake of FFA by tissues, *in vivo*. The overall goal of this project is to identify the mechanism by which the three isoforms exert different effects on the uptake of FFA by cells and to determine if these effects on FFA metabolism are related to the differential effects of the apoE isoforms on the development of vascular diseases.

W7

Pathways of catabolism of oxidized phospholipids

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Cigarette smoke increases the risk of cardiovascular disease, the leading cause of death in Western industrialized nations. Free radicals, which are found in cigarette smoke, can initiate oxidation and structural alteration of the unsaturated fatty acid-containing lipids of low-density lipoproteins (LDL). One of the first steps leading to atherosclerosis is the oxidation of LDL. Oxidized LDL (OxLDL) binds to macrophage scavenger receptors and is internalized giving rise to foam cells and eventually fatty streaks.

During the oxidation of LDL, phospholipids are oxidized yielding reactive species that can form covalent adducts with apolipoprotein B-100 (apoB), a large number of breakdown products are also generated. Both the modified protein and oxidized lipid fractions are recognized by macrophage scavenger receptors. However, little is known about the metabolism of these particles.

In order to more fully understand this process I have synthesized, purified and characterized a variety of oxidized phospholipids and phospholipid-peptide adducts. One of the established pathways of uptake of OxLDL is via the CD36 scavenger receptors. In a collaborative study, we have expressed this receptor in COS-7 cells and tested in competition studies the ability of various synthetic phospholipids to compete for the receptor binding against OxLDL. These experiments have yielded the putative structure of the epitope that is being recognized by the CD36 scavenger receptors. So far these experiments indicate the necessity of a particular presentation of the phosphorylcholine residue on the phospholipid.

I have also tested possible pathways for degradation of the synthetic oxidized phospholipids. The oxidized phospholipids were presented to various phospholipases and the products were tested for scavenger receptor recognition. Cytosolic and secreted phospholipase A_2 enzymes as well as bacterial phospholipase C and D were applied to these substrates. The effects of these phospholipids on the enzymes were also tested. The aldehyde containing oxidized phospholipids reacted with the enzymes and inhibited their respective activities. The phospholipid-peptide (non reactive) adducts were not recognized by the phospholipase A_2s but were modified by the phospholipase D. These results along with the results from the CD36 studies have lead to experiments where the structure of the OxLDL will be altered by the active phospholipases and consequent recognition by CD36 will be tested.

Using P388D$_1$ macrophage-like cells, which express the SRA1 and SRA2 scavenger receptors, I tested the ability of different synthetic oxidized phospholipids to associate with these cells. Preliminary experiments show only oxidized phosphatidylcholine-peptide adducts associated with these cells, even after extensive washes. Further experiments include determining the cause of the association between the oxidized phospholipids and the cells, i.e., whether it is an uptake mediated through a scavenger receptor pathway or nonspecific binding.

This work is relevant to understanding of the recognition, uptake and catabolism of the various products of oxidation by scavenger receptors – a pathway that leads to atherosclerosis.

W8

A mouse model for isoform-specific, apolipoprotein E-mediated atherosclerosis development and regression

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Atherosclerosis results from high levels of lipids in plasma, including low density lipoproteins (LDL) and remnant lipoproteins. Cigarette smoking contributes to atherosclerosis by raising plasma levels of remnant lipoproteins and by oxidizing plasma lipoproteins. Oxidized remnant lipoproteins and oxidized LDL are both potent promoters of atherosclerosis.

Apolipoprotein (apo) E is a critical mediator of plasma lipoprotein metabolism. One of the three common isoforms of this protein, apoE4, is associated with increased LDL levels and premature atherosclerosis, further increasing the risk of tobacco-related disease. How apoE4 raises LDL levels and predisposes to atherosclerosis is not fully understood.

To address these issues, we created a novel mouse model to study the isoform specific roles of apoE in atherosclerosis development and regression. Hypomorphic apolipoprotein E (hypoE) mice express reduced levels of an apoE4-like mouse mutant apoE referred to as Arg-61 apoE, in all tissues because of the presence of a neomycin (*neo*) cassette flanked by *LoxP* sites in *Apoe* intron 3. HypoE mice have reduced plasma apoE levels (2–5 % of wildtype) but display a near normal lipoprotein profile and do not develop atherosclerosis on a normal chow diet. HypoE mice crossed low density lipoprotein receptor (LDLR) mice, or hypoE/*Ldr*$^{-/-}$ mice, accumulate apoB100 and apoB48-containing remnant lipoproteins and display plasma cholesterol levels of 700 +/- 50 mg/dl when fed a normal chow diet. Unlike Arg-61 hypoE or *Ldr*$^{-/-}$ single mutant mice, but like *Apoe*$^{-/-}$/*Ldlr*$^{-/-}$ double mutant mice, Arg-61 hypoE/*Ldr*$^{-/-}$ mice spontaneously develop atherosclerosis when maintained on a normal chow diet. Assessment of atherosclerosis in the abdominal and innominate arteries revealed extensive atherosclerosis by 6 months of age. Histological analysis of atherosclerosis in the aortic sinus revealed complex lesions containing cholesterol crystals and cellular infiltrates. Spontaneous atherosclerosis in hypoE/*Ldr*$^{-/-}$ mice eliminates the need for feeding atherogenic diets containing cholic acid; a bile acid known to promote inflammatory immune responses which likely impact on atherosclerosis development. Cre-mediated excision of the *neo* cassette in hypoE/*Ldr*$^{-/-}$ mice crossed to Mx1-Cre transgenic mice permanently restores apoE expression in hepatocytes and in macrophages within 10 days of induction. This rapidly reverses hypercholesterolemia in these mice and leads to a lipoprotein cholesterol profile identical to *Ldr*$^{-/-}$ mice. Moreover, like *Apoe*$^{-/-}$/*Ldlr*$^{-/-}$ double mutant mice, the atherogenic lipoprotein profile in hypoE/*Ldr*$^{-/-}$ mice resembles the one seen in humans; derived form elevated plasma levels of both apoB100 and apoB48-containing remnant lipoproteins. We propose both Arg-61 and the recently derived wildtype hypoE mice crossed to *Ldlr*$^{-/-}$ Mx1-Cre transgenic mice, as models for apoE4 and apoE3 in atherosclerosis development and regression.

W9

Comprehensive evaluation of the ischemic leg

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The goal of this research project is to develop a set of magnetic resonance (MR) methods for the evaluation of vascular disease in the lower limb. Such disease represents one of the major tobacco-use-related health problems. Our set of MR methods includes those to assess 1) lower extremity vessel anatomy, 2) total blood flow, and 3) regional perfusion.

With MR, three-dimensional (3D) anatomic vessel imaging can be performed without injection of contrast agents. We are investigating a new MR vessel imaging method based on a so-called steady-state free precession (SSFP) sequence. With SSFP, blood appears bright compared to surrounding muscle owing to blood's unique relaxation parameters. SSFP also leads to a unique signal phase from surrounding fat, a property that can be exploited to suppress its otherwise bright signal.

Total blood flow in the leg can be measured with MR using either a phase-contrast (PC) method or a Fourier-velocity encoding (FVE) method. We are studying fast versions of these methods that do not require synchronizing the acquisition to the cardiac cycle, even in the presence of pulsatile flow. Both methods return an estimate of the flow averaged over the cardiac cycle. Our validation experiments on phantoms and human have demonstrated the accuracy and repeatability of these methods. FVE involves a longer scan time than PC but offers better ability to measure slower flow rates.

Regional perfusion assessment based on first-pass imaging of injected contrast material is the third area of study. For first-pass imaging in the lower leg, volumetric coverage with adequate time resolution is desirable. We have developed a fast 3D imaging method that is able to image 40 slices in a volume with 2.5 mm spatial resolution and 2.8 second time resolution. Our initial human studies, based on a cuff release in the leg, have shown the ability of the fast imaging method to track the signal enhancement indicative of a hyperemic response.

For assessing tobacco-related vessel disease in the lower leg, these MR methods are targeted to provide clinically useful information which, collectively, are not available with any other imaging modality. Much of our work has focused on new rapid imaging methods. These improvements in imaging speed will be important for application of these methods in a clinical environment. We plan to continue our validation studies and move to initial patient studies.

W10

Evaluation of the efficacy of LVRS in short term vs. longer-term LVRS animal models

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Introduction: Lung Volume Reduction Surgery (LVRS) has been evaluated for treatment of severe emphysema. Animal models are needed to investigate questions involving LVRS, including optimal surgical techniques and physiological mechanisms of response. Purpose: The purpose of this study is to assess whether short-term response is different from longer-term LVRS response in a rabbit model of moderate emphysema.

Methods: Emphysema was induced in 21 rabbits using 4 nebulizations of 11,000 units of porcine elastase (weeks 1,2,4,6), followed by LVRS at week 7. Animals were divided into 2 groups: (*1*) Short-term follow-up pulmonary function tests (PFT)'s at weeks 1, 7 (LVRS), and sacrifice week 8 and (*2*) Longer-term emphysema follow-up PFT's at weeks 1, 7 (LVRS), 8, and 3 month sacrifice. Static pulmonary compliance curves were obtained prior to emphysema induction (baseline), prior to LVRS (pre-SX) and prior to sacrifice (post-SX).

Results: Statistical analysis of short-term vs. longer-term LVRS rabbits showed no significant difference in compliance measurements between longer and short term treatment groups at baseline, emphysema, or post LVRS ($p=0.815$).

Conclusions: There is no apparent difference between short-term and long-term treatment of emphysema following LVRS in this animal model. Therefore, it may be possible to predict longer-term outcomes with relatively brief follow-up studies in this LVRS emphysema animal model.

W11

Mainstream and sidestream whole smokes affect primary human fibroblast proliferation and migration in a connective tissue-like culture

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The process of wound healing is composed of 3 stages: Inflammation, granulation tissue formation, and matrix remodeling. There is much clinical evidence to support the detrimental effect of tobacco smoke on wound repair during inflammation, but not much is known about its effects on the other stages of wound healing. Fibroblasts are important players at this stage during wound repair, they proliferate, migrate, and deposit extracellular matrix molecules (ECM) that contribute to wound contraction. If any of these processes is disturbed, wound repair will not proceed normally.

In this study, we investigate the effects of MSW ("first-hand smoke") and SSW (the main component of "second-hand smoke") on the development of granulation tissue in a system that mimics human connective tissue. Primary human fibroblasts are plated in collagen gels and cultured for 5-7 days to allow them to proliferate, deposit ECM, and secrete cytokines, contributing in this manner to the development of "connective" tissue. At the end of this period, the cultures are treated for 5 days, 6hrs/day, with the smoke solutions. We compared adult and fetal fibroblasts for their responses to the smokes in this system.

Our results indicate that both MSW and SSW adversely affect proliferation and migration of both fibroblasts. The lack of proliferation in adult fibroblasts is independent of an increase in expression in p21 (cell cycle inhibitor), whereas in fetal fibroblasts is p21 dependent. We also found that both types of smoke induce alpha smooth muscle actin expression, a protein characteristic of myofibroblasts which are cells that are important in wound contraction and closure. These findings may have important implications not just in wound repair but also in development. For example, babies that are born to smokers are observed to be either premature or weigh less than babies born to nonsmokers. We are currently investigating the molecular mechanisms underlying cigarette smoke inhibition of cell migration and proliferation and its effect on cell contraction.

W12

Environmental tobacco smoke exposure causes neuroplastic changes in NTS second-order neurons in lung afferent pathways in young guinea pigs

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Children exposed to ETS have increased respiratory symptoms, such as cough, mucus secretion, airway obstruction, and decreased pulmonary function. Previous studies have determined that ETS and mainstream smoke can increase the excitability of lung afferent fibers involved in defensive lung-CNS reflexes. What is less known is whether the changes in the pattern or frequency of lung afferent signals may cause plasticity in the lung-CNS reflex networks that could contribute to the symptoms. We tested the hypothesis that exposure to ETS in developing guinea pigs caused neuroplastic changes in second-order neurons in lung-CNS reflex pathways, specifically in the nucleus tractus solitarius (NTS), where lung sensory nerve input is first integrated. The extent to which the behavior of these NTS neurons is changed by ETS exposure will determine whether the lung-CNS reflexes are blunted, augmented, or remain unchanged. Young guinea pigs were chronically exposed to ETS or to filtered air (FA) for 5 weeks (from 1 to 6 wk of age). To identify NTS second-order neurons receiving synaptic input from lung afferent fibers, a fluorescent tracer (1,1'-dioctadecyl 3,3,3',3' tetramethylindocarbo-cyanine perchlorate [DiI]) was instilled in the airways through a tracheal cannula 2 weeks before experiments (at age of 4 wk) to allow for the anterograde transport to the central terminal boutons on the NTS neurons. Studies were performed on the labeled neurons in brainstems slices by using whole-cell patch clamp recordings to measure three indices of intrinsic neuronal excitability: 1) membrane potential (Vm), 2) membrane resistance (Rm), 3) spiking responses to injections of 2 sec depolarizing currents (40 -100pA) from a resting Vm of -50 mV. 167 neurons were recorded, 81 from FA-exposed and 86 from ETS-exposed animals. ETS exposure decreased the ability of the second-order lung afferent neurons to generate action potentials in response to depolarizing current injections, but did not have a profound effect on either Vm (-45.2±1.0 and -47.9±1.2 [mV]) or Rm (301 ±24 and 354±29 [M?], ETS and FA, respectively). The data suggest that ETS exposure may decrease the excitability of neurons in the lung-CNS reflex pathways, perhaps decreasing the ability of the reflexes to protect the lung against further injury from ETS.

W13

Prevalence of ETS exposure among adults with severe asthma

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Environmental tobacco smoke (ETS) exposure is widespread, affecting the majority of U.S. adults. Because it contains potent respiratory irritants, ETS is perceived as a potentially important aggravating factor for adults with asthma. Despite the importance of this question, existing data on the effect of ETS exposure on adults with asthma are surprisingly limited. In the current study, we evaluated the prevalence of ETS exposure in a cohort of 351 non-smoking adults with severe asthma who have been recently hospitalized. We used multiple techniques, including telephone survey-based and direct monitoring methods, to estimate the prevalence of ETS exposure in these adults with asthma. The survey-based method, which ascertains self-reported exposure in a variety of microenviroments, yielded the lowest overall prevalence of ETS exposure (15% during the past 7 days; 95% CI 12 to 19%). Direct ETS exposure monitoring revealed higher exposure estimates. Among 79 subjects with severe asthma who wore personal nicotine badge monitors for 7 days, the majority were exposed to ETS (89%; 79 to 95%). Using hair nicotine analysis, we estimated the prevalence of ETS exposure during a longer time period (i.e., 3 months) among 59 subjects. For each subject, we analyzed two hair segments to estimate ETS exposure during two time intervals: the previous month (first 1 cm of hair segment) and the preceding second / third months (2nd and 3rd cm hair segments). This method indicated that the majority of adults with severe asthma were exposed to ETS during the past 1 month (59%; 95% CI 46 to 72%) and past 3 months (75%; 95% CI 62 to 86%). In conclusion, the majority of adults with severe asthma are exposed to ETS. Direct exposure measurement techniques yield higher estimates of exposure than survey-based methods.

The majority of adults with severe asthma were exposed to ETS during the past 7-days, when direct ETS exposure measurement using a personal nicotine badge monitor.

W14

Cohort study of exposure to environmental tobacco smoke and risk of stroke

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The independent effect of exposure to environmental tobacco smoke (ETS, passive smoking) on the risk of stroke is not well established. We assessed the association of self-reported ETS exposure at home and outside the home with risk of ischemic stroke, transient ischemic attack and hemorrhagic stroke in adult men and women.

We performed a cohort study among 27,822 lifelong nonsmokers (10,522 men and 17,300 women) aged 30-85 years at enrollment. Self-reported ETS exposure (at home and outside the home, in hours/week) and stroke risk factors were collected at a health plan in San Francisco and Oakland between 1979 and 1985. Follow-up for hospitalizations and death was available through the end of 2000 (median=16 years).

In multivariate analysis adjusting for age, sex, race/ethnicity, educational attainment, marital status, hypertension, diabetes and serum total cholesterol, ETS exposure at home of 20 hours or more/week (in relation to ≤ 1 hour/week) was associated with a 1.46-fold (95% CI 1.12-1.91) increased ischemic stroke risk. No significant associations were found between ETS exposure outside the home and ischemic stroke or between exposure to ETS at home or out of home and the risk of transient ischemic attack or hemorrhagic stroke.

High-level ETS exposure at home was independently associated with increased risk of ischemic stroke among never-smokers.

W15

Evaluation of smoking room performance: survey results

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This study surveyed California workers in order to estimate the proportion of non-smoking workers employed in buildings with designated indoor smoking areas who are potentially exposed to environmental tobacco smoke (ETS) leaking from these areas. The survey was accomplished by including questions about indoor smoking areas at the workplace in the California Tobacco Surveys (CTS) and the California Adult Tobacco Survey (CATS), both funded by the California Department of Health Services. These ongoing surveys are statewide, cross-sectional surveys of smoking-related behavior. The survey results reported here are part of a larger research project whose primary objective is to investigate the effectiveness of the most important engineering design and operational parameters of smoking rooms affecting ETS exposures of occupants in adjoining workspaces.

Response data from the 1999 CTS and 2000 CATS were analyzed. Data about indoor smoking areas at the workplace were weighted to account for the probability of selection and adjusted to be representative of the California population for gender, race/ethnicity, age, education, and region. The effect of ordinances of local tobacco control jurisdictions on the operation of designated smoking rooms was also analyzed.

For 1999, we estimate that in California about 122,000 workers or 0.8% of the workforce potentially was exposed to ETS leaking from smoking rooms. This estimate is based on the 1999 CTS and the 1999 annual average employment for California. For the same year, we estimate that 265,000 employees or 1.7% of the work force worked in buildings that did not comply with the state's work place non-smoking statute (AB13).

Similarly for 2000, we estimate that approximately 100,000 workers (0.6% of the work force) were potentially exposed to ETS leaking from smoking rooms and 220,000 (1.4 % of the work force) were exposed to ETS from non-AB13-compliant smoking areas. This estimate is based on the 2000 CATS and the 2000 annual average employment for California. It is worth noting that the 2000 estimates are lower than the 1999 estimates, indicating a decline of smoking at the work place.

We also found that survey participants' reporting of smoking in various indoor settings was the same regardless of local ordinances. Indoor workers in work places with fewer than five employees were less likely to report that their workplace was smoke-free regardless of local tobacco control ordinances on the operation of smoking rooms.

Future activities include comparison of the above estimates to those derived from the 2001 and 2002 CATS as well as the 2002 CTS.

The results of this project may be used by policy makers to reduce unintentional exposure of non-smoking California workers to ETS and to obtain reliable estimates of non-compliance with AB 13.

W16

Persistent smoking in San Francisco bars: An ethnographic analysis

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Following passage of Assembly Bill 13, a ban on smoking in California worksites was extended to bars at the outset of 1998. In this study, we evaluated the effectiveness of the ban through several methods, including interviewing 60 bartenders, managers and patrons and sending pairs of observers to 120 randomly selected standalone bars in San Francisco. This poster focuses on the prevalence of smoking in these bars and an analysis of factors identified in the observations and interviews that were associated with defiance of the smokefree bar policy.

Face-to-face semi-structured interviews were conducted with 20 bartenders, 20 managers, and 20 patrons from the pool of randomly selected standalone bars. Pairs of trained observers were sent out on four separate occasions (on different days of the week and time periods) to randomly selected bars throughout 2002. The sample represents approximately one-third of the city's standalone bars. Following hour-long visits to each bar, the observers recorded their highly structured observations in and around the bars using handheld computers. They also wrote semi-structured narrative field notes to elaborate qualitatively what they had seen, with special attention to details of the bar environments and staff-patron interactions that were relevant to smoking behavior. The structured observations were downloaded to an Access database and the observation narratives and interview transcripts were compiled in ATLAS.ti, software facilitating text analysis.

Smoking in the bars was observed to varying extents across visits. In 62% of the bars, no smoking was ever witnessed, whereas in 24% of the bars, smoking was endemic. In the remaining 14%, smoking was rare or circumstantial (e.g., smoking in the doorway). Analysis of the structured and narrative observations revealed correlates of the bars in which smoking was endemic, including Asian and Irish ethnicity. The findings from this study should contribute to understandings of where and how outreach and enforcement may strengthen widespread adherence to the smokefree workplace policy in bars.

W17

Changes in population attitudes about where smoking should not be allowed: California vs. the rest of the US

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The decade-long California (CA) Tobacco Control Program is unique to the nation in its duration, emphasis and level of funding. Program emphasis is on changing social norms about smoking as a means to eventually discourage smoking and thus reduce the harmful effects of tobacco to the population. Attitudes about where smoking should not be allowed is one measure of population tolerance for smoking.

We used data from the 1992-92, 1995-96, and 1998-99 Tobacco Use Supplements to the Current Population Survey to examine changes in norms regarding where smoking should not be allowed in both CA and in the rest of the US. Venues queried were: restaurants, hospitals, work areas, bars/cocktail lounges, indoor sports venues, and indoor shopping malls. There were 228,308, 186,713 and 175,900 TUS self-respondents for each survey period, with 7-8% from CA.

For all but one venue (indoor shopping malls), the percentages for the rest of the US were lower in 1998-99 than in CA in 1992-93. The percentage of CA respondents saying smoking should not be allowed at all in 4 or more of the 6 venues increased from 58.5% in 1992-93 to 75.8% in 1998-99, compared to an increase from 46.5% to 57.2% in the rest of the US. Despite the initially higher levels in CA, the percentage change in was 29.6% for CA and 23.1% for the rest of the US. The most dramatic increases in the 4+ venue measure in CA occurred among smokers, from 30.0% in 1992-93 to 57.9% in 1998-99, mainly because never and former smokers already exhibited high levels by 1992-93 (68.4% and 57.5%, respectively). Another interesting finding was that, except for bars, younger persons (18-30 years), particularly in CA, showed greater increases in norm levels than older age groups. Further, while educational differences were apparent in 1992-93 they had disappeared by 1998-99 in CA but not the rest of the US. Having smokefree workplaces and homes was associated with the 4+ venue measure in both CA and the rest of the US.

We conclude that a strong, comprehensive tobacco control program such as CA's can drive population adoption of anti-tobacco norms. Since norm levels in CA in 1992-93 were on par with those in the rest of the US in 1998-99, effective tobacco control programs in other statues should be able to achieve the same attitude shifts seen in CA.

W18

Effect of smoking restrictions on smoking behavior in a cohort of young adults first interviewed as adolescents.

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Besides protecting nonsmokers from second-hand smoke, research indicates that smokefree homes and workplaces benefit smokers by encouraging reduced cigarette consumption, and to a lesser extent, quitting. There is less information about the impact of smoking restrictions on the smoking uptake process, which generally is completed during young adulthood.

Adolescents 12-17 years of age (n= 5,531) who responded to the 1993 California Tobacco Survey were interviewed again in 1996 (n=3,376, funded by Robert Wood Johnson Foundation), and in 1999 as young adults 18-23 years of age (n=2,445, funded by Tobacco Related Disease Research Program). We determined whether respondents lived in a smokefree home in 1993 and 1999, whether they still lived in the same household in 1999 as in 1993, and whether there were other smokers in the household. In 1999, respondents were asked if they worked in a smokefree indoor setting. In 1999, smoking status was assessed, and for current smokers, we determined daily cigarette consumption.

Respondents never subject to home smoking bans were significantly more likely to be current smokers in 1999 (34.8%), compared to those who lived in smokefree homes one (23.6%) or more (20.0%) years. Further, smoking prevalence was somewhat higher, regardless of smokefree-home status, for those who lived in a different household in 1999 than in 1993. Among smokers, there was little difference in daily cigarette consumption whether or not respondents lived in smokefree homes. However, daily cigarette consumption was slightly higher for those living in the same household as in 1993, particularly if there were other smokers in the home. In 1999, smoking prevalence tended to be higher for non-student workers in jobs not covered by a smoking ban than for non-workers, students (regardless of employment status) or workers in a smokefree workplace. Having both a smokefree workplace and home was associated with current smoking status in 1999; prevalence for those not subject to either ban was 44.8%, compared to only 19.3% for those subject to both types of bans. A smokefree home appeared more associated with current smoking than a smokefree workplace. Among smokers, daily consumption was slightly higher (mean 8 cigarettes/day) for respondents without bans, compared to having one or both types of bans (similar consumption levels, mean 5 cigarettes/day).

In contrast to published studies of adults of all ages indicating that smoking restrictions effect the level of cigarette consumption more than they do smoking status, the results of the present analysis of young adults suggest that smoking restrictions are more related to being a current smoker than to level of consumption among current smokers. However, daily consumption is generally still low during young adulthood. Quitting, and the influence of other potentially confounding socio-economic factors remain to be investigated. These preliminary findings provide some evidence that smoking restrictions may influence the smoking uptake process.

W19

Why the recent drop in California adolescent smoking prevalence?

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Preventing adolescents from smoking is an important goal of the California Tobacco Control Program. Nationwide, adolescent smoking prevalence began to increase in the early 1990s, but held steady in California. Then, a sharp increase in California occurred between 1993 and 1996, followed by a marked decrease by 1999 to below 1993 levels. We investigated the prevalence of known influences on adolescent smoking and factors related to the Program than might explain this pattern.

Using cross-sectional data on adolescent never smokers aged 12-15 years from the population-based cross-sectional California Tobacco Surveys (CTS) of 1993(n=2,708), 1996(n=3,157) and 1999(n=3,287), we investigated changes in the prevalence of various known and potential Program influences on adolescent smoking. Also, we examined changes in transition rates (from never smoker to any smoking) in 2 longitudinal cohorts of these adolescents from the 1993 and 1996 CTS followed up 3 years later (1993-1996 cohort [n=1,764]) and 1996-1999 cohort [n=2,119]) to see if the influence variables predict consistently in both cohorts.

Cross-sectional results showed that the prevalence of several key influences on adolescent smoking (e.g., smoking by best friends, lack of perception of strong anti-smoking norms among peers, receptivity to tobacco advertising and promotions) peaked in 1996, which should have led to an increase in adolescent smoking thereafter. However, while several Program influences (e.g., perception of the ease of getting cigarettes, perceived compliance with school no-smoking policies, smoke-free homes) were relatively unchanged between 1993 and 1996, they showed major changes that might discourage smoking between 1996 and 1999. The longitudinal data indicated that the transition rate to any smoking was lower in the 1996-1999 cohort (32.1%) compared to the 1993-1996 cohort (38.3%). Further, transition was suppressed in the 1996-1999 cohort much more in groups at lower (without an influence) rather than at higher risk (with the influence) to smoke, regardless of the type of influence (known predictor or Program-related). Not include in the analysis was the $1.37/pack cigarette price in 1998.

These observations suggest that California's Tobacco Control Program may have its greatest impact on adolescents already at relatively low risk for smoking. How to impact higher risk adolescents remains a challenge. Longitudinal surveys are important evaluative tools.

W20

Patterns of tobacco use from adolescence to young adulthood

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The goal of our research project is to understand the natural history of smoking behavior. Using data from the RAND Adolescent/Young Adult Panel Study, we will describe who initiates cigarette use, becomes a regular smoker, stops smoking, and relapses over time. We will also identify key predictors of those transitions.

This abstract describes results from the first completed study, which examines predictors of the transition to regular smoking among initial experimenters during middle adolescence (grades 8-10; N = 2,496), late adolescence (grades 10-12; N = 2,149), and young adulthood (grade 12-age 23; N = 1,534). Understanding the factors that contribute to smoking escalation is important because committed smokers are at higher risk than experimenters for academic difficulties, alcohol and drug misuse, and delinquent behavior. They are also less likely to quit or revert back to occasional use. Thus, reducing the likelihood that adolescents will transition from experimentation to regular smoking is an important goal for prevention programs.

Participants were originally recruited from 30 schools chosen to represent a wide range of community types, socioeconomic statuses, and racial/ethnic compositions. Self-report surveys were completed in grades 7-10 and 12, and at age 23. Participants were eligible for this study if they had smoked, but less than weekly at the beginning of each transition period. They were considered to have made the transition to regular use at each follow-up if they reported weekly or more frequent smoking. Huberized regression techniques, which adjust for weighting and clustering of observations, were used to determine the independent associations of predictor variables with subsequent smoking status.

Nineteen percent of the grade 8 experimenters had become regular smokers by grade 10, 16% of the grade 10 experimenters did so by grade 12, and 17% of the grade 12 experimenters did so by age 23. During middle adolescence, the risk factors for the transition to regular smoking included being white, having pro-smoking attitudes, having friends who smoke, weak academic orientation, and weak parental support. During late adolescence, being African American was protective, while pro-smoking attitudes, drinking, coming from a non-intact nuclear family, and having weak parental support acted as risk factors. Risk factors for becoming a regular smoker by age 23 included being young for one's cohort and having pro-smoking attitudes at grade 12.

These findings suggest several strategies for prevention efforts aimed at curbing the escalation of smoking. Altering pro-smoking beliefs may be particularly important for both adolescent and young adult experimenters. For adolescents, breaking associations with peers who smoke, encouraging a stronger academic orientation, strengthening resistance self-efficacy beliefs, and teaching alternatives to smoking in dealing with stress may all be important components of smoking prevention programs. Prevention efforts aimed at entire cohorts of adolescents may be particularly effective in meeting these goals..

W21

Teacher recruitment and participation for a classroom-based 7th grade tobaccoprevention program

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The current study examines the process of recruiting, training, and supporting 7th grade science teachers to lead a classroom-based tobacco prevention program. A curriculum was developed to address health effects of second hand smoke, tobacco advertising, and policy. 3 components each containing 2 sessions were developed. Each component was designed to stand on its own and to be inserted into the regular science curriculum.

16 schools were invited to participate in the program, which offered a one-day teacher training and a complete set of classroom materials. Initially, researchers met with all 16 principals to explain the goals of the program and obtain permission to approach teachers. Permission was granted unanimously and teachers at all 16 schools received an informational packet. Researchers then met with teachers to describe the curriculum and answer questions. Registration forms were distributed and teachers were told that their participation was voluntary. The initial registration deadline passed without a single sign up. Teachers indicated by word of mouth that the primary obstacle to registration was the substitute teacher costs that would be incurred during the training period. A second invitation was sent out offering to cover this expense and provide teachers with a $250 honorarium. 14 teachers at 6 schools subsequently enrolled in the program.

At the end of each training, teachers were asked to select which components they would implement in their classrooms. Over 78% of teachers signed up to deliver all three components. Trained program experts observed each teacher twice and completed an evaluation form. Teachers were also encouraged to seek support and advice from program staff. After each session, teachers completed a session evaluation form. At the completion of the program, teachers completed a program evaluation form.

Process data was collected including likeability, ease of implementation, language appropriateness, conflicts with other school priorities, percentage of activities completed, and amount of time to complete each activity,. Overall, teachers liked the program, felt that activities were easy to implement, felt that language was too difficult for some English as second language (ESL) classes, felt that the amount of time spent delivering each activity conflicted with other school priorities such as standardized testing, completed at least 66% of the activities and took more time than expected to complete each activity. Results of this study indicate that teacher led programs are feasible but that efforts must be made to accommodate school needs. In particular, schools may be unwilling to expend the additional costs, may feel that prevention conflicts with academic goals and may have special needs populations that require tailored program materials.

W22

Can eating and exercise habits moderate the relationship between smoking status and emotional health?

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Adolescent smoking has been associated with negative affect, including depression. By contrast, high fruit and vegetable intake and regular physical activity have been associated with reduced risk of negative affect, including reduced risk of depression. We sought to examine in a large California adolescent database whether high fruit and vegetable intake might moderate the relationship between smoking and negative affect.

Sample: The sample consisted of approximately 360,000 7th, 9th, and 11th graders in California schools who completed the California Healthy Kids Survey during the period: 1998-2001. Primary ethnic affiliation of this sample was: 2% American Indian, 2% Pacific Islander, 11% Asian, 5% African American, 32% Latino, 38% White, and 10% Other. Girls comprised 53% and boys comprised 47% of the sample.

Measures: Three smoking outcome measures were assessed. Exposure to experimental smoking was assessed by: "During your life have you ever tried a cigarette, even one or two puffs?" Exposure to current smoking was assessed by: "During the past 30 days, on how many days did you use cigarettes?" Exposure to regular smoking was assessed by: " Have you ever smoked cigarettes regularly, that is, at least one cigarette every day for 30 days?"
The exercise question was: "On how many of the past 7 days did you exercise or do a physical activity for at least 20 minutes that made you sweat and breathe hard?" The fruit question was: "During the past 24 hours (yesterday), how many times did you eat fruit? (do not count juice)?" The vegetable question was: "During the past 24 hours (yesterday), how many times did you eat vegetables (including salad and nonfried potatoes)?" The mood state question was: "During the past 12 months, did you ever feel so sad and hopeless almost everyday for two weeks or more that you stopped doing some usual activities?

Results: Girls were more likely to report feeling sad and hopeless than boys (OR = .61, ± .03). All non-white ethnic groups were more likely to report feeling sad and hopeless than whites (OR's varied from 1.12 to 1.32). As expected, all smoking questions were related to the mood state question (OR's varied from 2.1 to 2.4), with stronger effects for current and regular smoking than for experimental smoking. All multivariate models predicting mood state included sex, primary ethnic affiliation and one of the smoking status measures. Adding the fruit, vegetable, or exercise measures consistently yielded small but significant coefficients, with increases in fruit and vegetable intake and increases in exercise associated with small reductions in risk of feeling sad and hopeless.

Conclusion: Fruit and vegetable intake and exercise habits may moderate the relationship commonly observed between smoking and risk of negative mood state. To the extent that other studies report that smoking is used to modulate negative affect, increased intake of vegetables and fruits and increased exercise may help to reduce the need for smoking. Prospective research on the impact of changes in diet and physical activity on smoking status is warranted.

W23

Perceived risks and benefits of smoking: Differences between adolescents who have and have not smoked

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Explanations of adolescent risk behavior typically make reference to adolescents' belief that they are invulnerable to harm. However, there has been little empirical examination concerning whether adolescents who have smoked differ from adolescents who have never smoked in their perceptions of smoking-related physical and social risks. Further, few studies have considered perceived benefits (including both physical and social benefits) in adolescents' behavioral decisions. The purpose of this study was to examine whether adolescents who have at least tried a few puffs of a cigarette differ from adolescents who have never smoked at all in their perceptions of: 1) smoking-related risks, 2) smoking-related benefits, 3) their ability to quit smoking, and 4) the effects of second-hand smoke.

Two hundred and twenty eight 9th graders (mean age 13.98, SD = .42; 57.1% females) completed a self-administered survey concerning their smoking experiences and perceived risks and benefits of smoking. Participants indicated the percent chance that they would look cool, be popular, look more grown-up, get lung cancer, get a bad cough, have trouble catching their breath, have a heart attack, and get wrinkles if they smoked. Participants also indicated the percent chance that non-smokers who are often in the presence of smokers would get a number of smoking-related negative outcomes.

Adolescents who have smoked at least a few puffs of a cigarette estimated their chance of experiencing a smoking-related negative outcome as less likely than did adolescents who have never smoked at all (mean percent differences ranged from 6% to 17%; t-values: 1.72 to 4.33, p's ≤ .05 to .000). Smokers also believed the effects of second-hand smoke were less than did non-smokers (t-values = 2.16, 3.5, p< .05). In addition, smokers reported being less likely to still be smoking in 5 years and more likely to be able to quit than did non-smokers (t=2.65, 2.92; p< .009, .004, respectively). In contrast, adolescents who have smoked perceived the chance of experiencing a smoking-related social benefit (e.g., looking cool, being more popular) as more likely than did non-smokers (mean differences between 6% to 9%; t-values: 1.74 to 1.89, p's ≤ .06 to .09). A similar pattern of results was found between adolescents who intend to smoke sometime in the next 6 months, and those who do not intend to smoke.

The findings indicate that adolescents who have smoked and intend on smoking perceive fewer risks and more benefits of smoking than do non-smokers and those without intentions to smoke. It is not clear from this cross-sectional data whether such perceptions motivate smoking behaviors or are reflective of their experiences. We are colleting longitudinal data to address this question. The data do suggest that rather than solely focusing on risks as a way to deter adolescent smoking, the role of perceived benefits in adolescents' smoking may be an additional critical focus for intervention. In addition, efforts should be made to increase adolescents' awareness of the addictive nature of cigarettes and the effects of second-hand smoke.

W24

Adolescent smoking: A unique correlate to aggressive behavior among classmates

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Problem behavior theory suggests that several diverse health risk behaviors, including aggression and smoking, may occur simultaneously among adolescents. Studies have shown associations among a constellation of health risk behaviors. However, few studies have examined the relationship between health risk behaviors after accounting for the effects of various peer influences.

The purpose of this study was to examine whether smoking was more prevalent among bullies, victims, and aggressive victims (e.g., those who are victimized and are also aggressive). Using an ethnically diverse sample of healthy 6th graders (n=1393; mean age= 11.3) in Southern California, this study used a self-administered survey to assess lifetime smoking, bullying behaviors, victimization, friendship networks, and demographic variables. Controlling for the effects of gender, ethnicity, friends' bullying behaviors, and popularity (e.g., friendship network variables), separate odds ratios for lifetime smoking were calculated for adolescents who were classified as bullies, victims, and aggressive victims.

Logistic regression results revealed that bullies (p< .0001) and aggressive victims (p< .05) had higher rates of lifetime smoking relative to victims and non-aggressive, non-victimized students. Such findings suggest that early experimentation with smoking, independent of peer behaviors and social status, may be an important marker of aggressive behaviors and other health risk behaviors. Conflict resolution strategies may effectively reduce adolescent smoking by developing healthy alternatives to aggression. Future longitudinal studies should determine common and unique determinants of various health risk behaviors among a diverse population of youth.

W25

Bonding to school, neighborhood, and parents among adolescent self-identified peer groups as a protective against smoking

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Theories of adolescent bonding predict that those who bond with normative groups will engage in normative behavior. On the other hand, those who bond with deviant or high risk groups will exhibit deviant and high risk behavior. Several studies have documented the association between being members of high risk peer groups and engaging in high risk behavior, for example smoking. It is therefore logical to assume that those who are members of high risk groups have not bonded with normative groups, and thus are at an increased risk for smoking and other such high risk behavior.

As part of a longitudinal study of the smoking behavior of adolescents in the greater Los Angeles area, this study sought to examine the relationship between parental, neighborhood, and school bonding and membership in various self-identified peer groups and how this relationship is associated with smoking behavior.

All three domains of bonding were found to be protective against smoking, with school bonding having the strongest relationship. There were also significant differences in the levels of parental, neighborhood and school bonding among the various self-identified groups. Parental bonding was lower among skaters, rockers, newly arrived immigrants, popular kids, gamers and gangsters, and higher among artistic kids, smart kids, and religious kids. Neighborhood bonding was lower among rockers, newly arrived immigrants, and gangsters, and higher among jocks, popular kids, and smart kids. Skaters, rockers, newly arrived immigrants, gamers, and gangsters all showed low school bonding, while artistic kids, and smart kids scored higher in school bonding. There were additional variations in smoking behavior among these groups as well. Skaters, rockers, newly arrived immigrants, and gangsters were at increased risk for smoking, while being an artistic kid, smart kid, or religious kid was protective. The relationship between bonding and smoking did not decrease when peer group membership was controlled for.

These results add to the literature on adolescent peer group affiliation as a risk or protective factor for smoking behavior. In particular, it replicates findings that peer group affiliation strongly associated with smoking, even when taking bonding into account. This indicates that bonding to school, neighborhood and parents, and peer group membership are independent factors which influence adolescent smoking.

W26

Parental job loss and adolescent smoking

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Previous studies of adolescents have identified associations between stressful life events and health risk behaviors such as smoking. Family-related stress and conflict also can increase the risk of smoking among adolescents. Most studies of stress and smoking have combined numerous stressful life events into general indices of life stress, obscuring the effects of individual stressors on adolescents' risk behaviors. Therefore, the impact of specific stressors on adolescent smoking is not known.

The recent economic recession has resulted in corporate downsizing and increases in unemployment rates. Consequently, many adolescents have experienced the stress associated with family members' unemployment. This study examined one particular stressful life event, a parent's job loss, as a predictor of adolescent smoking initiation over a one-year period.

As part of a longitudinal study of culturally relevant smoking prevention curricula in ethnically diverse Southern California schools, middle school students completed surveys in 6th grade and again in 7th grade. Of the 2168 students with complete data at both timepoints, 252 (12%) reported that an adult in their household had lost a job within the past 6 months.

Logistic regression analyses were conducted to assess the effect of parental job loss on smoking initiation among 6th grade never-smokers. Controlling for demographic, socioeconomic, and educational variables and parenting characteristics, never-smokers who reported parental job loss between 6th and 7th grade were more likely to have initiated smoking between 6th and 7th grade (Odds ratio = 1.68, 95% confidence interval = 1.08, 2.60).

Additional logistic regression analyses assessed the effect of parental job loss on past-month smoking among 6th grade ever-smokers. Parental job loss was not a risk factor for past-month smoking among students who already were ever-smokers in 6th grade.

Results indicate that the job loss of a parent or other adult in the household can increase the risk of smoking initiation among adolescents in the household. The high prevalence of job loss in the recent economic recession could lead to an increase in adolescent smoking unless prevention programs are developed to address this issue. School-based and community-based curricula could be useful in helping adolescents and their parents cope with the stress of unemployment in ways that are more healthy and productive than tobacco use.

W27

Outcomes from a teacher led tobacco prevention curriculum designed for infusion into seventh grade science classes

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The current study examines outcomes of a teacher led tobacco prevention curriculum that addressed environmental tobacco smoke and tobacco advertising and was specifically designed for infusion into a regular 7th-grade science class. Each curricular component addressed a specific prevention objective as well as a traditional academic objective. For example, students studied the effects of environmental tobacco smoke while learning the stages of the scientific method. Each activity consisted of two classroom sessions and a homework assignment and could be implemented alone or taught in a sequence. Three activities were developed in total. Teachers were given full choice as to which activities they would like to implement and in what order.

Sixteen schools were invited to participate, and teachers were actively recruited through presentations and flyers. A total of 14 teachers from 6 schools enrolled in the program and received a one-day training on all three tobacco prevention activities. At the end of the training, teachers were asked to indicate which activities they planned to teach. Over 78% of teachers planned to teach all three activities.

Students were pretested on attitudes toward and knowledge of curriculum components prior to the implementation of activities. Teachers were supplied with all necessary materials and were encouraged to seek assistance and support during the implementation. Every teacher was observed twice during the course of the implementation by trained observers. All students were post tested on attitude and knowledge items and on likeability of the activities at the program's conclusion.

For those students who received the target activity there were statistically significant increases from pre to post test in knowledge of tobacco risks and knowledge of legal restrictions on tobacco advertising, and statistically significant reductions in positive attitudes toward ETS exposure and pro-tobacco marketing. Almost 90% of students receiving at least one activity believed the lessons would help them abstain from tobacco use, 84% expressed liking for the program, and 86% reported learning something new. Of participating teachers, 91% believed the program effective or very effective in preventing tobacco use, 73% liked the program very much, and 82% felt that an infused curriculum for smoking prevention is a very good idea.

This evaluation of a teacher-led tobacco prevention curriculum provides preliminary evidence that anti-tobacco education can be taught in the context of regular 7th-grade science classes. Adding anti-tobacco education to existing school curricula across multiple academic subjects could increase students' exposure to prevention messages without taking class time away from required academic subjects. Longitudinal research is needed to determine the effect of this method on students' smoking behavior.

W28

Dissuading at-risk youths from smoking via mass media

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Background: More than twenty US states, including California, fund mass media antismoking campaigns aimed at youth. Many approaches are being used because no one is certain what works. In our research, we are conducting theory-based, experimental research to determine the causal impact of various types of mass media messages on adolescents' smoking-related beliefs and behavioral intentions.

Method: We conducted a study using antismoking ads as stimuli. We identified nine types of antismoking ads communicating one of three major message themes that are now commonly used: Health Theme: Disease and Death, Second-hand Smoke, Dying Parent, and Addiction; Social Theme: Cosmetics and Social Acceptance; and Marketing Theme: Selling Disease and Death, Marketing Tactics, and Tobacco Marketing Activism. We then developed testable predictions based on Protection Motivation Theory. For instance, we predicted that Disease and Death ads would strengthen beliefs about the severity of, and/or youths' vulnerability to, smoking's health risks. For each ad type, we then identified 3 representative ads that were similar executionally in terms of message content, spokesperson, and emotional valence (e.g., all three ads may have used youth, rather than adult, spokespeople). We wanted to ensure homogeneity within ad condition, and also to investigate whether certain executional styles might work better than others. We recruited 1949 subjects from 9 public schools, balanced in gender and ethnically diverse. Subjects were randomly assigned to view a tape containing a TV show with embedded filler ads, and either 3 antismoking ads representing one of the aforementioned ad types or 3 control ads. The control ads were PSAs unrelated to smoking, and provided the baseline condition from which antismoking ad effects were statistically assessed. Prior to viewing the tape, subjects completed measures of smoking initiation risk drawn from an extensive literature review. These measures included: sensation seeking, self esteem, attention deficit, aggression, delinquency, depression, anxiety, consumer susceptibility to interpersonal influence, past smoking behavior, gender and ethnicity. The main dependent variable, intent to smoke, was measured after exposure to the stimulus tape. An ad type was deemed effective if it significantly lowered subjects' intent to smoke relative to the control condition ($p \leq .05$). Mediational or cognitive process measures were also assessed after the stimulus tape was viewed. These measures were based on Protection Motivation Theory and included: perceived severity of and vulnerability to the health and social risks of smoking, perceived ability to resist pressure to smoke from peers and tobacco marketers, and perceived efficacy of tobacco marketing activism.

Conclusions: We found that the Disease and Death and Addiction ads successfully lowered youths' intent to smoke. None of the other ads influenced intent. A follow-up study ($n = 246$) focusing on the Disease and Death and Addiction ads verified that these ads reduced intentions to smoke, and that they did so by increasing youths' perceptions that they were vulnerable to the health risks of smoking. Executional factors such as youthful spokespeople were not predictive of ad effectiveness, except humor that was found to be ineffective. None of the moderating variables were found to be significant in terms of predicting youth response to the ads.

W29

Project EX: Classroom-based tobacco use prevention/cessation program in continuation high schools

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The current research project involves the refinement, implementation, and evaluation of a novel approach to teenage tobacco use cessation among continuation high school (CHS) youth. Project EX was a continuation high school-based clinic tobacco use cessation program that was funded through TRDRP (6RT-0182;1997-2000). This clinic program achieved a 17% quit rate at a 5-month post-quit day follow-up, compared to 8% for a control group, through use of a experimental design (Sussman, Dent, & Lichtman, 2001). This program also has shown promise of generalizability among high school youth in China (Zheng et al., conditionally accepted; 14% versus 3% quit rate at a 5-month post-quit day follow-up, in a multiple baseline single group design). Project EX thus far is being delivered as a school-based clinic. While this school-based clinic version of Project EX is effective, its reach is limited to those who attend the clinic. Both tobacco users and non-users are found in the classroom setting. The main goal of the present research is to improve cessation work by adapting the clinic program to the classroom context in continuation high schools.

There are four reasons to bring cessation education into the classroom setting. First, CHS youth are at very high risk for regular tobacco use. A total of 85% of these youth have tried tobacco, 71% have an intention to try tobacco in the future or are monthly users, 57% are monthly users, and 48% are daily users. Youth who do not smoke are confronted with smoking among their peers on a daily basis. Second this modality greatly increases the reach of programming and, therefore, may increase the total number of quitters. Third, such programming can be framed so as to exert a preventative function among youth that are not current tobacco users. Motivation enhancement, social skills, and life skills materials, contained in Project EX, is likely to be more relevant. In fact, program development studies for Project EX took place in the CHS classroom setting, composed of users and non-users. The non-users enjoyed the programming as much, or more, than the tobacco users. Also, they perceived that the activities developed would help themselves not to use tobacco in the future. Finally, cessation as classroom programming can be a useful practical health science educational tool in the high school classroom setting. Tobacco use addiction and cessation is an important topic to be instructed by the time a youth graduates high school, because of intrinsic academic importance in health behavior science and to appreciate the practical, societal costs of addiction.

In Year 1, Project EX will improve tobacco use cessation research by adapting the Project EX clinic program to the classroom context in CHS's. Material will be edited and piloted in classrooms. The material will be made appropriate as a means of cessation and indicated prevention. In Years 1, 2, and 3, implement and assess the effects of the classroom program at 6 CHS's. This 8-session program will be compared to a standard care control condition (classroom assessments only) at 6 other CHS's with immediate pretests, immediate post tests, 6 and 12-month follow-ups, in a two-group experimental design. We expect that the quit rates achieved will be at least as high as those previously obtained with the Project EX clinic-based program.

W30

Experimental evaluation of minors' access to tobacco (Project EEMAT)

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The standard method for measuring youth access to tobacco entails sending youth confederates to attempt to purchase cigarettes in a random sample of stores. Studies using this method have found that youth access to tobacco in California has decreased significantly since 1994 – from 60-90% to a current access rate of 6-14%. Simultaneously however, youth report that their access to tobacco remains high in the communities where their measured access is low. This measured vs. reported access discrepancy has yet to be explained, and raises questions about actual access rates. Project EEMAT is the first scientific analysis of this discrepancy. The hypothesis tested is that the measured vs. reported access discrepancy reflects the discrepancy between the tobacco purchase-strategies used by youth within versus outside of access studies.

In Year 1, a random sample of youth smokers was interviewed about the strategies they use to obtain tobacco from stores. The majority of youth reported using two strategies: The Familiarity Strategy in which they purchase tobacco only from clerks who recognize them as "good customers" (buyers of non-tobacco products) and hence are willing to sell tobacco to them, and the Lie About Age Strategy in which youth lie about being too young to make the purchase. Hence, in the subsequent years of this ongoing project, youths have been attempting to purchase tobacco from the same set of stores using three different methods: (1) In the Standard Research Protocol (used in access studies), youths appear at each store once to purchase tobacco, are strangers to the clerks, and are truthful about their age. (2) In the Familiarity Protocol youth are rendered familiar "good customers" by appearing at each store four consecutive times to purchase non-tobacco items and chat with the clerk, and then request tobacco on their fifth appearance. (3) In the Lie Protocol, youths appear at stores once to purchase tobacco but lie about being too young to make the purchase. Hence, we are modeling and comparing the behavior of youth within vs. outside of access studies.

Because this project is ongoing, at present we have data only on the Familiarity vs. Standard Protocol. Those data indicate that sales of tobacco to youths in the Familiarity Protocol are 5.5 times higher than in the Standard Protocol: Access rates for familiar youth (e.g., 62.5% for 17 year olds, and 42.9% for Latinos in Familiarity) are significantly above, whereas access rates for youth strangers (e.g., 6% for the same 17 year olds, and 14% for the same Latinos in Standard) are significantly below the state- and federally-mandated 20%. Thus, our data to date strongly suggest that the methodology for assessing youth access to tobacco lacks ecological validity insofar as it does not match how youth actually obtain cigarettes. Access has decreased and is low for the youth strangers within, but remains high for the familiar youth outside of compliance studies – and hence the measured vs. reported access discrepancy. The implications of these findings are obvious and serious.

W31

The spatial distribution of lung cancer in California

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Objective: To examine the relationship between lung cancer and economic/demographic variables across zip codes while controlling for spatial confounding.

Method: 1900 zip code areas in California were examined for rates of lung cancer taking into consideration population characteristics of persons living in those areas. Population characteristics were assessed using 2000 Census data. Lung rates were abstracted from hospital discharge data for the year 1999.

Results: Significant spatial auto-correlation exists between zip codes for lung cancer. When controlling for spatial auto-correlation, we found that unemployment, population density, adjacent area population, female head of household, older age, public assistance, and residential stability were positively related to lung cancer. Hispanic ethnicity was negatively related to lung cancer.

Conculsions: The current study suggests that spatial autocorrelation is a factor when examining the relationship between socio-economic/demographic measures and lung cancer and COPD, respectively. This analysis allows for a more precise understanding of typical SES/demographic measures than is possible without taking into account spatial characteristics. "Hot spots" for rates of lung cancer, (per zip code), above and below expected will also be presented and discussed.

W32

Use of network analysis to structure smoking prevention programs

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This study was designed to test whether network methods for identifying peer opinion leaders and their assignment to groups is an effective strategy for school-based tobacco prevention programs.

Three leader and group conditions, opinion leader, teacher, and network, were compared by randomly assigning them to 87 6th grade classrooms within 16 schools. Pre and post curriculum data were collected from 1,960 students on attitudes to smoking, refusal self-efficacy, perceived social consequences, and intention to smoke. The network condition consisted of identifying peer leaders using student nominations and matching those leaders to students who nominated them. Effect sizes were estimated using OLS and logistic regression controlling for within school co-variation, and repeated at the classroom level.

Students in the network condition liked the prevention program more. Relative to the opinion leader condition, students in the network condition had improved attitudes ($\hat{a}$=-0.07; $p<.01$), improved self-efficacy ($\hat{a}$=-0.09; $p<.001$), and decreased intention to smoke (adjusted odds ratio = 0.44, 95% CI = 0.38, 0.56). We conclude that network data can be used to structure tobacco prevention programs in schools. The network condition was the most effective method for structuring the curriculum and can be improved and further tested in multiple settings to improve health promotion efforts.

W33

Trandisciplinary scientific collaboration: A grounded theory of the research process

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Transdisciplinary scientific collaborations have the potential to solve real-world health and social problems such as tobacco-related disease. Over the past decade, scientists and scholars have received increasing encouragement and funding to join teams to work jointly on the problem of tobacco use and other health problems. Yet little is known about how to promote the success of scholars working collaboratively, and few if any studies have examined that circumstances that influence the process and success of interdisciplinary collaboration. This dissertation study provided a systematic investigation of the interactions and synergies (or intellectual bridging of ideas, theories, methods, or findings among different disciplines) among the members of the Transdisciplinary Tobacco Use Research Centers (TTURC) at the University of California, Irvine (UCI) and the University of California (USC).

The primary purpose of this study was to create a grounded theory of transdisciplinary scientific collaboration that identifies key antecedents and processes that facilitate and hinder cross-disciplinary collaboration. This study extends the research currently being conducted by the UCI TTURC Transdisciplinary Core project. Faculty USC and UCI TTURC members, supportive staff members, and administrators from each university were among the individuals interviewed and surveyed regularly. The content, affect, and scientific potential outcomes of informal communication and meetings were recorded and coded to reveal barriers and facilitators of collaboration between scientists of different disciplinary backgrounds. Web-based forms were developed and utilized to collect attitudinal, evaluative, and outcome-related data from TTURC members after meetings and other important events.

Quantitative and qualitative data were converged and interpreted to reveal a model of transdisciplinary scientific collaboration that might facilitate future collaborations. Study findings suggested that a narrow disciplinary scope, a strong shared work history, and spatial proximity among researchers on a transdisciplinary team leads to smooth-running and rapidly-progressing collaboration and production of transdisciplinary outcomes. Conversely, transdisciplinary scientific collaborations starting with a broad disciplinary scope, a lack of shared history, and spatial distance among collaborators develop transdisciplinary outcomes more slowly and with less ease; and a mediating social capital-building phase will be necessary before a shared model and other transdisciplinary innovations can be created. Implications for transdisciplinary scientific collaborations are discussed.

W34

A dynamic model of smoking and healthcare expenditures in CA

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This study develops a series of related models that permit understanding of the impact of smoking on health and healthcare costs as a dynamic process. It will take into account smoking behavior, quitting behavior, and the impact of aging on mortality, on the current treatment status of people with two classes of smoking related diseases, and on poor-health status. The models will be used to analyze the impact of changing smoking behavior over time. The models of smoking attributable mortality, likelihood of current treatment status for smoking related diseases, and poor health status are estimated with national survey data, and will be applied to California to estimate the annual and lifetime healthcare expenditures that can be attributed to smoking in the state. The models will then be used to evaluate the net effect of the California Tobacco Control program on the health of its citizens and healthcare costs.

To date, work has focused on the theory and estimation of the three models discussed. Theoretical models have been developed, datasets have been identified, and empirical estimation is underway. The estimation of the dynamic models involves multiple steps in an iterative process. We have determined that we must control our current treatment models for the sample selection bias introduced by mortality, and we must control our poor health status models for the sample selection bias introduced by mortality and current treatment status. These sample selection bias effects have necessitated a change in the order of the estimation of our models. While our plan was to estimate poor health, then current treatment, and finally mortality, we are now estimating models in the reverse order. Starting values for the process have been derived, and the different models are at different stages of completion. Preliminary results show that longer duration of smoking is associated with poorer health, the more packs per day currently smoked the poorer the health status, and the longer the time since quitting the better the health status.

Models of the impact of smoking on nursing home expenditures in California will incorporate the institutionalization of sick smokers and also the institutionalization of people who live alone due to the smoking-related death of a spouse. Finally, the net impact of California's tobacco control programs will be analyzed by comparing simulations of health expenditures that follow from smoking attributable health effects under different smoking behaviors of the state's residents based on the estimated models using yearly prevalence rates to simulations designed to describe California's smoking behavior had the programs not existed. For example, a comparison of current health effects and expenditures to health care expenditures based on the status quo prior to 1989 will be made.

This study will provide estimates of the economic success, health status improvement, and the smoking disease and mortality reductions attributable to California's tobacco control programs. This knowledge will be valuable for charting future directions for the program and for other states that are considering implementing or altering their own tobacco control efforts.

W35

Financial analysis of smoking cessation therapy in men age 18 to 64 years

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This poster presents simulation estimates of the medical cost savings following smoking cessation in men age 18 to 64 years. Medical cost savings are defined to be the excess direct medical costs from fourteen tobacco-related illnesses following smoking cessation: heart attack, stroke, emphysema, chronic bronchitis, pneumonia, Parkinson disease, Crohn disease, ulcerative colitis, and cancers of the lung, larynx, esophagus, pancreas, oropharynx, and bladder. Savings are calculated for a randomly chosen healthy smoker who quits, from one of two age groups: 18-39, and 40-64 years. The quitter is followed until entry into Medicare or death. Two types of smokers are modeled: the 'lifelong smoker' who would have smoked until death if he had not quit, and the 'typical smoker' who has the average probability of quitting in future years if he had not quit this year.

The expected cumulative undiscounted cost savings for 40-64 year olds are $11,881 for lifelong smokers and $7,706 for typical smokers. Discounted at 3% per year, the cost savings are $8,360 for lifelong smokers and $5,612 for typical smokers. The cumulative undiscounted savings for 18-39 year olds are $18,885 for lifelong smokers and $10,501 for typical smokers. The discounted savings are $8,628 for lifelong smokers and $5,094 for typical smokers.

There are two types of uncertainty in the estimates: uncertainty about the epidemiological parameters, and uncertainty about the costs of treatment following diagnosis of a disease. The uncertainty from epidemiological parameters is minor and produces a relative standard error between 5% and 12% of the mean savings. The uncertainty from the cost of treatment is greater and more difficult to quantify since there are too few estimates of the cost of treatment to determine a well-defined distribution of cost estimates. Sensitivity analysis using lower bound estimates of the costs of treatment resulted in a reduction of between 35% and 40% in the expected cumulative cost savings reported above.

From the perspective of the commercial health care sector, the expected discounted cost savings following cessation in a typical smoker are large relative to the expected cost of producing an ex-smoker. In other words, many modalities of smoking cessation are largely self-financing and could be provided free to the enrollee or at a fraction of the cost of services and materials. These modalities include extended smoking cessation counseling by health care professionals and adjunctive pharmaceutical therapies such as nicotine gum, nicotine patch and Bupropion.

From the perspective of the individual health care provider or managed care organization, enrollee turnover rates are relatively high and most cost savings following cessation occur after the patient has a high probability of disenrollment. Therefore only a small fraction of these cost savings can be can be captured, and for the typical male smoker, only counseling is self financing at a zero or low enrollee co-payment. Therefore some program for pooling the cost savings among individual providers, or a cooperative effort at smoking cessation by all providers is needed in order to capture the potential cost savings from cessation in currently healthy smokers.

W36

Tobacco industry efforts to undermine the American Stop Smoking Intervention Study (ASSIST)

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To describe how the tobacco industry responded to the National Cancer Institute's American Stop Smoking Intervention Study (ASSIST); to demonstrate how the tobacco industry's aggressive efforts to defeat ASSIST could have contributed to the limited success of the program.

In 1991 the National Cancer Institute (NCI) in collaboration with the American Cancer Society (ACS) initiated the ASSIST program, a seven-year, seventeen-state project which was the largest and most comprehensive tobacco control intervention ever undertaken in the United States. State health departments and local ACS divisions formed coalitions with health organizations and community groups to implement the program. The tobacco industry considered this initiative a major threat because of its scope, its emphasis on public and private policy change, and its creation of a local tobacco control infrastructure.

We searched the tobacco industry document websites and the U.C.S.F./Legacy Tobacco Documents Library (http://legacy.library.ucsf.edu/). We identified and reviewed an estimated ninety internal tobacco industry documents using search terms including "ASSIST," "American Stop Smoking Intervention," and "Cancerscam." We also interviewed key individuals at the National Cancer Institute and elsewhere who participated in the development, implementation and evaluation of the ASSIST program.

The tobacco industry responded to ASSIST with a carefully coordinated effort. The documents describe a range of strategies the tobacco industry planned and implemented, including a media and public relations campaign, numerous Freedom of Information and audit requests, monitoring and infiltrating ASSIST coalitions, mobilizing legislative and other allies, and attempts to pre-empt local initiatives with weaker state laws. Messages conveyed by the tobacco industry focused on allegations of "illegal lobbying" by ASSIST coalition members but also included "waste of taxpayer dollars," "tax grabs," "greedy" health professionals and charities, and discrimination against low-income smokers.

We intend to investigate further the extent to which strategies planned by the tobacco industry were actually implemented. We also plan to assess the role the tobacco industry played specifically in the devolution of ASSIST from a large-scale, policy-focused NCI intervention study to a series of state and local grants under the Centers for Disease Control.

This study will demonstrate the serious threat the tobacco industry sees in large-scale, population-based, policy-focused interventions which foster local tobacco control infrastructures. It will shed light on how tobacco companies respond to this type of threat, including the various strategies they used to derail ASSIST, specific resources and allies they mustered in these efforts, and the motives behind various attacks on the ASSIST program and those participating in it. This information will provide useful lessons for future tobacco control interventions.

W37

The effect of over-the-counter sales of the nicotine patch and nicotine gum on quit attempts in California

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Changes in tobacco-related public policies have been a major component of both state and federal tobacco control strategies. Because policy level interventions can have a large impact on smoking cessation and disease reduction, it is important to evaluate which interventions work, and of the interventions that work, determine how well these interventions are working. The purpose of this population-based study was to evaluate whether quit attempts utilizing nicotine replacement products (gum and the patch) increased after these products became available to California smokers over-the-counter. During two different months in 1996, both nicotine gum and the nicotine patch became available for sale over-the-counter. The co-occurrence of this public policy intervention with the administration of the 1996 California Tobacco Survey (CTS) provided an opportunity to conduct a "natural experiment" examining whether obtaining these products without a prescription increased the likelihood of making a quit attempt using these products.

For this study, we utilized data collected as part of the 1996 CTS that was conducted between September 1996 and January 1997. In this survey, smokers were asked to indicate whether they had made a quit attempt that lasted one day or longer during the past year. If they had made a quit attempt, they were asked to give the start of their most recent quit attempt that lasted for a day or longer. Smokers were also asked whether they used nicotine gum or the nicotine patch during this recent quit attempt.

We calculated the monthly rate of quit attempts (successful and unsuccessful) that included the use of nicotine gum or the patch between October 1995 and November 1996. Next, we used multiple regression to model the rate of monthly quit attempts weighted by the sample size. Because smokers will more readily recall quits occurring closer to when they were surveyed than quits occurring further back in time, we included a term in the model that accounts for this biased recall of quits. This term represents the number of months elapsed between the month of the quit attempt and the month of the survey interview. We then included a term in the model indicating whether the quit attempt that included NRT occurred before or after these products were available for sale over-the-counter.

After controlling for the number of months between the quit attempt and survey month, there was a significant increase in the rate of quit attempts with the use of the patch after the patch was available over-the-counter (beta = 0.348, $p<0.01$). The rate of increase in quit attempts using nicotine gum after gum was available over-the-counter was marginally significant (beta = 0.196, $p<.081$). These results suggest smokers in California increased their use of nicotine replacement products when making a quit attempt after these products became available for sale over-the-counter in 1996.

W38

Participatory research in school-based tobacco cessation

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University of Southern California (USC) and the Humboldt County Office of Education (HCOE) are jointly conducting a study that would provide a test of the effectiveness of nicotine replacement therapy in the context of a school-based tobacco cessation program. This participatory research study is utilizing the Project EX curriculum, a USC research-validated behavioral component. The Project EX program consists of a repertoire of theoretically and empirically derived motivational strategies presented during ten one-hour sessions. An over-the-counter nicotine gum or herbal gum is randomly assigned to the subjects.

The California Tobacco-Related Disease Research Program and the California Department of Education jointly fund this study under the School-Academic Research Award (SARA) mechanism, with the intent to stimulate and support collaborations between schools and academic investigators in the performance of scientific research into tobacco control issues. In addition to researchers from USC and HCOE working in partnership, high school staff nurses and health educators actively participate in conduct of the study.

The study will be conducting cessation clinics through the 2002-2003 school year. Student subjects are recruited from comprehensive, continuation and community high schools throughout Humboldt County, California.

Elements of this study are presented, with preliminary quit and retention rates as well as ratings of the Project EX sessions. Successes and barriers that were experienced using the participatory research method are also identified.

W39

Anti-tobacco programming: Reaching the deaf and hard-of-hearing
Partnering for tobacco control research among deaf youth
School-based anti-tobacco programs for deaf/HH youth

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Effective prevention strategies are a critically important element of the tobacco-control agenda in California and nationwide. We report here on a first-ever, multi-step program of research, funded by TRDRP, to design and develop such interventions for Deaf and Hard-of-Hearing (Deaf/HH) youth, a unique culturally linguistically understudied and underserved population. We describe:

- *collaboration among tobacco control researchers at UCLA; the California School for the Deaf, Fremont (CSDF), a key educational institution serving Deaf/HH youth; and leading community-based organizations serving the Deaf community in California [Greater Los Angeles Council on Deafness (GLAD), the California Coalition of Agencies Serving the Deaf and Hard-of-Hearing (CCASDHH)].*

- *data collection among deaf youth to gain the necessary understanding of the patterns and determinants of tobacco use in this distinct socio-cultural population. We include findings from: a quantitative survey conducted among Deaf/HH youth and young adults (N=467); in-depth open-ended in-person interviews conducted among a randomized sample of students drawn from our survey respondents (N=40); and in-depth telephone interviews conducted among educators serving deaf youth in California=s residential and day schools, and mainstream schools and colleges (N=45).*

- *the school-based tobacco prevention curriculum for Deaf/HH youth that has been developed. The curriculum builds on a life-skills and modular approach, is designed for use among youth in grades 7-12, includes extra-curricular and community adjuncts and elements, and is tailored to the linguistic and cultural requirements of this population. We outline the theoretical underpinnings of the intervention, and the steps taken to craft this program, including the role of educators, students, community leaders and tobacco researchers.*

Finally, we will outline our plans for next steps: application to TRDRP support through the Full SARA funding mechanism for a randomized controlled trial to test the impact of this educational program on the tobacco-related knowledge, perspectives and behaviors of this population.

W41

Smoking cessation on the internet: Recruitment method, motivation, and quitting smoking

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The specific aim of this study is to determine whether recruitment method is associated with the characteristics of smokers participating in smoking cessation efforts on the Internet. With the growing number of clinical research studies being conducted on the World Wide Web (WWW), the recruitment strategies utilized by investigators may prove to appeal to specific groups of users. Published research on conducting clinical trials using the Internet indicates it can be very cost-effective when dealing with large populations. Subject recruitment, obtaining informed consent, randomization, administration of the intervention, data collection, and case management can be greatly automated with web based processes. However, various recruitment methods may result in differentially motivated subjects.

"Tomando Control – II" is a smoking cessation study conducted over the WWW using a standard smoking cessation self-help intervention with adult smokers (n=3,550). Volunteer smokers took part in a one-group pre- and post-test outcome study. Three recruitment strategies were utilized: 1) a direct e-mail was sent from a large health information website to more than 78,000 members, 2) a nationally broadcast news story was televised in over 60 cities throughout the USA, and 3) postings of the site on Internet search engines. Readiness to quit was assessed using items paralleling the Transtheoretical Model/Stages of Change measure (Prochaska, DiClemente, & Norcross, 1992). Outcome data was collected via the Web at 1-month and 6-months post intervention.

We will examine whether the three recruitment methods resulted in differences in 1) the demographic characteristics of participants, 2) the stages of change of the participants, and 3) the rates of serious quit attempts and 7-day abstinence found in each of the three cohorts recruited. We will also examine whether recruitment method and readiness to quit interact to predict a serious quit attempt and 7-day abstinence.

Diverse recruitment methods and group-specific sites may help in reaching each of the many segments of the smoking population. Identifying recruitment methods best able to attract those smokers most likely to quit smoking and stay quit would have great implications for resource allocation in efforts to reduce smoking rates.

W42

Major depressive symptoms in smokers trying to quit via the web

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Depression has been found to influence smoking rates. The World Wide Web is being used increasingly to provide smoking cessation information to smokers worldwide. The objective of the "***Tomando Control – II***" study was (1) to estimate rates of lifetime and current major depressive episodes and rates of depression symptoms in smokers trying to quit on the World Wide Web, and (2) to determine the relationship between current major depressive episodes and ability to quit. Data were collected from 4598 smokers (3550 English speakers and 1048 Spanish speakers) recruited into a smoking cessation research study conducted over the World Wide Web. Rates of lifetime and current major depressive episodes (MDEs) were estimated using the MDE Screener (a questionnaire based on items from the Diagnostic Interview Schedule) and rates of depressive symptom levels were measured using the Center for Epidemiological Studies-Depression Scale. Serious quit attempts (24-hour periods without smoking), and quit rates (7-day abstinence) were obtained via the web at 1- and 6-month follow-ups.

Lifetime prevalence of major depressive episodes (MDEs) for English- and Spanish-speaking smokers were 37.9% and 43.6%, respectively. Current (past 2 weeks) MDE rates were 16% and 23.2%, respectively. English-speaking smokers with current MDEs at entry into the study are less likely to have serious quit attempts and to achieve 7-day abstinence at 1-month and 6-month follow-ups when participants with missing follow-up data are considered to be smoking.

We conclude that smokers trying to quit smoking over the WWW have high rates of major depressive episodes, and current MDEs significantly reduce their ability to quit, at least for English-speaking smokers. These findings suggest that smoking cessation efforts (at least those offered via the WWW) should seriously consider including a mood management component as an essential element in their interventions.

The ***"Tomando Control – II"*** study is now conducting a randomized controlled trial comparing the standard smoking cessation guide tested in the above study with a combination of the guide plus a mood management intervention. If the addition of the mood management component results in significantly higher quit rates, future smoking cessation programs that include such a component will have a greater probability of reducing the number of smokers in our communities.

W43

Two approaches to referral for smoking cessation treatment

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Despite 35 years of smoking cessation efforts, the prevalence of smoking remains high. There have been many clinical trials of different treatment approaches to help patients stop smoking and a large number have been shown to be effective. However, very little is known about how to get smokers to attend these programs. This study is an analysis of two different primary-care based methods for counseling and referring smokers. Our primary goals are to compare the effectiveness of two methods of referral to smoking cessation programs, to determine the incremental cost-effectiveness of having an on-call smoking cessation counselor as compared to standard care, and to evaluate patient/provider satisfaction with each of the referral methods.

One of the two primary care teams at the Sepulveda VA was randomly assigned to be the control team, in which physicians are responsible for counseling their patients and, when appropriate, referring them to the Smoking Cessation Clinic (SCC). On the intervention team, the physician has the additional option of providing limited counseling and then paging an on-call counselor. She provides extensive counseling and assists in referring the patient to either the on-site SCC or to a telephone counseling helpline. She also tracks all referred patients, performing limited case management.

To evaluate the effectiveness of on-call counseling versus standard care, we are following a population-based sample of smokers for 18 months to assess their smoking status, quit attempts, and the type of counseling received. We have identified and recruited into the study 482 smokers who have completed baseline surveys. Of the 482 study participants, 187 (39%) had tried to quit at least once in the past year and 335 (70%) had been counseled on cessation by a VA provider within the last year. 158 (33%) of them had been referred to a cessation program in the past 12 months. Of these, 50 (10%) had attended a program. Only 12 (3%) of the study participants had been referred to a telephone counseling program, of which 1 (0.2%) person actually called.

On-call smoking cessation counseling has been available to the intervention team since July 2002. The primary study outcome will be the number of smokers who use a smoking cessation program.

Despite the high success rates at helping smokers quit, organized smoking cessation programs are vastly underused. This study will provide useful original data on how effective each referral method is in actual clinical practice. It will also yield practical information about costs that can be expected by using these referral methods for smoking cessation.

W44

Self-hypnosis for smoking cessation

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While smoking continues to be the largest cause of preventable death and disability, smoking cessation remains a difficult task. Fewer than half the smokers who enroll in smoking cessation programs are abstinent at one year. Antidepressant medications have recently been added to self-help literature, cognitive-behavioral counseling and nicotine replacement therapy as approved treatments. However, additional effective smoking cessation interventions are needed. Self-hypnosis has been shown to be helpful for pain management and, when combined with behavioral therapy, for the treatment of obesity, insomnia, anxiety and hypertension. Recent studies of self-hypnosis for smoking cessation have yielded quit rates comparable to those for multi-component treatments combining counseling and nicotine replacement. Use of self-hypnosis combined with nicotine replacement warrants further investigation.

We are conducting a randomized clinical trial in which all smokers receive 2 months of transdermal nicotine patches. In addition, approximately 50% of the participants are randomly assigned to receive training in self-hypnosis, whereas the remaining 50% of participants are randomly assigned to receive self-help literature and cognitive-behavioral counseling. Based on treatment assignment, participants receive either instruction in self-hypnosis in two forty-five minute sessions or behavioral counseling in two forty-five minute sessions. If training in self-hypnosis results in biochemically-confirmed smoking cessation rates comparable to those of behavioral counseling at six months and again at one year, it may become a useful addition to conventional smoking cessation approaches.

The study is currently enrolling and following participants. A total of 135 participants, 35% of them veterans, have been enrolled and are being followed. Thirty-seven percent are women. Seventy percent are white. The smokers' mean age is 47 years and 67% were educated beyond high school. They smoked an average of a pack of cigarettes per day upon entering the study. At present, 68 participants have been assigned to self-hypnosis and 67 have been assigned to behavioral counseling. Six-month follow up data are available for 66 participants. The self-reported six-month quit rates are 29% for the self-hypnosis group and 25% for the behavioral counseling group. If these quit rates are confirmed biochemically, self-hypnosis combined with nicotine patches would appear to compare favorably with standard behavioral counseling and patches six months after treatment.

W45

Dissemination of a smoking cessation program for inpatients

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This project focused on issues related to the implementation and dissemination of existing approaches to tobacco control. The goal of this project was to disseminate a scientifically-tested inpatient smoking cessation program (*Staying Free*) to a wide range of hospitals in the South San Francisco Bay Area. We hoped to demonstrate that the program was comparably successful when used in these hospitals as it was when implemented in clinical trials.

Dissemination was conceptualized as having two phases. In year one, the Stanford research team assisted each hospital with program implementation and provided on-going assistance. In year two, the Stanford research team withdrew in order to assess the degree to which the program was institutionalized. The program was implemented in six hospitals, including a Veterans Administration, county, university, managed care, small and large community setting. Each adapted the original Staying Free model in order to capitalize on local hospital resources and needs. These models addressed systems for identifying smokers and for providing educational, counseling, pharmacological, and follow-up components. Intervention materials developed in partnership with the participating hospitals included a relaxation CD, posters for patients' rooms, training materials for staff, and interventions for Hispanic and Vietnamese populations. Approximately 200 professionals received intensive four-hour training while an additional 300 professionals were oriented to the program.

The transition from implementation to institutionalization combined with changes in the delivery model at some hospitals resulted in increased or decreased patient recruitment in the second year/phase. These results will be discussed more fully in December. Overall, 317 individuals (63% men, 37% women) were enrolled during the implementation phase and 424 (57% men, 43% women) during the institutionalization phase. Participants were middle-aged (M = 49, SD = 12.91), and moderately diverse (58% Caucasian, 12% African American, 20% Latino, 7% Asian American, and 3% Other/Unknown). During the implementation phase, the program resulted in a self-reported cessation rate of 24%% (18% to 45%) at six-month follow-up, with patients lost to follow-up counted as smokers. Preliminary data for the institutionalization phase (final outcome will be available in December) suggest that the quit rate remained stable 24% (14% to 48%). These results are comparable to controlled trials.

While our final conclusions will need to be based on complete follow-up data, we feel that our results thus far demonstrate that: (1) it is possible to implement and institutionalize an inpatient smoking cessation program in any hospital. (2) Dedicated staff time is essential to running the program and results in higher patient recruitment, even with identical tobacco use measures in place. (3) Dedicated staff time, however, is not sufficient for high cessation outcomes—intervention providers must be highly skilled and must receive on-going feedback and training. These results are critically relevant to the proposed NCQA, AMA and JCAHO tobacco use measures and to the provision of tobacco cessation programs to all hospitalized smokers in the state of California.

W46

Determination of mercapturic acid metabolites of toxic substances in urine of smokers using liquid chromatography -tandem mass spectrometry

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The objective of this project is to develop methods for measuring human exposure to toxic substances present in tobacco and tobacco smoke.

Despite advances in smoking cessation research and new pharmacotherapies for tobacco addiction, many people who want to quit are unable to do so. Consequently, there is considerable interest in "harm reduction," the possibility of reducing the harmful effects of continued tobacco use in persons who are unable to quit. Tobacco companies are developing products that are claimed to deliver lower levels of toxic substances to the user than do conventional cigarettes. Tobacco addiction treatment researchers are investigating the possibility that smokers' cigarette consumption can be reduced, for example with nicotine gum or patches, and that this may lead to reduced health risk.

A major issue is how to determine whether significant reduction in exposure to toxic substances has occurred. For example, smokers who reduce the number of cigarettes that they smoke may smoke them more intensively, extract more nicotine and other toxic substances from each cigarette, and not significantly reduce their daily intake. On the other hand, smokers using nicotine patches may inhale less smoke from their cigarettes, thereby reducing their daily exposure. Therefore, simply counting cigarettes is not reliable for estimating exposure to toxic substances. Methodology for determining intake of specific toxic substances is needed to evaluate these possibilities.

Tobacco and tobacco smoke contain numerous toxic substances (for example. about 50 carcinogens have been identified), and measuring exposure to each individually would not be practical. Our approach is to develop methods for measuring specific toxic substances, or metabolic break-down products, or other tobacco-derived substances in biologic fluids (such as blood or urine) from smokers. Concentrations of these substances, called "biomarkers" can be used as a measure of exposure.

Many toxic substances, including most carcinogens, exert their toxic effects by chemically reacting with and altering the structure of macromolecules such as DNA and proteins. In some cases, a metabolite of the toxic substance is the species that reacts with the macromolecule. These reactive substances are usually of a class known as "alkylating agents," which, in addition to reacting with macromolecules, are also metabolized to non-toxic substances called mercapturic acids. Mercapturic acids are useful as biomarkers for assessment of exposure to toxic substances.

This presentation will describe our progress in the development of a new analytical method for measuring concentrations of several mercapturic acid metabolites excreted in the urine of smokers, including metabolites of benzene, a carcinogen, and acrolein, a pulmonary toxin. It is anticipated that these methods will be used by tobacco addiction treatment researchers in clinical trials to evaluate potential harm reduction, to measure toxic substance exposure in persons using new tobacco products claimed to be less harmful, and to determine toxic substance exposure in persons using low nicotine-content, potentially non-addictive cigarettes.

T47

Cohort study of exposure to environmental tobacco smoke and risk of stroke

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The independent effect of exposure to environmental tobacco smoke (ETS, passive smoking) on the risk of stroke is not well established. We assessed the association of self-reported ETS exposure at home and outside the home with risk of ischemic stroke, transient ischemic attack and hemorrhagic stroke in adult men and women.

We performed a cohort study among 27,822 lifelong nonsmokers (10,522 men and 17,300 women) aged 30-85 years at enrollment. Self-reported ETS exposure (at home and outside the home, in hours/week) and stroke risk factors were collected at a health plan in San Francisco and Oakland between 1979 and 1985. Follow-up for hospitalizations and death was available through the end of 2000 (median=16 years).

In multivariate analysis adjusting for age, sex, race/ethnicity, educational attainment, marital status, hypertension, diabetes and serum total cholesterol, ETS exposure at home of 20 hours or more/week (in relation to ≤ 1 hour/week) was associated with a 1.46-fold (95% CI 1.12-1.91) increased ischemic stroke risk. No significant associations were found between ETS exposure outside the home and ischemic stroke or between exposure to ETS at home or out of home and the risk of transient ischemic attack or hemorrhagic stroke.

High-level ETS exposure at home was independently associated with increased risk of ischemic stroke among never-smokers.

T48

Smoking-induced subclinical atherosclerosis detected by ultrasound: media atrophy and luminal diameter enlargement in women

Fan, Amy
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Cigarette smoking is an established causal factor for atherosclerosis. However, the association between cigarette smoking and carotid atherosclerosis in terms of arterial wall composition and luminal diameter assessed by ultrasound is not clear.

The objective of the current study is to examine whether there is a dose-response relationship between cigarette smoking and carotid artery intima-media thickness (and its composition) in middle-aged men and women; whether the atherogenic effects of smoking diminished following smoking cessation; whether there are gender differences among these relations.

The cohort of the Los Angeles Atherosclerosis Study consists of 573 middle-aged adults (men, age 40-60 yr; women, age 45-60 yr) who had no history of cardiovascular heart disease (CHD) or stroke at baseline. A follow-up examination was completed at about 3 years. A sub-cohort was obtained from those with IMT measurement ³ 0.7 mm. This criterion was proposed because of the difficulty to obtain accurate thickness measurements for IMT composition if overall IMT is thinner than 0.7 mm. A total of 413 subjects (men, n=231; women, n=182) were thus included in the study. Among the included sample, about 49% (n=89) of women and 62% (n=143) of men were former or current smokers. The intima-media thickness, echogenic- (intima) and echolucent-layer (media) thickness were measured with B-mode ultrasound. The carotid luminal diameters were obtained at the same site for arterial systole and diastole. The cumulative cigarette exposure (*i.e.,* smoking pack-years) was calculated by daily packs smoked multiplied by the number of years smoked. The cumulative cigarette pack-yrs were categorized separately by sex into 4 groups, *i.e.*, never smokers, light smokers, moderate smokers, and heavy smokers. The last three groups were obtained by the tertiles of smoking pack-year history. Former smokers were smokers who had already quitted smoking before enrollment and remained abstinent during the follow-up. Random effects models and individual growth models in SAS were used to examine the effects of cigarette smoking and smoking cessation on the baseline and progression of IMT, IMT composition and luminal diameter.

The results showed that current smoking was associated with thicker echogenic layer (intimal thickening) in both men and women. Smoking-induced carotid atherosclerosis in women was also associated with thinned media, enlarged luminal diameter; while in men, there were curvilinear associations between medial thickness, luminal diameter, and smoking pack-years. In both women and men, smoking cessation was associated with decreased intimal thickening. Particularly, the medial thickness in women increased in former smokers and the mixed effects of smoking cessation on IMT composition resulted in dissociation between smoking cessation and total IMT in women.

It was concluded that the atherogenic effects of smoking on the carotid arterial wall is gender-related; overall IMT *per se* is not a valid measure for detecting smoking-induced atherosclerosis in women; part of the adverse effects of smoking is reversible following cessation. The findings need to be verified by other epidemiologic studies with larger smoker samples. The recognition of the atherosclerotic progression and regression associated with cigarette smoking and smoking cessation may provide motivation for early cessation and delay or reverse the disease process.

T49

Smoking, estrogen metabolites and cognitive function

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Previous studies reported effects of cigarette smoking on endogenous hormone levels including an antiestrogen effect and an earlier age at menopause in women smokers. Some studies have reported that smoking is associated with negative effects on cognitive function. However, whether this association is due to the antiestrogen effects of smoking is unclear because the literature on the association of estrogen with cognitive function is inconsistent. One possible mechanism by which smoking may affect cognitive function is through an alteration in levels of endogenous estrogen metabolites. In the body, 17β estradiol is metabolized into 16α-hydroxyestrone (16α-OHE_1), which is considered to be the active form, and into 2-hydroxyestrone (2-OHE_1), which is the inactive form. The goal of this project is to examine the cross-sectional and prospective associations of smoking, and of the estrogen metabolites 16α-OHE_1, (active form), 2-OHE_1 (inactive form), and their ratio (16α/2-OHE_1) with cognitive function in older women.

Subjects for the present study consisted of 426 women aged 65 and older who were participants in the Rancho Bernardo Study and attended a follow-up clinic visit in 1988-91. None of the women were current estrogen users. Information on cigarette smoking history, past use of estrogen replacement therapy, and educational level was obtained. Cognitive function was assessed with the twelve tests of cognitive function. Estrogen metabolites were assayed in samples of blood frozen from this visit in 2000.

The average age of these women was 74 years (SD=8); 47% had used estrogen in the past and 58% had completed at least some college education. Of these women 45.1% reported a history of smoking; mean pack years was 26 (SD=23). Comparisons of ever smokers and never smokers indicated that never smokers were significantly older ($p=0.03$), but there were no significant differences in education, past use of estrogen, body mass index, estrogen metabolites or their ratio. After adjustment for age, pack years of smoking was significantly associated with higher levels of 16α-OHE_1, (active form; $p=0.03$), but there were no significant differences in levels of 2-OHE_1 (inactive form), or their ratio ($p<0.10$). Regression analyses adjusting for age and education indicated that levels of 16α-OHE_1 were significantly and positively associated with scores on the Minimental Status Examination ($p=0.05$) and world backward ($p=0.05$). The 16α/2-OHE_1 ratio was negatively associated with Blessed scores ($p<0.01$). Pack years was associated with slightly higher scores on serial sevens ($p=0.05$) and world backward ($p=0.04$) but lower scores on Trails B ($p=0.05$). A significant interactive effect was found for pack years with 2-OHE_1 on Serial Sevens ($p=0.05$) and World Backward ($p=0.03$), and for pack years with the 16α/2-OHE_1 ratio on Serial Sevens ($p=0.04$).

The results of this study are important because they show that smoking can have direct effects on cognitive function as well as indirect effects through an association with estrogen metabolites. Our future work will examine the associations of smoking and estrogen metabolites with cognitive function over time.

T50

Effect of maternal smoking on human placental development

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Our work is to understand why maternal smoking impairs fetal development. A logical explanation is that the ill effects are due to placental damage. The placenta, a transient organ that attaches the baby to the uterus before birth, directs pregnancy outcome. Our group discovered that in normal pregnancy placental development is closely coupled to oxygen tension. Specifically, we found that cytotrophoblasts proliferate in hypoxia and differentiate when they encounter a more highly oxygenated environment, i.e., the uterine wall.

These findings suggest that placental cells have an unusual molecular mechanism of sensing and responding to changes in oxygen tension. We found important roles for the von Hippel-Lindau tumor suppressor protein (pVHL) and the transcription factors with which it interacts, primarily hypoxia-inducible factor-2 alpha (HIF-2 alpha). The downstream targets they regulate include members of the vascular endothelial growth factor (VEGF) family that play important roles in placental development. Our previous immunolocalization studies in situ showed that pVHL expression declines in cytotrophoblasts that invade the uterine wall, allowing HIF-2 alpha translocation to the nucleus, which triggers a concomitant upregulation of the cells' VEGF expression. Functional studies in vitro showed that this important chain of events is also regulated by oxygen tension: in hypoxia, HIF-2 alpha and pVHL expression in the cytoplasm increases, and, by inference, a decrease in cytotrophoblast VEGF expression occurs. The present studies used our knowledge of this placental oxygen-sensing system to gain information about the effects of smoking on the placenta.

First, we collected samples of placental tissues from women who smoked while they were pregnant. The donors were as follows: group 1, women who smoked <10 cigarettes/day; group 2, women who smoked 10-20 cigarettes/day; group 3, women who smoked 20 cigarettes/day; group 4, women who did not smoke but were exposed to secondhand smoke. The controls were gestationally matched samples from women who did not smoke during pregnancy. Immunolocalization on tissue sections was the method of analysis. Relative to control samples, placentas from smoking mothers and mothers exposed to secondhand smoke showed evidence of the dysregulation of oxygen-sensing mechanisms, particularly in cytotrophoblasts, the epithelial cells of the placenta that carry out its specialized functions. Specifically, in samples from very early pregnancy (6-8 weeks of gestation) we found an increase in pVHL expression that was associated with a parallel increase in expression of HIF-2 alpha and a decrease in VEGF staining. These findings are direct evidence that smoking during pregnancy produces a very hypoxic environment that results in abnormal placentation.

The results of our study show that maternal smoking starves the placenta of oxygen and changes the developmental fate of cytotrophoblasts. To our knowledge, this is the first molecular explanation for the link between smoking and pregnancy complications. This finding is highly significant, because understanding the causes is the first step in designing interventions that will reduce the terrible human and financial toll that smoking-induced pregnancy complications take on citizens of California.

T51

Pyridines that inhibit diverse biological processes are more concentrated in sidestream than mainstream smoke solutions from commercial brand cigarettes

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Several pyridine derivatives that have adverse effects on oviductal functioning, chick chorioallantoic membrane growth, and angiogenesis at picomolar doses have been identified in cigarette smoke. The purpose of this study was to determine the concentrations of 2-methylpyridine, 2-ethylpyridine, and 3-ethylpyridine in mainstream and sidestream smoke solutions of various types of cigarettes The cigarettes selected included regular and light U.S. filtered cigarettes (Marlboro and Camel), several new U.S. brands that claim to have reduced carcinogens (Omni and Advance), and University of Kentucky research 2R1 and 1R4F cigarettes. The relative absorbance of each smoke solution was first measured using a spectrophotometer (λ=300 nm). Sidestream smoke solutions showed a 6- to 10-fold higher absorbance than mainstream solutions. The Omni sidestream smoke solution had the highest absorbance values compared to the other commercial brands. Next, the smoke solutions were extracted, and 2-methylpyridine, 2-ethylpyridine, and 3-ethylpyridine were identified and quantified by gas chromatography-mass spectrometry. The concentrations of all three pyridine compounds were higher in sidestream than in mainstream smoke solutions. In the sidestream smoke solutions, Advance cigarettes had the highest 3-ethylpyridine concentration, Omnis had the highest concentration of 2-methylpyridine, and 1R4Fs had the highest concentration of 2-ethylpyridine. This is the first time that this particular group of toxicants has been shown to be present in higher concentrations in sidestream than in mainstream smoke solutions, suggesting that there are previously unrecognized risks associated with exposure to sidestream smoke. Purified standards of the pyridine compounds identified in the smoke have been tested in dose-response studies on the oviduct and shown to affect oocyte pickup rate, ciliary beat frequency, and smooth muscle contraction at picomolar doses. Further study has compared the percent inhibition of oviductal functioning using whole mainstream and sidestream smoke solutions made using the commercial cigarettes. Previously mainstream smoke of 2R1 cigarettes showed 40 to 60% inhibition of oviductal functioning. Preliminary results show the highest inhibition with mainstream smoke of Marlboro, Camel, and Advance (45-80% inhibition) cigarettes, but significantly lower levels of inhibition with Omni cigarettes (2-6

T52

Do girls and boys smoke for the same reasons?

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While increased research attention has been paid to gender differences in adolescent smoking prevalence, relatively few studies have examined gender differences in motives for smoking. Teenage girls may take different approaches in developmental tasks. They may desire a different identity at home and require different social representation in the society. Among children in immigrant families, girls may adapt to the host culture differently from their male counterparts. Using an ethnically diverse sample of 8th and 9th grade adolescents attending schools in three Southern California districts (n = 3370, mean age = 14.03, 48.6% female, 43% Hispanic/Latino, 26% Asian, 15% White, 6% African-American), this study will examine gender differences in associations among smoking status (never smoked, experimental smoking, current smoking, and established smoking) and the following psychosocial measures: acculturation (including three constructs: language use, media, and social relation), self-image (including 5 constructs: physical appearance, anxiety, intellectual status, mood, satisfaction, and popularity), and restrained eating (using a subscale in the Dutch Eating Behavior Questionnaire). This study will also investigate gender differences for associations between smoking and psychosocial factors among various ethnic groups, especially between two immigrant populations: Hispanic/Latino and Asian adolescents.

Preliminary results using a subset (Asian-Americans) of the total sample demonstrated no significant gender differences in smoking status (R^2 = 0.309 for experimental smoking, R^2 = 0.443 for 30-day smoking, and R^2 = 0.924 for established smoking), a surprising result given the dramatic gender discrepancy in smoking in the countries of origin. Separate odds ratios were calculated and compared for girls and boys. Logistic regression revealed that acculturation, self-image, and weight concerns were associated with 30-day smoking for girls (p = 0.050 for acculturation, p = 0.004 for self-image, p = 0.032 for weight concerns), but not for boys (p = 0.097 for acculturation, p = 0.208 for self-image, p = 0.410 for weight concerns). For girls, high self-image was associated with a significantly lower rate of smoking, and high scores in weight concerns and acculturation were significantly associated with increasing probability of smoking.

Further analyses will examine ethnic variation in gender differences for associations among acculturation, self-image, weight concerns, and smoking. Gender differences among ethnic groups with respect to identity crises, the acculturation process, and physical attributes will be examined. This study may elucidate the unique psychological and cultural forces leading girls and boys to smoke. It may also allow the consideration of gender-specific needs in tailoring smoking prevention programs.

T53

Genderd ifferences in predictors of smoking cessation via an internet site

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Mounting evidence suggests that nicotine dependence is different for men and women, with seemingly greater relative risk of negative health outcomes for women. The objective of this study was to examine gender differences in factors that predict self-reported quit rates among smokers trying to quit on the World Wide Web. Data were collected from 4603 smokers recruited into a smoking cessation research study conducted over the World Wide Web. Baseline predictors included participants' self-report of the number of cigarettes smoked daily over the past year, whether there were any other smokers in their home, and confidence in the ability to quit over the next three months. In addition, psychological dependence on nicotine was measured with the Fagerstöm Test for Nicotine Dependence and depressive symptom levels were measured using the Center for Epidemiological Studies-Depression Scale. Quit rates (7-day abstinence) were obtained via the web at 1- and 6-month follow-ups.

Hierarchical logistic regressions were used to examine predictors of quit rates by gender. Missing baseline data were handled by case-wise deletion, leaving 2400 females and 1470 males in the analyses. Participants with missing follow-up data were considered to be smoking. Five demographic variables were controlled by entering them at the first step, and five psychosocial predictors were entered at the second step. With ten predictors in the final model, a criterion alpha of $p < .005$ was used to control for Type I error rate. Overall, tests of the full model against a constant-only model were statistically reliable, and there was reliable improvement in model fit with the addition of psychosocial predictors. For both women and men, greater quit confidence at baseline predicted 7-day abstinence at the 1-month follow-up. For men, there were no significant predictors of abstinence at the 6-month follow-up. For women, greater quit confidence *and* lower psychological dependence on nicotine at baseline predicted abstinence at the 6-month follow-up.

We conclude that confidence in one's ability to quit smoking is an important predictor of quit rates for both women and men over the short term (1-month follow-up). Both quit confidence and, particularly, nicotine dependence appear to be important predictors of quit rates for women over longer periods of time (6-month follow-up). These results suggest that psychosocial interventions should attempt to increase cessation self-efficacy for both men and women and, in addition, focus on methods for women to overcome psychological dependence on nicotine.

T54

Bayview-hunters point project toward smoking cessation

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Among all racial/ethnic groups, African Americans currently have the worst death rate from major tobacco related diseases. Because this group bears a disproportionate burden of tobacco-related health problems, it is particularly urgent that researchers focusing on tobacco control partner with African American communities to ensure that research is culturally relevant and useful. This project seeks to establish a strong, sustainable community-academic partnership, to gain experience working together, and to collect preliminary data and plan a larger proposal for rigorously-designed, community-based research aimed at promoting smoking cessation among adult African Americans (age 35 and older) in the Bayview-Hunter's Point community of San Francisco.

The specific aims of this pilot project are to educate academic personnel about the community's concerns related to tobacco and smoking cessation; to educate community research partners about the research process, tobacco's disproportionate effects on African Americans, and tobacco industry behavior; to conduct co-designed, partnered pilot research in the community, and to mutually develop a proposal for the community's intervention study.

We will present findings from community focus groups with smokers and former smokers, discuss capacity building efforts and planning for the partnership, and present findings from our community survey and Town Hall Meeting. The outcomes of this project will be a sustainable community-academic research partnership, preliminary studies, and a full proposal for an innovative yet rigorous community-partnered study.

T55

Status-seeking themes used to market cigarettes to African Americans

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In the past, advertisers marketed cigarettes to African-American smokers using status-seeking symbols. We investigate whether cigarette manufacturers continue to use these themes of sophistication in magazine advertisements to market cigarettes differently to African-American smokers.

We collected information on cigarette advertisements appearing in thirteen popular magazines circulated from 1980 to 1996. Among other information, we obtained the date and name of the magazine issue, the brand(s) of cigarettes advertised, and the themes depicted in the ads. We categorized the themes into three categories – sophistication, people and other – and confined the analysis to those advertisements that used the themes of sophistication and/or people. The Sophistication category included those themes that described people or activities pictured in the advertisements in which the people were elegant, glamorous, sophisticated, or sultry. The People category comprised all themes that described people or activities pictured in the ads in which the people pictured were not "sophisticated." The analysis was restricted to the menthol cigarette brands popular among African Americans and the cigarette brands most popular among white smokers. Each theme category was compared by the brands popular among African Americans and the brands popular among whites.

The association between themes and type of cigarette brand was investigated using a chi-squared analysis. Multiple logistic regression analysis was performed looking at the effect of ethnicity on thematic content of advertising, while controlling for magazine type, magazine periodicity, and publication year of magazine issue.

The advertisements that marketed brands smoked by African Americans were more likely to contain themes of *sophistication* and less likely to contain *people* themes than those ads for brands popular among white smokers. Future analysis will include the comparison of these same themes in magazines popular among African Americans and magazines popular with the general population.

T56

Tobacco use and acculturation: California residents of Korean descent

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Center for Behavioral Epidemiology and Community Health

Koreans have one of the highest smoking prevalence rates in the world. Despite their growing presence in California, Koreans are consistently underrepresented in all areas of behavioral research, and effective tobacco prevention and treatment have not yet targeted them due to a lack of systematic behavioral data. Most studies do not differentiate between subgroups of Asian and Pacific Islanders. Data in this study are drawn from a large population based survey of Korean descent (N=2756 adults and 561 adolescents) and show that acculturation to American society is one of the major determinants of smoking among this rapidly growing group. Half of the respondents reported "they have never smoked a cigarette, even a few puffs," (95% CI = 48.6, 52.4) and eighty percent of those surveyed are strongly committed to not smoke in the future. Approximately 18 percent of females and 80 percent of males reported having smoked in their lifetime (95% CI 77.3, 81.8), with 9 percent of these females (95% CI 7.4, 10.6) and 71 percent of these males (95% CI 68.4, 73.6) categorized as addicted smokers (smoking at least 100 cigarettes in lifetime).

A majority of the respondents identified themselves as either Korean or bicultural with Korean self-identity. 53 percent of the respondents identified as Korean and 20 percent of the respondents identified as bicultural with Korean self-identity. Only 4 percent of the sample identified themselves as bicultural with Western identity. 22 percent of the respondents reported them as bicultural with both Korean and Western self-identities. Gender and acculturation interact so that more acculturated males are less likely and more acculturated females more likely to smoke.

A sample of 2756 adults and 561 adolescents of California residents of Korean descent were interviewed by phone for approximately 30 minutes. First, a list of Korean surnames was developed using published sources and scanned into machine-readable form. Second, a subset of all telephone subscribers with Korean surnames was selected from all listed telephone subscribers in California. Third, the Korean subset of listings was randomly sampled and people of different ethnicity were screened out from initial calls. Questions on smoking uptake and cessation, family history of smoking related diseases, level of acculturation, levels of family satisfaction, exercise, diet, etc, were asked in the language of preference (Korean or English).

T57

Conducting school and community-based participatory research among Asian/Pacific Islanders and Hispanic/Latinos: Meeting the challenge of the Integrated Research Program (IRP) in multiethnic populations

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Over 50% of the population in the state of California is of Asian/Pacific Islander (API), Hispanic/Latino (H/L), American Indian or African American origin. Over 196 languages are spoken in the Los Angeles Unified School district alone. These various groups combined make California the most diverse state in the nation. To meet the health promotion needs of this population, the University of Southern California, Institute for Health Promotion and Disease Prevention Research (USC-IPR) obtained funding for an Integrated Research Program from TRDRP for conducting tobacco prevention research in a community and school based participatory fashion in Pacific Rim populations. With additional funding from NCI's USC TTURC, the program has been successful in engaging middle schools and their respective communities in the API and H/L population in an active participatory research process that is a model for the nation. This poster focuses on the process by which the USC-IPR research team engaged ethnically diverse youth, their families, teachers, cultural experts and key community members in this successful endeavor. , Collaboration from participating schools and communities was sought at every stage of the process from refining the research questions, developing questionnaires and curriculum, problem solving with an Advisory Committee as issues were raised, to disseminating early study findings via a newsletter. Advisory Committee members, parent groups, school personnel, community opinion leaders and cultural experts were brought in, as needed, to set the stage, enhance and maximize subject participation, facilitate the research and disseminate results in a culturally relevant and community appropriate manner. Needs for enhancing recruitment where met through participation of key cultural consultants, exchanges with key opinion leaders, and interventions made at strategic points in the recruitment effort. Retention of subjects was secured through continued exchanges of information with teachers, parents, and community in a culturally grounded manner, and the participation and a continuous presence of USC representatives in the schools involved in school activities beyond the specific research. Special meetings with school personnel took place in order to provide them with preliminary results and information, and obtain their feedback as we moved through the next steps of the research. Focus groups were conducted with key school personnel and community opinion leaders to assess process, provide feedback, and focus on how and when the information could be best disseminated to ensure that communities and the schools received maximum benefits from the research. Not only has this been a fruitful outcome in terms of conducting the research, but the USC-IPR research team enhanced the lives of the students and their families by delivering special classes, clinics, and developing a version of the curriculum to accommodate new English learners. In one participating school, we conducted a needs assessment, which resulted in the development of a new proposal to NIH to meet other needs of the school and the families living in that immediate community. The establishment of a two-way system of communication early on ensured our focus on the importance of community and school involvement in the research, identifying and managing barriers to implementation and setting the tone for next steps. This poster will show the interweaving process of the participatory research from start to finish and the necessary steps to secure participation at every level of the research enterprise in a multicultural setting.

T58

School-level differences in ethnic composition, academic performance, and SES by level of participation in a smoking prevention program

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A trial of a culturally-tailored smoking prevention program and its culturally-neutral counterpart aimed at early adolescents has been conducted over the past two years in Los Angeles-area public and parochial schools. In order to assess whether public schools at various levels of participation (main trial participation, pilot participation, or declined to participate/ruled out from participation) differed in demographic composition, analyses of variance were conducted of percentage of African-American, Asian, Hispanic, White, and Other ethnicity, as well as academic performance (as measured by API) and SES (as measured by percentage of students receiving free lunch). Tukey tests were used to further compare the means for scores that yielded significant results.

A total of 86 schools were considered non-participating, 20 were pilot schools only, and 9 participated in the pilot and the main trial. Currently only public schools are included in the analyses. Compared with non-participating schools, pilot schools had significantly more Asian and "Other" students, fewer Hispanic students, had a higher level of academic performance and a higher SES. Pilot schools also had a higher proportion of "Other" students than did main trial schools. There were no other differences between main trial and pilot only schools, and no differences between main trial and non-participating schools.

Since the trial targeted Asian and Hispanic students in equal proportions, the higher proportion of Asian students and lower proportion of Hispanic students in the pilot group is not surprising, as Asians were approximately 10% of the non-participating schools' composition while Hispanics comprised approximately 73%. While pilot schools differed from non-participating schools on several measures, there were no differences between the non-participating schools and the main trial schools. This indicates that despite recruiting schools that were thought to be able to provide maximum student participation, the findings of the study are still generalizable to students in California public schools.

T59

The influence of depressive symptoms on smoking behavior & intention to smoke in acculturating youth

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Depressive symptoms have been shown to be predictive of smoking initiation in several studies, but there has been considerable debate recently about the temporal relationship between depression and smoking. Some theorists contend that depression is a consequence of smoking. We explored the association between smoking and depression in the earliest stages of smoking among ethnic groups that are underrepresented in the literature. This paper looks at intention to smoke because it is one of the best predictors of actual smoking, precedes experimentation and regular smoking. We were able to assess the mindset of a young adolescent as they consider which habits they will or will not adopt. This study explored the relative impact of depression on smoking among two acculturating ethnic groups in Los Angeles area-Chinese, & Latino adolescents.

Over 800 seventh graders in the Los Angeles area participated in this cross-sectional analyses and completed measures of acculturation, depressive symptoms (CES-D), ever smoking, and intention to smoke. The ethnic background of participants were 19% white, 40% Latino, and 41% Chinese. The Chinese students were the least acculturated, and had the lowest levels of depressive symptoms. Latinos reported the highest levels of depressive symptoms, were most likely to intend to smoke in the next year, and were the most likely to have started experimenting with cigarette smoking. Acculturation, as measured by language use, was not predictive of smoking behavior or intention. Depressive symptoms remained a significant predictor of intention to smoke when acculturation, SES, gender and ethnicity were controlled for, but was predictive of actual smoking for Latinos only. Across ethnic groups those in the highest quartile of the CES-D were at two times the risk for smoking as compared to those in the lower quartiles.

Our findings suggest that depression is predictive of smoking and in turn may be a more robust risk factor for Latinos than for other groups. Some notable gender differences were also found. These results provide some support for depression preceding regular smoking. We are studying intention to smoke and early experimentation in order to better understand the initial stages of smoking. Most of this sample is not regular smokers. In this sample depression is correlated with experimental smoking. If nicotine effects brain chemistry in such a way as to cause depression these students have not experienced enough smoking to experience that kind of effect. Rather we see evidence that depressive symptomatology is related to the early adoption of unhealthy habits. These cross-sectional results suggest that future longitudinal studies should address ethnic differences in the impact of depression on smoking.

T60

Assessing project FLAVOR: a culturally tailored tobacco prevention program

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Project FLAVOR's goal was to develop a tobacco prevention curricula tailored by content and method of delivery for California's multicultural classrooms. The program was designed to address several factors that have been shown to be associated with tobacco use including perceived prevalence, the social consequences of tobacco use and refusal skill self-efficacy. Schools were randomly assigned to either receive Project Flavor or a standard tobacco prevention program that addressed the same factors without being designed to be culturally relevant. We administered a pretest just before implementation and an immediate posttest to 1800 students in 16 schools in Southern California.

The two groups did not differ in terms of ethnic composition, or ever smoking at pretest. Preliminary results show that Project FLAVOR produced several changes in the hypothesized direction. FLAVOR reduced perceived prevalence from time 1 to time 2; students at post were more likely to say that smoking would affect their relationship with their parents, and were less likely to endorse cognitive distortions. Comparisons between the programs at immediate post were less clear. At post, the two groups did not differ on intention to smoke, or in changes in perceived prevalence when all students were analyzed. Because FLAVOR focused particularly on Latinos and Asians, we looked at whether Latinos or Asians were more impacted by FLAVOR than by the standard program. For Latinos, we found a trend for a larger change in perceived social norms. Ethnic and gender differences were found in receptivity of program. Asian girls were least likely to enjoy both programs and Latino girls were most likely to respond that would like to do the program again.. These preliminary results demonstrate that project FLAVOR was able to affect the factors of greatest interest, and suggest that FLAVOR may over time have more impact for Latinos than a standard program.

T61

Implications of multiethnic categorization on risk behavior susceptibility and prevalence rates.

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Analyses were conducted to investigate how lifetime smoking behavior, alcohol use and smoking susceptibility rates in multiethnic participants changed with distinct definitions of multiethnicity. Data were analyzed from a survey that was conducted as part of a longitudinal trial of culturally relevant smoking prevention programs for ethnically diverse middle school students in Southern California. Multiethnicity was first defined to include participants who endorsed the following racial/ethnic combinations on the questionnaire: Black + White, Black + Asian, Black + Hispanic, Asian + Latino, and Asian + Black + Latino, for which the percentage of the total sample was 1.08%. The percentage rose to 11.70% when the combination of Latino + White was added to the definition of multiethnicity and to 14.05% when Latino and any other ethnic category was included in the multiethnic coding scheme.

Lifetime smoking rates calculated by ethnicity using the initial multiethnic coding scheme resulted in Black 11.7%, White 6.4%, Asian/PI 4.5%, Latino 10.9%, Other 9.1%, and Multiethnic 9.1% When multiethnicity was coded to include Latino and White, Multiethnic lifetime smoking rate maintained at 9.2% When multiethnicity was coded to include Latino and any other ethnicity, multiethnic smoking rates increased to 10.7% In terms of smoking susceptibility, when participants were asked if they thought they would smoke in the next year 9.1% of multiethnic participants belonging to our first multiethnic classification scheme were observed to indicate susceptibility. This number increased to 12.9% when the classification was expanded to include Latino + White, and maintained at 12.4% when the classification was further expanded to include Latino and any other ethnic category. When asked if participants had ever used or tried at least one drink of alcohol, 12% in the first multiethnic category indicated they had. This number increased to 23.3% when Latino + White was added to the category and 21.8 when Latino and any other ethnicity was added to the definition of mutiethnicity

The multiethnic endorsements obtained in this study were seen to be similar to those obtained in other studies (Unger, et al., 2000) looking at health risk behaviors in ethnically diverse adolescents. Results from these analyses indicate the importance of taking into consideration the distinct ways of coding multiethnic categorization. Decisions regarding the coding of the ethnic category of multiethnicity may have direct implications for conclusions about smoking prevalences, smoking susceptibility rates, and other health risk behaviors, all of which could drive epidemiologic results, and affect general research conclusions regarding health risk behaviors in ethnically diverse population samples which may in turn influence public policy decisions.

T62

Biculturalism is protective against psychosocial risk factors for adolescent smoking

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Many California adolescents are immigrants or children of immigrants. Immigrants and their families face unique challenges as they acculturate to the United States. Some of those challenges could cause stress, family conflict, and rebelliousness, which might increase adolescents' risk of smoking. Biculturalism—retaining aspects of one's culture of origin while also learning a new culture—could give adolescents a wider array of coping skills and sources of social support. Therefore, biculturalism might have protective effects against adolescent risk behaviors such as smoking.

As part of a longitudinal study of culture and adolescent smoking, this study examined biculturalism among Hispanic and Asian-American adolescents in California and assessed the effects of biculturalism on their smoking behavior and psychosocial correlates of smoking. Hispanic (n=1039) and Asian-American (n=525) students in Southern California completed surveys of their acculturation, cultural values, tobacco-related attitudes and beliefs, and lifetime smoking behavior in 6th grade and again in 7th grade. Path analyses were conducted to determine the direct and mediated effects of biculturalism on smoking over a one-year period.

Biculturalism did not have a direct effect on lifetime smoking among Hispanics or Asian-Americans. However, biculturalism did have an indirect effect on smoking, mediated by psychosocial variables. Controlling for age, sex, and socioeconomic status, biculturalism was associated with a lower probability of having friends who smoke among Asians and Hispanics. Having fewer friends who smoked, in turn, was associated with a lower risk of smoking. In both ethnic groups, biculturalism was associated with higher levels of parent-child communication; among Asians, biculturalism also was associated with less unsupervised after-school time (latchkey). Among Hispanics, biculturalism was associated with the values of interpersonal harmony (similar to the traditional Hispanic value of simpatia), and family support (similar to the traditional Hispanic value of familism).

The results of this study indicate that biculturalism has potentially protective effects against adolescent risk behaviors such as smoking. Bicultural adolescents are less likely to choose smokers as friends, perhaps because they have better communication with their parents and/or less unsupervised time. Among Hispanics, biculturalism also is associated with protective traditional cultural values that decrease the risk of smoking. In the diverse society of California, feelings of belongingness to two cultures might provide adolescents in immigrant families with additional coping skills and social support. If bicultural competence skills can be taught to adolescents in acculturating families, they might provide adolescents with the social resources they need to avoid health risk behaviors such as smoking.

T63

Ethnicity, immigration, and spontaneous self-concept: implications for smoking interventions.

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The spontaneous self-concept is self-related information that dominates cognitions and consequent behaviors. Previous studies suggest that individuals from Eastern cultures are "collectivist", and tend to describe themselves in relation to others, whereas individuals from Western cultures are "individualist", and tend to provide ego-centered self-descriptions.

The purpose of this study was to determine whether spontaneous self-concept varied according to ethnicity and immigrant status in a multiethnic, multicultural sample of 1,391 6th grade adolescents attending Los Angeles schools. Students' spontaneous self-concept was assessed with a modified, shortened version of Kuhn and McParland's (1954) Twenty Statements Test. Ten blank lines were provided to answer the question, "Who am I?" Responses that referred to social roles (e.g., "I am a student") or relations to others (e.g., "I like my Mom") were coded as "collectivist." Responses that referred to abstract self-descriptions (e.g., "I am nice") or specific self-attributes (e.g., "I have brown hair") were coded as "individualist."

Proportions of Collectivist and Individualist responses were compared by ethnic group (Latino, Asian, and White respondents) and by immigrant status (immigrant, first generation US-born, at least second generation US-born). Asian and Latino students generated a larger proportion of collectivist responses and a smaller proportion of individualist responses relative to White students. Students who were at least second generation Americans had a higher proportion of individualist responses than immigrant students. However, the proportion of collectivist responses did not differ across generations. Such findings suggest that the adolescent self-concept varies by ethnicity and immigration status, and may have important implications for framing smoking prevention programs.

T64

Parenting characteristics and adolescent smoking in an ethnically diverse longitudinal sample

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The purpose of this study was to determine whether ethnic differences in smoking exist, and if parenting characteristics in the 6th grade predicted smoking in the 7th grade. Data was collected from a sample (Asian, Hispanic/Latino, Multiethnic and Non-Hispanic White) of middle school students in urban Southern California. Students completed self-report questionnaires twice approximately 1 year apart. Lifetime smoking, parenting characteristics (parental smoking status, adolescents' perceptions of parent-child communication, and parental monitoring) and demographics were assessed. Logistic regression analyses of lifetime smoking controlled for demographics (SES, generation status, family structure, age, and sex).

Of 922 students at time 2 the mean age was approximately 12.3 years. At time 1, Asians and Whites had lower rates of smoking relative to Latino/Hispanics. During time 2 Asians had significantly lower rates of smoking initiation relative to Latino/Hispanics. Parental monitoring was protective of smoking at time 1 (OR=0.63; $p<.0001$) and at time 2 (OR=0.63; $p<.01$). Parental communication was protective at time 1 (OR=0.73; $p<.0001$) but not at time 2. Parental smoking was a risk factor at time 1 (OR=1.48 $p<.01$) but only marginally significant at time 2 (OR=1.54; $p=.09$). At time 2, living with both parents (OR=0.34; $p<.01$) was the strongest protective factor.

These findings suggest that ethnic differences in the age of smoking initiation should be considered when designing smoking prevention curricula. Even though parental influences often decline with age, parental monitoring remained important for smoking. Future studies should investigate how family structures shape differences in adolescent smoking.

T65

The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and drug-naive mice

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Research efforts in our laboratory are focussed in understanding the neurobiology of nicotine reinforcement and nicotine dependence in order to assist in the development of novel therapeutics for smoking cessation. Glutamate is an excitatory brain neurotransmitter that has been implicated in the rewarding effects of nicotine, one of the main ingredients in tobacco smoking that leads to dependence. Nicotine increases glutamate release in the ventral tegmental area and the nucleus accumbens, and thus enhances dopamine neurotransmission in the mesolimbic system that has been implicated in mediating the rewarding effects of drugs including nicotine. Metabotropic glutamate receptors 5 (mGluR5) are found in the nucleus accumbens and may play a role in modulating the post-synaptic response to glutamate and dopamine. The aim of the present study was to investigate the effects of the mGluR5 antagonist 2-methyl-6-(phenylethynyl)-piperidine (MPEP) on intravenous nicotine self-administration in Wistar rats and DBA/2J mice.

Rats were allowed to self-administer nicotine ((0.01, 0.03 mg/kg/inf) or respond for food on a fixed-ratio reinforcement schedule. Drug-naive mice were acutely exposed to nicotine (0, 0.016, 0.048, 0.16, 0.48 ug/inf) self-administration using a yoked-controlled procedure and a fixed ratio schedule; analyses indicated that self-administration was obtained only at the 0.048 ug/inf dose by the mice. MPEP ((0, 1, 3, 9 mg/kg; i.p.) dose-dependently reduced nicotine self-administration, with no effect on food-maintained responding in the rats. Further, MPEP ((0, 5, 10, 20 mg/kg; i.p.) decreased nicotine self-administration in the mice.

These results indicate that blockade of mGluR5 decreases the rewarding effects of nicotine in both rats and mice; and are consistent with findings from other laboratories showing a role of mGlu5 receptors in cocaine self-administration. In conclusion, glutamate appears to positively modulate the rewarding effects of drugs of abuse, such as nicotine, through mGlu5 receptors. It is hypothesized that mGluR5 plays an essential role in mediating the reinforcing effects of acute nicotine, possibly via modulation of mesolimbic dopaminergic neurotransmission. The present studies were conducted in non-dependent rats that had access to nicotine for 1 hour per day, five days per week. Future work will focus in the development of rat models of nicotine dependence involving intravenous nicotine self-administration in dependent rats that best mimic the human drug use patterns. Then, we will investigate whether pharmacological treatments, such as mGluR5 antagonists and other behavioral manipulations, would decrease nicotine intake in dependent animals also. In conclusion, the results of the present study suggested that antagonists at mGluR5 receptors may be useful pharmacological treatments for nicotine dependence and tobacco smoking.

T66

The antidepressant bupropion enhances brain reward function and reverses nicotine withdrawal in the rat

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Bupropion is an atypical antidepressant and the only non-nicotine-based therapy approved for smoking cessation. Its use has raised debate as to how a non-nicotine-based drug can aid in smoking cessation. The present studies were conducted to assess the effects of bupropion on brain reward function under baseline conditions and subsequent to withdrawal from chronic nicotine administration using the intracranial self-stimulation paradigm in rats in order to understand the factors that contribute to bupropion's therapeutic properties.

Acute bupropion administration, similar to nicotine, dose-dependently lowered reward thresholds in non-withdrawing subjects indicating an increase in reward. Interestingly, a subeffective dose of bupropion blocked the threshold lowering effects of nicotine. Rats withdrawn from chronic nicotine exhibited increases in somatic signs of withdrawal and elevated brain reward thresholds reflecting "diminished interest or pleasure" in the rewarding stimuli. Bupropion reversed both the reward deficit and the somatic signs, with the highest dose inducing a protracted reversal of the threshold elevation.

These data provide evidence that bupropion acts on at least three levels to alter brain reward circuits influenced by nicotine, in addition to reducing the expression of somatic signs. First, bupropion increases brain reward function under baseline conditions in non-withdrawing subjects. Second, at low doses bupropion blocks the rewarding effects of nicotine. Third, bupropion reverses the negative affective aspects of nicotine withdrawal. Such actions are likely to act in concert to mediate the unique anti-smoking properties of bupropion. Future research will focus in exploring the brain sites involved in nicotine dependence and in mediating the therapeutic effects of bupropion in rats. Such studies will promote our understanding of the neurobiological and behavioral mechanisms of nicotine dependence and lead to the development of better therapeutics for the treatment of nicotine withdrawal and addiction.

T67

Nicotine withdrawal decreased prepulse inhibition and decreased sensitivity to self-administered nicotine in DBA/2J mice

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Smoking cessation in humans leads to an abstinence syndrome that includes affective and somatic symptoms that have been hypothesized to contribute significantly to craving and relapse to tobacco smoking. The present studies were designed to characterize the somatic symptoms of spontaneous and mecamylamine-precipitated nicotine withdrawal, and to evaluate the effects of nicotine withdrawal on intravenous nicotine self-administration, acoustic startle response and prepulse inhibition (PPI) in DBA/2J mice. Intravenous nicotine self-administration reflects the rewarding effects of acute nicotine. The startle response reflects reactivity to environmental stimuli, while PPI reflects sensorimotor gating processes. The aims of the present studies were to examine the effect of nicotine withdrawal in these behavioral processes.

Nicotine dependence was induced by continuous nicotine infusion via osmotic minipumps (18 mg/kg/day for 14 days). Intravenous nicotine self-administration was studied across a range of unit doses (0.016-0.48 mg/infusion) before and after the induction of dependence. Mice acquired nicotine self-administration at the unit dose of 0.048 mg/inf before the induction of nicotine dependence. After minipump removal, a decreased sensitivity to the reinforcing effects of self-administered nicotine in dependent mice was found during withdrawal. Neither spontaneous nor mecamylamine precipitated (0.3-3 mg/kg, s.c.) nicotine withdrawal affected the expression of somatic signs, such as rearings, head, body or forelimb shakes. Jumping activity was significantly increased in nicotine-withdrawn mice during spontaneous nicotine withdrawal, but not during mecamylamine-precipitated withdrawal. Nicotine withdrawal decreased PPI, tended to increase startle reactivity and increased general activity while in the startle chamber. The decreases in PPI were reversed by self-administered nicotine.

The results suggest that acute nicotine withdrawal is characterized by decreased PPI and decreased sensitivity to the reinforcing effects of acute nicotine reflecting tolerance-like changes in the sensitivity to acute reinforcing effects of nicotine. In mice, these changes were observed in the absence of an overt somatic abstinence syndrome. The PPI deficits were reversed by further nicotine self-administration. In conclusion, these results indicate that nicotine withdrawal is associated with deficits in sensorimotor gating processing in mice that can be reversed by further nicotine administration. Finallly, the results suggest that continuous nicotine infusion leads to tolerance to the aversive effects of a high nicotine dose. Future work will focus on the study of antipsychotic drug treatments in nicotine dependence, nicotine self-administration and nicotine withdrawal signs in both rats and mice in order to investigate the factors that contribute to the high smoking rates seen in schizophrenia patients.

T68

Characterization of α4β2 nicotinic receptor-fluorescent protein chimeras

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Nicotine addiction underlies directly or indirectly most of the tobacco-related diseases, which are one of the leading preventable causes of mortality. Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand gated cation channels formed by various combinations of α (α2-α10) and b subunits (β2-β4). α4β2 nAChRs constitute the most abundant high affinity nicotinic receptors in the brain, and their activation in tobacco smokers play a major role in mediating nicotine addiction. Although the mechanisms of nicotine addiction remain unclear, altered trafficking of neuronal nicotinic receptors (nAChR) may be one contributing mechanism that modulates neuronal excitability.

To study such mechanisms, we made fluorescently tagged nAChR subunits: yellow fluorescent protein (YFP) at the N-terminus (N) or intracellular loop (M3-M4) of α4 and cyan FP (CFP) at the C-terminus (C) or M3-M4 loop of β2. Electrophysiology and fura-2 recording on transfected HEK293 cells showed that N-α4-YFP and/or C-β2-CFP function less than wild-type (WT) α4β2. Happily, M3-M4 α4-YFP β2-CFP had near-WT ACh dose-response relations, maximal responses, and Ca^{2+} flux. We examined fluorescence energy transfer (FRET) to compare the relative proportions of assembled α4β2 subunits in different subcellular and neuronal compartments. Multi-spectral fluorescence imaging and linear unmixing confirmed that α4-YFP associates with β2-CFP: when YFP was bleached, CFP was dequenched, showing robust FRET. PKC-transfected, PMA treated cells displayed larger ACh responses, more surface fluorescence, and larger FRET efficiency (E, 37 ± 4%) than untreated cells (22 ± 2%). Like WT α4β2 in untransfected midbrain neurons, transfected M3-M4 α4-YFP and β2-CFP nAChRs were distributed in the soma and dendrites; and E was greater in dendrites (41 ± 11%) than soma (29 ± 3%). Thus, cell surface and dendrites express a greater proportion of assembled nicotinic receptors than intracellular compartments or neuronal somata.

We plan to produce an α4-YFP nAChR knock-in mouse in order to examine altered subcellular localization in neurons and quantify changes in expression of nicotinic receptors in neurons in a variety of brain regions during prolonged nicotine administration and withdrawal. Secondly, we will further examine the mechanism by which PKC effects increased surface expression of α4b2 nicotinic receptors. We anticipate that more detailed knowledge will be garnered on the mechanism of α4b2 nicotinic receptors in mediating nicotine addiction.

T69

Differences between smokers and nonsmokers in tests of selective attention and working memory: effects of abstinence and cigarette smoking

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Cognitive enhancing effects of nicotine may play a role in the initiation and maintenance of smoking. In fact, differences in cognition have been observed between smokers and nonsmokers, and such differences may precede and contribute to nicotine dependence. This study compares the performance of smokers and nonsmokers on tests of selective attention and working memory, and evaluates how nicotine abstinence and smoking affect performance in smokers.

Thirty-one subjects participated in two groups: nonsmokers (n=15, 33.9±2.4 (s.e.m.) years; ≤5 cigarettes/lifetime) and smokers (n=16; 31.6 ±2.5 years; ≥15 cigarettes/day). They performed the Stroop Color-Word Interference Task, which tests selective attention, and the N-Back Task, which tests working memory. The Stroop Task requires participants to ignore the meaning of a written word (a color name) that is presented while saying the color in which it appears. The color and meaning of the word match in the congruent condition, and do not in the incongruent condition, when the subject must suppress the tendency to read while reporting the color they see. In the N-Back Task, a sequence of letters is presented, and the subject must respond when seeing a letter that appeared "N-Back" in the sequence. There were four levels of difficulty: in the "0-Back" condition, the subject was to respond whenever the letter "X" appeared; for "1-Back", a response was required whenever a letter was repeated immediately. Similarly, in the 2-back and 3-back conditions one or two letters (respectively) intervened before the target letter and its repetition. There were four tests (on 2 days): two administered after the smokers had smoked to satiety and two others 16–24 h after initiating abstinence from smoking. Tests on each day were conducted before and after smoking. Nonsmokers participated at corresponding times.

On the Stroop Task, smokers had a longer mean reaction time (RT) than nonsmokers in congruent and incongruent conditions, in each of the 4 tests (pre- and post-smoking at satiety and during abstinence). The difference between groups was greater when the smokers were tested in the abstinent state.

On the N-Back Task (0-Back, 1-Back, 2-Back and 3-Back), smokers exhibited longer RT compared with nonsmokers, and when tested in abstinence vs. satiety (each N-Back condition) smoking reduced RT (1- and 2-Back). Smokers made more errors of commission (possibly reflecting impulsivity) than nonsmokers (1- and 2-Back) and more errors of this type during abstinence than satiety. Smoking on either test day generally did not reduce errors of commission. Smokers made more errors of omission (presumably reflecting lack of attention) than nonsmokers (2- and 3-Back) and smoking reduced the number of errors, primarily during abstinence.

The findings indicate that smokers and nonsmokers differ in selective attention and working memory, even during smoking satiety. Impaired performance accompanied smoking cessation, and smoking improved performance. We are applying functional magnetic resonance imaging in a subset of the research participants discussed here in order to elucidate the neurobiological bases of the cognitive differences observed.

T70

Pharmacology of nicotinic receptors in PreBötzinger Complex that mediate modulation of respiratory pattern

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Some prevalent disorders of respiratory control such as sudden infant death syndrome (SIDS) and sleep-disordered breathing (sleep apnea) are known to correlate to cigarette smoking, but the underlying biological mechanisms describing how smoking causes SIDS and sleep apnea are not understood. Nicotine from cigarette smoke acts on nicotinic acetylcholine receptors (nAChRs) in the brain affecting respiratory pattern. The goal of this project is to understand the cellular and synaptic mechanisms underlying regulation of breathing by nicotine and to identify subtypes of nAChRs mediating these nicotine effects.

The preBötzinger Complex (preBötC) in the brainstem is the putative brain site for respiratory rhythm generation in mammals. We have delineated the mechanisms underlying regulation of respiratory pattern by nicotine. Nicotine acts on nAChRs in the preBötC to regulate respiratory frequency and pattern. Activation of nAChRs modulates excitatory neurotransmission by potentiating tonic excitatory input into preBötC inspiratory neurons and inhibiting excitatory coupling between these neurons (Shao & Feldman, 2001), a subset of which is believed to generate respiratory rhythm. Using a medullary slice preparation containing the preBötC from neonatal rat, we examined the effects of subtype selective nAChR antagonists on nicotine-induced responses in inspiratory neurons in the preBötC and on the respiratory-related motor activity from the hypoglossal nerve (XIIn). The α7 nAChR antagonists α-bungarotoxin or methyllycaconitine (MLA) had little effect on the actions of low concentrations of nicotine (0.5 μM, approximately the arterial blood nicotine concentration immediately after smoking a cigarette) which included: an increase in respiratory frequency, a decrease in amplitude of XIIn inspiratory bursts; a tonic inward current associated with an increase in membrane noise, an increase in the frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs), and a decrease in the amplitude of inspiratory drive current in voltage-clamped preBötC inspiratory neurons. These nicotinic actions were completely reversed by nicotinic antagonists dihydro-β-erythroidine (DH-β-E) or hexamethonium and reduced by d-tubocurarine. RJR-2403 (0.5-1 μM), an agonist selective for α4β2 nAChRs, increased respiratory frequency to 186% and decreased the amplitude of XIIn inspiratory bursts to 83% of baseline. In voltage-clamped preBötC inspiratory neurons, RJR-2403 induced a tonic inward current of –15.2 pA associated with an increase in membrane noise, increased the frequency to 157% and amplitude to 106% of spontaneous EPSCs and decreased the amplitude of inspiratory drive current to 80% of baseline. MLA had little effect on RJR-2403 actions, while DH-β-E completely reversed them.

In this study, we have demonstrated that the predominant subtype of nAChRs in preBötC in neonatal rats that mediates the regulation of respiratory pattern by nicotine is an α4β2 combination and not an α7 subunit homomer. The parallel changes of the cellular and systems level responses induced by different nicotinic agonists and antagonists support the idea that modulation of excitatory neurotransmission affecting preBötC inspiratory neurons is a mechanism underlying the cholinergic regulation of respiratory pattern. This study provides a useful model system for evaluating potential therapeutic cholinergic agents for their respiratory effects and side effects. We have identified compounds which antagonized the acute effects of nicotine on respiratory frequency and pattern (Shao & Feldman, 2002).

The future direction of this project is to further study the mechanisms underlying the differential modulation of excitatory neurotransmission by nicotine in preBötC neurons. Insight into these cellular processes will provide a physiological basis for prevention, diagnosis and treatment of SIDS, sleep apnea and respiratory failure during organophosphate poisoning, in particular, nerve gases. SIDS and sleep apnea are a substantial public health burden due to their widespread prevalence.

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T71

Cadherin-related receptors at nicotinic synapses in the chick ciliary ganglion

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Nicotine, the primary bioactive substance in tobacco, exerts its effects in the body by acting on nicotinic acetylcholine receptors (nAChRs). These receptors are present throughout the nervous system and are normally activated by the endogenous neurotransmitter acetylcholine (ACh). Both ACh and nAChRs appear very early during vertebrate development, and early nicotine-exposure has long-lasting effects on the developing nervous system. One class of nAChRs containing the α7-subunit is particularly abundant in the nervous system (α7-nAChRs), and has a high relative permeability to calcium. Depending on their subcellular localization these receptors exert multiple functions including modulation of synaptic transmission at early stages during development.

We are interested in molecules that might regulate nAChR function and distribution at nicotinic synapses. A family of cell surface receptors termed cadherin-related neuronal receptors (CNRs) has been proposed to contribute to synapse formation and specification in the brain. We use the chick ciliary ganglion (CG) as a model system to test the hypothesis that CNRs may contribute to the development of nicotinic synapses. Here we report cloning of two CNR family members and several splice variants from the CG by screening a phage cDNA library. Using non-radioactive in situ hybridization we analyzed the mRNA distribution in embryonic CG and brain. To determine CNR protein expression and subcellular distribution we generated several antibodies. Immunohistochemistry suggests that CNRs are concentrated on somatic spines in CG neurons, specialized post-synaptic structures that are also enriched in α7-nAChRs. Developmental analysis shows that CNRs appear early during synapse formation. Interestingly, the CNR expression pattern closely parallels that of α7-nAChRs. To address the function of CNRs at the synapse, we prevented synapse formation onto ciliary neurons by ablating the midbrain region containing neurons normally projecting to the CG, and found that CNR and α7-nAChR expression and localization are altered drastically in the absence of presynaptic input.

The expression and localization of CNRs as well as their dependence on innervation suggest that they play important roles in the formation of nicotinic synapses in the CG. Revealing the molecular mechanisms underlying the development of nicotinic synapses may suggest developmental events at risk by prolonged exposure to nicotine, e.g. during pregnancy.

T72

Altered spinal expression of nicotinic receptor subunit gene cluster on chromosome 8 may be linked to augmented responses to spinal nicotinic agonists in genetically hypertensive rats.

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Spontaneously hypertensive rats (SHR) exhibit enhanced pressor, heart rate and nociceptive responses to spinal nicotinic agonists. This accompanies a paradoxical decrease in spinal nicotinic receptor number, particularly in the superficial dorsal horn region, in SHR compared to normotensive rats. The congenic strain, SHR-Lx, with an introgressed chromosome 8 segment from the normotensive Brown-Norway (BN)-Lx strain exhibits reduced blood pressure. This segment contains a gene cluster for three nicotinic receptor subunits ($\alpha3$, $\alpha5$ and $\beta4$) expressed in the nervous system. We examined the implication of this gene cluster in the enhanced responsiveness of the SHR. Pressor and nociceptive responses to spinal cytisine, a nicotinic agonist, were diminished in SHR-Lx. Moreover, with repeated administration, these responses desensitized faster in SHR-Lx and progenitor BN-Lx, than in progenitor SHR/Ola. This implicates the gene cluster, in both cardiovascular and nociceptive responses to spinal nicotinic agonists. Immunohistochemical studies with affinity purified polyclonal antibodies against specific nicotinic receptor subunits identify expression of $\alpha3$, $\alpha4$, $\alpha5$, $\beta2$ and $\beta4$ subunits in various spinal neurons and terminals. Interestingly, $\alpha3$, $\alpha5$ and $\beta4$ subunits were identified to be present presynaptically in the superficial dorsal horn region where the primary afferent terminals project. Our present studies are aimed to determine if there is an association between the lowered expression of nicotinic receptor sites and the expression of $\alpha3$, $\alpha5$ and $\beta4$ subunits in the superficial dorsal horn region in SHR compared to normotensive rats. Since diminished responsiveness to agonist stimulation is greater than the basal blood pressure differences between the strains and the introgressed rat chromosome maps to a QTL in human hypertension, polymorphisms in the three nicotinic receptor genes become candidates for altered central control of blood pressure.

T73

PDZ-domain proteins interact with nicotinic receptors

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The behavioral effects of nicotine depend on its binding to and activating specific receptors, called nicotinic receptors, found on neurons in the nervous system. Nicotinic receptors are positioned on the surfaces of neurons where they normally respond to the neurotransmitter acetylcholine when released at the synapse (the sites where signals from one neuron are communicated to another). Localizing the nicotinic receptors to specific regions on the neuron allows for precise activation of subsets of receptors, distinct signaling to the interior of the cell, and separate regulatory control of receptor populations. Thus, how and where nicotinic receptors are positioned on the neuron determine their role in the behavioral effects of nicotine. The proteins and mechanisms that position nicotinic receptors at these locations are not known. It is the goal of this project to identify the proteins that interact with nicotinic receptors and determine the roles of these proteins in clustering and targeting nicotinic receptors to specific locations on neurons.

I have identified a family of proteins containing specific protein-interaction domains called PDZ domains that interact with nicotinic receptors and may play a role in organizing and positioning nicotinic receptors at specific locations on neurons. Using neurons from the chick ciliary ganglion as a model system I have found that PDZ-proteins co-localize with a subset of nicotinic receptors at synaptic sites. The PDZ-proteins and nicotinic receptors were identified by specific antibodies and visualized by fluorescence microscopy. Expressing combinations of nicotinic receptors and PDZ-proteins in non-neuronal cells demonstrated specific PDZ-protein/receptor interactions that could be confirmed by co-immunoprecipitation of the components. The interactions depended on the form of PDZ-protein expressed and were subunit-specific for nicotinic receptors. Association of PDZ-proteins and native nicotinic receptors from neuronal preparations was also observed. Experiments designed to disrupt the interaction of the nicotinic receptors and the PDZ-proteins in non-neuronal cells have been done and the results suggest that a similar strategy would be useful in determining the role of the PDZ-protein/receptor interaction in neurons.

PDZ-domain proteins have additional protein interaction domains that allow them to interact with a variety of cellular regulators in addition to the nicotinic receptors. Thus, these proteins may play a key role in organizing nicotinic receptors and providing a protein scaffold for bringing additional regulatory components in proximity to the receptors. These studies are providing basic information regarding the function and regulation of the receptors that mediate nicotine's behavioral effects.

T74

Synthesis and biological evaluation of heteroaromatic compounds as novel smoking cessation agents

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In the State of California, approximately one out of five adults smoke tobacco. Many of these individuals are desirous of quitting smoking because they are aware of the evidence that smoking is a leading cause of preventable death. However, counseling and other self-help programs to help smokers decrease smoking or decrease the ingestion of nicotine are not one hundred percent successful. Therefore, an urgent need exists to develop new therapeutic agents that could work in conjunction with these counseling and self-help programs.

(*S*)-Nicotine is a prominent pharmacologically active substance present in tobacco. Nicotine causes complex central nervous system, behavioral, cardiovascular, endocrine, neuromuscular and metabolic effects in humans. Nicotine is one of the most addictive substances known. After administration to humans, nicotine undergoes extensive metabolism. The large majority of nicotine metabolism goes through one key metabolic step: the cytochrome P-450 2A6-dependent formation of nicotine $\Delta^{1',5'}$-iminium ion. In the presence of aldehyde oxidase, nicotine $\Delta^{1',5'}$-iminium ion is converted to cotinine. Cotinine has a long half life and is a useful marker for nicotine exposure and also is a functional indicator of cytochrome P-450 2A6 activity. Cotinine can be further metabolized, but the vast majority of nicotine (i.e., as much as 80% of a normal dose) is primarily dependent on cytochrome P-450 2A6 for initial oxidation that leads to eventual decrease in pharmacological activity. If humans modulate their smoking to control nicotine consumption as has been previously suggested, it is possible that individuals with decreased nicotine metabolism will be predisposed to ingestion of lower amounts of nicotine and reduced addiction liability. Evidence points to modulation of cytochrome P-450 2A6 as a selective means to pharmacological intervention of smoking.

A library of heteroaromatic compounds have been designed and chemically synthesized. An assay of the inhibition of coumarin hydroxylase (a high-throughput assay for cytochrome P-450 2A6 inhibitors) is used to determine the lead candidates within the library. The lead compounds are subsequently assayed as inhibitors of cytochrome P-450 2A6 by HPLC and radiometric assays previously described in our laboratory. The synthesis, lead optimization and inhibitory effect of the new compounds on cytochrome P-450 2A6 activity will be discussed.

Development of novel compounds to act to decrease cigarette smoking or to treat nicotine dependence is directly relevant to the goals of the University of California Tobacco-Related Disease Research Program. Successful elaboration of a smoking cessation medication would reduce the human and economic cost of tobacco use by decreasing smoking and decreasing lung cancer.

T75

Chemical synthesis and pharmacological evaluation of inhibitors of nicotine metabolism: novel smoking cessation agents

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The major pharmacologically active chemical in tobacco is (S)-nicotine. People smoke cigarettes to obtain a certain concentration of nicotine in the blood stream and the brain. Some individuals smoke a great deal more than others and ingest a good deal more nicotine. For example, men metabolize nicotine much more rapidly than women and on the whole, men consequently smoke more cigarettes per person than women. This type of difference in smoking behavior has also been observed in different ethnic groups as well. The amount of nicotine in the blood is dependent on the rate it is ingested as well as the rate it is eliminated from the body. If humans modulate their smoking behavior to control nicotine consumption, it is possible that individuals with decreased nicotine metabolism will be predisposed to smoke less and ingest lower amounts of nicotine and eventually reduce their addiction liability. Less smoking leads to lower exposure to other toxic materials in tobacco smoke and this may lead to less adverse health risks as well. The goal of our work is to develop selective smoking cessation agents that could help reduce the number of cigarettes smoked. By treating smokers with agents that decrease the number of cigarettes smoked per day and at the same time providing counseling and self-help programs, individuals would be given an opportunity to decrease smoking altogether.

We have studied the metabolism of nicotine for some time now and have developed approaches to selectively inhibit this metabolic pathway. In particular, we have begun to understand the specific structural requirements for inhibition of cytochrome P-450 2A6 (CYP2A6), the key first metabolic step in the overall cascade leading to the elimination of nicotine. CYP 2A6 is an NADPH-dependent hemoprotein that functions in a classical reduction-oxidation cycle involving a perferryl oxygen complex (i.e., $FeO3^{+}$) and an odd electron abstraction/rebound mechanism. Recognizing the fundamental mechanism of the enzyme and utilizing the currently known (albeit sparsely described) substrate specificity, we designed several classes of CYP2A6 inhibitors. The compounds were chemically synthesized (in many cases by multi-step synthetic pathways) and pharmacologically evaluated in vitro. Inhibitors containing centers of chirality were separated and individually tested. Some of the initial compounds were not highly active, but iterative structure-function studies and lead optimization provided refined lead compounds that were highly potent inhibitors. Currently, we are evaluating the potency and selectivity of the most active lead compounds as inhibitors of CYP2A6. We are also evaluating selected inhibitors as agonists and antagonists of other pharmacological targets to determine the pharmacological profile of the most promising agent.

This work is responsive to the California State Tobacco Related Research Disease Program goal of reducing the human and economic cost of tobacco use. Our work has already resulted in the identification and characterization of a number of agents that inhibit nicotine metabolism and potentially blunt the effects of nicotine on the central nervous system. Elaboration of such an agent if elaborated into a medication could be very useful in decreasing smoking addiction and preventing lung cancer.

T76

The fate of nicotinic receptors in aging and nerve damage

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Determining the fate or role of nicotinic receptors in aging is of general public health interest. Nicotinic receptors in the brain are thought to be involved in the addiction to the nicotine present in tobacco smoke. Elderly tobacco users often report the smoking-related alleviation of symptoms related to neurological afflictions including Alzheimer's and Parkinson's diseases, as well as schizophrenia. In addition, some long term effects of smoking involve peripheral autonomic nervous functions involving nicotinic receptor dysfunction.

We have shown that communication across a cholinergic synapse (a synapse that utilizes the neurotransmitter acetylcholine and nicotinic receptors for chemical neurotransmission) in the autonomic nervous system is severely attenuated in advanced age, as seen in synapse elimination and reduced electrical activity (usually referred to as a postsynaptic potential). An important question to answer is whether the reduction in synaptic efficacy originates in the pre- or postsynaptic cell. By measuring the frequency and magnitude of miniature postsynaptic potentials (the small basic units of synaptic transmission) it is possible to get a first glimpse at the relationship between events in the two communicating cells. Initial results indicate no cause and effect relationship between events in the two cells. Rather, it is more likely that changes in both neurons originate downstream from an event that triggers synaptic withdrawal and possibly nicotinic receptor downregulation. In addition, our data suggests that the process of synapse elimination occurs at individual boutons of the same input axon in an independent manner. Recently, we have been able to visualize the nicotinic receptors on individual neurons from young mice. We hope to soon measure changes in the number of nicotinic receptors on old neurons.

One of our goals is to mimic the effects of aging on synaptic transmission with a model using postsynaptic nerve damage. We now have established a baseline for this model by showing that crushing, but not severing, the postsynaptic nerve causes synapse elimination reminiscent of that seen in aging with a nadir of minimum function at around 1 week after the procedure and complete recovery approximately 2 weeks later. We will use this model to track changes in the regulation of nicotinic receptors during the injured state and in recovery. Gaining an understanding of the role of nicotinic receptors in aging and nerve damage should facilitate the development of therapeutic interventions for a number of directly or indirectly related neurological disease states including tobacco addiction.

T77

Nicotinic receptors on hippocampal GABAergic neurons

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Tobacco smoking is the most frequent form of substance abuse and is a major health problem estimated to cause 4 million deaths a year worldwide. Several studies have shown that the addictive process is strongly driven by the pharmacological actions of nicotine in the central nervous system (CNS). This alkaloid exerts its effects by binding to a family of cell surface proteins called nicotinic receptors. In the CNS, nicotinic receptors normally respond to the chemical acetylcholine (ACh) and this interaction produces electrical events in the cells. The receptors represent the initial step in the pathway of nicotine addiction. One of the most abundant nicotinic receptors in the nervous system is a species containing the α7 gene product (α7-nAChR). Such receptors bind α-bungarotoxin and have a high relative permeability to calcium which, together with their strategic placement on neurons, enables α7-nAChRs to control a variety of cellular events. Examples include neurotransmitter release, neurite extension, second messenger cascades, and neuronal survival. When inappropriately activated or regulated, nicotinic receptors may contribute to diverse neuropathologies including schizophrenia and Alzheimer's disease.

The hippocampus is one of the most interesting brain structures for examining the role of nAChRs and nicotine in CNS synapse formation because of the central role it plays in learning and memory. We have used fluorescent α-bungarotoxin to image α7-nAChRs on hippocampal neurons and examine their regulation in culture. Highest levels of α7-nAChR staining were found on GABAergic interneurons after 2 weeks in culture. The α7-nAChRS were concentrated in clusters on the soma and dendrites, and were localized in part at GABAergic synapses on the cells. The clustering of such receptors was dependent on glutamatergic activity, since blockade of either total neuronal activity by tetrodotoxin, or glutamate NMDA receptors by APV reduced both the number of clusters and the number of neurons expressing detectable levels of α7-nAChRs. The treatments did not alter either the total number of surviving neurons or the number of GABAergic interneurons. Immunological inactivation of the endogenous neurotrophins NGF and BDNF produced decrements equivalent to those of tetrodotoxin and APV, whereas addition of BDNF and NGF each increased staining levels. In contrast, blockade of GABAergic or nicotinic signaling by bicuculline and d-tubocurarine, respectively, had no effect, though added nicotine increased the levels of α7-nAChRs. Neurons with the highest levels of α7-nAChRs often displayed clusters of the receptors at the distal tips of filopodia emanating from the cell body and dendrites. These structures were observed during the initial 1-3 weeks in culture, suggesting an important role for nicotinic receptors in synapse formation or dendritic branching.

The studies outlined here may provide useful insights into the physiological significance of nicotinic receptors and indicate some of the likely consequences of exposure to nicotine during CNS development, as may occur from maternal tobacco usage. Since α7-nAChRs have been implicated in a variety of behaviors and neuropathologies, understanding were the receptors are located and how they are influenced by nicotine exposure is likely to have significant biomedical implications.

T78

Assessing nicotine place conditioning in a mouse model

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Nicotine plays a pivotal role in mediating the addictive nature of tobacco in humans. Animal models have been developed to study the rewarding properties of nicotine. Indeed, because of the well-characterized library of genes and mutations in the mouse, the mouse model has received much attention. Thus, in the following experiments the place-conditioning (PC) paradigm was used to measure nicotine-induced behaviors and reward in Swiss Webster mice. The PC paradigm measures the incentive motivational properties of stimuli that become associated with drug effects through classical conditioning. The drug is administered in a distinct environment. After several pairings, the environment becomes associated with the effects of the drug, thereby acquiring incentive-motivational properties. Thus, the environment becomes a cue eliciting approach (i.e., conditioned place preference; CPP) or avoidance (i.e., conditioned place aversion; CPA) depending on whether rewarding or aversive properties of the drug have been conditioned. Nicotine-induced PC has been examined in rodents, yet it is difficult to establish nicotine PC reliably across various experiments. Therefore, we sought to understand the experimental parameters that maximize the formation of an association of the unconditioned stimulus (nicotine injection) with the conditioned stimulus (environment paired with nicotine). The PC apparatus consists of rectangular chambers divided into two distinct equal-sized compartments that differ in olfactory, visual, and sensory stimuli. During PC training, one of the compartments was paired with an injection of nicotine such that animals received an injection of their respective dose of nicotine (0.5-2 mg/kg) or saline and were confined to one of the compartments for 30-min. The alternate compartment was paired with an injection of saline using the same experimental parameters. Also, a group of mice received saline in both compartments and served as controls. In experiment 1, the saline and nicotine sessions occurred on the same day separated by four hours, whereas in experiments 2, 3, and 4 the saline and nicotine sessions were on separate days. During conditioning, the behavioral effects of acute and repeated nicotine administration were assessed. Twenty-four-hr after the last experimental session, the animals were tested for PC such that the animals were given access to both compartments simultaneously for 15-min and the amount of time the animals spent in each compartment was recorded. Various experimental parameters were manipulated in order to attempt to establish nicotine CPP in mice. The modifications in experimental parameters included: (1) Pre-exposing the animals to both compartments simultaneously to disclude some novelty effects; (2) Limiting the animals' food intake to enhance motivation; (3) Increasing the number of nicotine-pairings to attempt to maximize the formation of the association of the nicotine injection and the environment. In all the experiments, dose-dependent nicotine behavioral effects were observed, whereas nicotine CPP was absent. In further studies we will modify the experimental parameters and examine the effects of nicotine in different strains of mice (including ICR and C57 BL/6J strain).

T79

Nicotine exposure during development impairs spatial learning in adult rats.

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Cigarette smoking during pregnancy can produce a number of adverse effects on the brain and behavioral development of the child. To better understand the effects of developmental nicotine exposure on behavior, rats were exposed to nicotine during the 2nd and 3rd trimester equivalents. Pregnant dams were implanted with subcutaneous time-released nicotine pellets on gestational day 8. Three doses were used: 0, 25 and 35 mg, which were released steadily over a period of 21 days. Thus, nicotine exposure occurred during late gestation (in utero) as well as the early postnatal period (via mother's milk). A non-treated control was also included. During adulthood, offspring were tested on a Morris spatial learning water maze, a task that is sensitive to the functional integrity of the hippocampus. Locomotor activity was also tested in automated open field activity chambers. Subjects exposed to 35 mg nicotine during development exhibited significant deficits in spatial learning. During acquisition of the Morris maze test, 35 mg nicotine subjects took longer path lengths to find a hidden platform compared to all other groups. During a probe trial, subjects exposed to 35 mg nicotine exhibited faster swimming speeds, made fewer passes through the target position and spent less time in the target area compared to control groups, demonstrating significant spatial memory impairments. Performance of subjects exposed to 25 mg nicotine was intermediate during the probe trial, not differing significantly from either the 35 mg nicotine or controls. Interestingly, there were no significant differences in ability to swim to a visual platform, nor were there significant differences among groups in locomotor activity in the open field. These data suggest that nicotine exposure during late gestation in humans can lead to spatial learning deficits, which may indicate hippocampal dysfunction.

T80

Differential changes in nicotinic receptor subtypes after nigrostriatal damage in mice

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Although tobacco use is generally linked to detrimental effects on health, epidemiological studies overwhelming demonstrate a reduced incidence of Parkinson's disease in smokers. While the active reagent in tobacco responsible for this apparent neuroprotective effect remains to be identified, nicotine has be suggested as a potential candidate. This hypothesis is based on the observation that (1) nicotine interacts with the striatal dopaminergic system that degenerates in Parkinson's disease and (2) nicotine exerts a neuroprotective effect both in cultured cells and animal models against nigral neuron degeneration. In addition, nicotinic receptor stimulation may alleviate some of the motor deficits following nigrostriatal degeneration.

Previous studies have shown that several nicotinic receptor subtypes are present in human, monkey and rodent basal ganglia. Here we investigate how these subtypes are altered in a mouse of Parkinson's disease. To induced nigrostriatal damage we used the selective dopaminergic neurotoxin MPTP. We used the nicotinic receptor radioligands ^{125}I-α-conotoxin MII (binds to α6* receptors), ^{125}I-epibatidine (α2-α6*), ^{125}I-A85380 (β2*) and ^{125}I-α-bungarotoxin (α7*) to assess changes in striatal receptor distribution in both moderately (35 mg/kg MPTP s.c.) and severely (20 mg/kg MPTP i.p. 2x daily for 3 d) lesioned MPTP-treated mice. Animals with a moderate lesion had a 67% decline in the dopamine transporter, while animals with a severe lesion showed an 85% depletion, with similar decreases (47% and 75%, respectively) in ^{125}I-α-conotoxin MII binding. Declines in ^{125}I-epibatidine and ^{125}I-A85380 sites were less dramatic (22% and 28%, respectively for ^{125}I-epibatidine; 28% and 33%, respectively for ^{125}I-A85380). Inhibition studies showed that only 60% of the striatal ^{125}I-epibatidine sites decreased after nigrostriatal damage were α-conotoxin MII sensitive. ^{125}I-α-bungarotoxin sites were unchanged after lesioning.

These results suggest that in mouse striatum there is a loss of other nicotinic receptor in mice with nigrostriatal damage. Interestingly, the effects of nigrostriatal damage on nicotinic receptor sites in the mouse differed significantly from those in the monkey. In mice, both α6* and (α2-α5) sites were decreased after MPTP treatment with a somewhat greater decline in α2-α5* receptors. By contrast, striatal α6 sites in monkeys appear to be selectively vulnerable to nigrostriatal damage, while other nAChR subtypes are lost only after severe lesioning. These data show that there are species-dependent differences in receptor changes after nigrostriatal damage that must be considered when extrapolating results from animal studies to the human condition. Identification of the nicotinic receptor subtypes through which nicotine exerts its effects is important as the different subtypes may serve as therapeutic targets for prevention and treatment for Parkinson's disease and other neurodegenerative disorders.

T81

Anxiogenic profiles in adult rats exposed to nicotine during adolescence

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Although a high percentage of teens and young adults continue to smoke and use tobacco products, relatively few studies have assessed the lasting behavioral and neurophysiological consequences of adolescent nicotine exposure. In previous studies, our laboratory has reported that adolescent nicotine exposures induces a lasting decrease in motor activity and signs of hyperarousal in the central nervous system in adult rats. This combination of neurobehavioral effects suggests that there may be a lasting anxiogenic profile associated with adolescent nicotine exposure. The present study was designed to assess anxiety in adult rats exposed to nicotine during adolescence. Thirty day old male Sprague Dawley rats were exposed to nicotine for 5 days using transdermal nicotine patches. After 3-4 weeks of abstinence from nicotine behavior was assessed in two animal models of anxiety, the open field test and the "food conflict" test. In both tests of anxiety, rats exposed to nicotine displayed an anxiogenic profile compared to control rats. In the open field test, nicotine exposed rats moved to the perimeter of the open field more rapidly when 1st placed in the open field. Nicotine exposed rats also had significantly lower overall activity in the open field. In the "food conflict" test, nicotine exposed rats made significantly fewer approaches to food, spent significantly less time in contact with food, and ate less food which was placed in the exposed region of the testing apparatus in comparison to control rats. Overall, these data indicate that rats exposed to nicotine during adolescence display an anxiogenic profile. Importantly, this anxiogenic profile is observed weeks beyond the period of acute nicotine withdrawal. This finding is consistent with previous interpretations of the lasting neurophysiological changes resulting from adolescent nicotine exposure which have been reported by our laboratory. Taken together, these persistent neurobehavioral changes observed following adolescent nicotine exposure clearly indicate that this is a time of extreme susceptibility to the detrimental effects of nicotine on neurobehavioral function. Future studies are being focused on the potential neurochemical systems perturbed by nicotine which may contribute to these effects.

T82

A DNA vaccine against vascular endothelial growth factor receptor 2 (FLK-1) prevents effective angiogenesis and inhibits growth and metastasis of lung carcinoma

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Lung cancer cells remain an elusive target for immunotherapy due to their heterogeneity and genetic instability. We developed a novel strategy with an oral DNA vaccine targeting instead stable, proliferating endothelial cells in the tumor vasculature via their upregulated vascular endothelial growth factor receptor 2 (FLK-1). Our vaccine effectively protected mice from lethal challenges with lung carcinoma cells and reduced growth of established metastases in a therapeutic setting. The vaccine effectively induced cytotoxic $CD8^+$ T cell killing of endothelial cells by breaking peripheral tolerance against the FLK-1 self-antigen resulting in vastly reduced dissemination of spontaneous and experimental pulmonary metastases. Angiogenesis in the tumor vasculature was suppressed without impairments of fertility, neuro-muscular performance or hematopoiesis, but with a slight delay in wound healing. This strategy of targeting proliferating endothelial cells in the tumor vasculature aids in circumventing problems when targeting genetically unstable and heterogeneous tumor cells directly since endothelial cells are easily accessible and homogeneous. Unlike tumor cells, they are not moving targets since they are genetically stable and do not downregulate MHC antigens required for T cell recognition of tumor cells. This approach provides a new strategy for the rational design of novel therapies for lung cancer.

T83

Developing new gene therapy approaches to lung cancer treatment.

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Lung cancer is the leading cause of smoking related deaths and new approaches for treatment of the disease are needed. We have addressed this by combining immunotherapy and vaccination strategies to develop an efficient therapy for lung cancer. The primary goal of our study is to develop an immunotherapy approach where the immune inducing cells (Dendritic cells, DC) are enhanced *in situ* for induction of effective immune response. In combination with the enhanced DC *in situ* we are also testing a new kind viral vector for gene therapy purpose for effective anti tumor response.

We have used two growth factors, GM-CSF and IL-4 to enhance the population of DC *in situ* in a mouse model. This treatment, and the resulting DC *in situ*, is being evaluated for their ability to inhibit lung tumor. We have also combined this therapy with a vaccination strategy where a new kind of viral vector, known as "Gut less Adenovirus" vector is being evaluated for anti tumor response.

Our results have demonstrated that when GM-CSF and IL-4 are delivered continuously (10μg/day of each cytokine for seven days) by osmotic pumps, it results in enhanced DC in spleen of mice. In the spleen of GM-CSF/IL-4 treated mice, the cells expressing DC marker CD11c, and it increases from 5% of control mice to 20% in the treated mice. This treatment results in increase of two main sub-populations of DC i.e. myeloid DC ($CD11c^+/CD11b^+$) and lymphoid DC ($CD11c^+/CD118\alpha^+$). The DC generated by this method expresse increased MHC I and MHC II and they display increased ability for antigen uptake *in vitro*. We have further analyzed the capacity of these DC for their ability to uptake antigen *in vivo*. These studies reveal that the different DC sub populations generated in situ are efficient in antigen uptake and can present them to antigen specific CD8 T cells much efficiently than DC of normal mouse.

These results indicate that DC can be generated in situ and are able to efficiently present antigen to the immune system. We further tested the ability in situ generated DC for their anti tumor activity. Our results indicate that in a therapeutic model of anti-tumor response, where DC were generated in situ a significant tumor reduction takes place, only when a conventional replication deficient adenovirus is used for gene therapy purpose.

However, since complete regression of established tumor was not achieved, we hypothesized that a more efficient adenoviral viral vector may be needed for effective anti-tumor response. To this effect we are working with a new type of adenoviral vector known as "Gutless Adenoviral Vector". This virus lacks all extra viral components that may hinder multiple viral injection for induction of effective immune response to target tumor antigen. Studies with the gutless adenoviral vectors have revealed that they are efficient in transduction of DC and are able to express higher level of transgene-antigen expression in DC than conventional adenoviral vectors. In vivo studies with gutless adenovirus have also revealed their superior ability to induce cytotoxic T cell against a trangene-target-antigen of interest. Currently we are evaluating the ability of the gutless adenoviral vector in combination with *in situ* generated DC for their ability to induce anti-tumor effects.

In summary, our study indicates that growth factors (GM-CSF and IL-4) can be effectively used for generation of functional dendritic cells *in situ* and enhanced anti tumor immune response. Currently we are evaluating gutless adenoviral vectors in combination with *in situ* generated dendritic cells for effective gene therapy for lung cancer.

T84

Growth inhibition of small cell lung cancer (SCLC) cell lines mediated by IFN-γ activated human monocytes and H22xBN-Antagonist in combination with paclitaxel

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Monoclonal antibody-based immunotherapy alone or in combination with chemotherapy has shown efficacy in the treatment of various cancers. H22xBN-Antagonist is a bispecific molecule (BsMol) consisting of a humanized monoclonal antibody against FcγRI and an analog of the bombesin (BN) antagonist, (D-Phe^{6}, Leu-NHEt^{13}, des-Met^{14})-BN(6-14). The analog was created by the addition of a Cys residue to the N-terminus of the antagonist, so as to create a free-SH, which was necessary for construction of the BsMol. By flow cytometry analysis, the BsMol bound to several SCLC cell lines. Growth inhibition of SCLC cells and Swiss3T3 cells was observed in the presence of the synthetic BN-antagonist with an added Cys residue. The presence of the BsMol enabled targeted cytotoxicity of SCLC by IFN-γ activated human monocytes. In order to enhance tumor cell killing, we combined a chemotherapy agent with targeted immunotherapy for BN/GRP receptor positive SCLC cells. Tumor cells were first exposed to sublethal concentrations of paclitaxel for one hour and then washed to remove the drug. The cells were then cultured with human monocytes and BsMol or un-conjugated H22 for 72 hr. Inhibition of tumor cell proliferation was measured by a standard thymidine incorporation assay. The exposure to paclitaxel for 1 hr usually resulted in 30-50% growth inhibition in paclitaxel-sensitive SCLC cell lines, H69 and DMS273. The presence of the BsMol and the effector to target (E:T) ratio 10:1 resulted in an additional inhibition of 10-50% for H69; 40-80% for DMS273 (n=4). This targeted tumor cell inhibition is largely dependent on the E:T cell ratio and the presence of the BsMol. After paclitaxel exposure, SCLC cells incubated with monocytes alone showed increased thymidine incorporation in some experiments, suggesting a growth stimulatory effect by cytokines produced from the monocytes. Growth inhibition by the presence of the BsMol and monocytes was observed in some paclitaxel-insensitive SCLC cell lines as well. In conclusion, the BsMol can effectively mediate targeted SCLC cell inhibition. Since the mechanism of tumor cell killing is different from that of paclitaxel, the combination treatment results in an additive

T85

Vascular targeting of lung tumors

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Lung cancer is a direct consequence of smoking and requires a new approach to treatment that will improve efficacy and decrease the deleterious side effects associated with current therapies. The broad focus of this project is to investigate at the molecular level the differences between the vasculature of normal and neoplastic tissues in order to create diagnostic and therapeutic probes that may prove to be useful in improving both the early detection and treatment of cancers caused by tobacco use. We isolated luminal endothelial cell plasma membranes directly from various normal and lung tumor-bearing tissues and used 1-D and 2-D gel electrophoresis to resolve the membrane proteins to create vascular endothelial protein (VEP) maps. Comparative VEP map analysis has reveals extensive heterogeneity of cell surface protein expression between normal and tumor tissues as well as the induction in tumors of tumor-induced endothelial markers. At least 5 tumor-induced targets (which we call TE1-5) were identified by mass spectrometry. Western analysis of subcellular fractions and immunohistochemical staining of various normal and tumor tissues with antibodies against these candidate targets confirmed their up-regulation in the endothelia of tumor blood vessels compared to the normal tissue. Using antibodies to TE3, we have discovered that TE3 appears lung tumor-specific by Western analysis, tissue immunostaining, and in vivo targeting. Biodistribution studies revealed significant accumulation of TE3 in lung tumors with 25% of the injected dose was detected in the lung tumors within 60 min (60-fold more than in normal rat lung). Tumor imaging was also successfully performed. Gamma scintigraphy performed in vivo in rats bearing lung tumors using radiolabeled TE3 antibodies revealed significant accumulation of TE3 antibodies in the tumors. We quantified TE3 antibody targeting to the lung tumors but not normal lung tissue by dissecting regions containing visible tumors away from normal regions of the lung and measuring the amount of TE3 antibody probe in each fraction by gamma scintillation. TE3 antibodies accumulated only in the areas of the lung containing tumors and not in the normal tissue. Thus, TE3, discovered through our proteomic analysis of lung tumor endothelial cell plasma membranes appears to be an exceptional candidate for pursuing further preclinical testing as a tumor vascular target.

T86

The role of mast cells in lung cancer Angiogenesis

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Several lines of evidence suggest that mast cells promote tumor angiogenesis. The receptor tyrosine kinase EphB4 and its co-receptor ephrinB2 have been implicated as key regulators of angiogenesis during development. These receptors are also expressed in adult mouse vascular tissue in normal and in tumor angiogenic vessels, apparently also playing a role in pathogenic angiogenesis. We show that mouse and human mast cells express the gene transcripts of several of the B-class Eph and ephrin family members implicated in angiogenesis. Furthermore, mast cell EphB4 is activated by a soluble form of ephrinB2 (ephrinB2-Fc), resulting in differential mast cell gene expression. By immunohistochemistry, mast cells expressing either EphB4 or ephrinB2 are present in tissues of a mouse tumor model of squamous cell carcinoma of the skin (K14-HPV16) and of bronchoalveolar carcinoma (CC10-SV40Tag). Thus we show for the first time the presence and activation of a new class of potential angiogenic modulators in mast cells.

T87

Repair of Irofulven induced DNA damage

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Irofulven is a novel chemotherapeutic agent developed under support from the TRDRP. Once inside tumor cells the drug is converted to unstable active metabolites that damage DNA by producing poorly characterized lesions. We performed a detailed survey of DNA repair-defective mutants to characterize factors involved in the repair of irofulven-induced DNA lesions. We demonstrated that > 90% of irofulven's lethal effects in human cells can be repaired by the nucleotide excision repair (NER) pathway.

The core NER enzymes XPA, XPF, XPG and TFIIH are essential for recovery of cells from irofulven-induced damage. Surprisingly, in contrast to other DNA damaging agents, the presence of the global NER initiator factors XPC, HR23A/HR23B, and XPE are not required for repair of irofulven-induced lesions to proceed. Cell survival is instead dependant upon the repair and recovery from transcription inhibition, and requires the presence of CSA, CSB, and UVS, the factors critical and specific for transcription-coupled repair. Base excision repair and non-homologous end-joining of DNA breaks does not lay a major role in processing of irofulven lesions. Active RAD18, however is required and indicates that the lesions also block replication forks.

We conclude that irofulven-induced DNA lesions (in contrast to other chemotherapeutic agents) are exceptional in that they are ignored by all of the known global repair systems, and can only be repaired when trapped in stalled replication of transcription complexes. The production of these highly unusual DNA lesions may explain some of the drug's specificity towards tumor cells.

T88

An explanation of the delay of mitosis following cdc6 overproduction

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Many forms of cancer are clearly related to tobacco use and exposure. The abnormal proliferation of cancer cells is triggered by many oncogenes that encode growth factors, receptors, or proteins of signal transduction pathways. These pathways are inherently redundant, so that no single oncogene can be a reliable marker for all proliferating and/or neoplastic cells. However, signalling pathways converge at the point of initiation of DNA replication. Cdc6 is an essential protein for the key regulatory step of initiation of DNA replication in all eukaryotes investigated so far. Cdc6 is present throughout the cell cycle of proliferating cells, but not in nonproliferating quiescent cells. This offers potential for improved early diagnostic and differential diagnosis of cancer by using Cdc6 as a marker of pre-malignant (dysplastic) and malignant cells in clinical samples. Our goal is to understand why eukaryotic cells, from yeast to humans, regulate the function of Cdc6 during the cell cycle and how this is achieved at the molecular level.

In the yeast *Saccharomyces cerevisiae*, Cdc6 participates in the formation of the pre-replicative complexes (pre-RCs) at the end of mitosis and G1. At the transition of G1 to S, and until late mitosis, Cdc6 becomes highly unstable and it is degraded via the SCF pathway. Cdc6p contains several potential target sites for the Cdc28 protein kinase, some at the N-terminus and the others at the C-terminus. The mutation of all these sites, a subset of them, or just one site at C-terminus into non-phosphorylatable sites results in the stabilization of Cdc6p. We wanted to gain a better understanding in the role of Cdc6p phosphorylation in the G2 and M phases of the cell cycle since other groups and our own laboratory had shown that ectopic expression of Cdc6p results in a cell cycle delay at the G2/M stage.

We show here that cells expressing stable phosphorylation site mutants of Cdc6p are defective in degradation of Pds1p at the metapahase to anaphase transition as well as degradation of Clb2p at the exit of mitosis. In addition, expression of these mutants in a *cdc16* background results in synthetic lethality, suggesting that Cdc6p participates in the same process as the APC. Expression of stable mutants of Cdc6p in *rad24*?, *mad1*? and *bub2*? strains did not suppress the elongated morphology of the cells neither the delay in Pds1p degradation, indicating that Cdc6p ectopic expression does not trigger the DNA or the spindle checkpoints. The G2/M delay due to Cdc6p ectopic expression is enhanced in an *mih1*? background and expression of stable mutants in this background results in synthetic lethality, suggesting the delay is due to decreased Cdc28p/Clb2p activity. However, disruption of *SWE1* only suppresses the G2/M delay in *mih1*? cells expressing wild-type Cdc6p, and does not suppress the delay in cells expressing stable mutants. In addition, some of the stable mutants that delay G2/M do not interact with Cdc28p. These data suggest that Cdc28 inhibition must be achieved by mechanisms other than inhibitory phosphorylation and interaction with Cdc6p. We will present data showing that Cdc6p participates in a pathway that bypasses the spindle checkpoint.

T89

Anti-apoptotic paracrine effects of parathyroid hormone-related protein in lung cancer cells

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Parathyroid hormone-related protein (PTHrP) was discovered from a lung carcinoma as the factor responsible for humoral hypercalcemia of malignancy, and the majority of the work on PTHrP expression in lung cancer has focused on its effects on calcium homeostasis. However, PTHrP is a growth factor for many carcinoma cells and PTHrP expression reflects more advanced and aggressive tumors, suggesting that PTHrP may play an active role in cancer progression. PTHrP is also known to have effects on apoptosis in many malignant and non-malignant cells. The purpose of this study was to investigate the effects of PTHrP on apoptosis of BEN lung cancer cells, a human squamous bronchial lung carcinoma line, exposed to ultraviolet radiation

Cells were grown to 70% confluency in DMEM with 5% fetal bovine serum, washed in PBS, and incubated in the same media for 24 hrs with 100 nM PTHrP 1-34, PTHrP 38-64, PTHrP 67-86, PTHrP 107-139, or PTHrP 140-173. Cells were then exposed to 50 mJ/cm^2 UV-B radiation over 30 minutes and cultured for another 24 hr. Apoptosis was assessed by measuring caspase 3, caspase 8, and caspase 9 activities with fluorescent substrate assays in cell lysates or by TUNEL staining of fixed cells.

UV-B exposure caused a five to six-fold increase in caspase 3, caspase 8, and caspase 9 activities in BEN cells. Both PTHrP 1-34 and PTHrP 140-173 caused dose-dependent reductions in caspase 3 activity after UV compared to untreated cells. The other peptides did not have significant effects. Caspase 3 activity was reduced 19 ± 4% and 29 ± 7% after PTHrP 1-34 and PTHrP 140-173 treatment, respectively, in irradiated cells. Caspase 8 and 9 activities were reduced to a similar extent. Total cell protein was increased __% suggesting improved cell survival. Finally, the % of TUNEL-positive cells was reduced from 5 ± 2% in control irradiated cells to 0.3 ± 0.8 % and 2 ± 2% in cells treated with PTHrP 1-34 and PTHrP 140-173, respectively ($P \leq 0.05$).

Increased caspase 9 activity following UV-B irradiation in BEN lung cancer cells suggests activation of a mitochondrial-dependent apoptosis pathway. Increased caspase 8 activity suggests activation of either BID or a mitochondrial-independent pathway. PTHrP 1-34 and PTHrP 140-173 both protect against UV-induced apoptosis as evidenced by reductions in caspase activities, increases in cell survival, and decreases in the % of TUNEL-positive cells. The mechanism for this protective effect remains to be determined.

T90

The role of P450s in tobacco-mediated lung Cancer

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Smoking is a chronic condition affecting more than 46 million Americans. People who smoke are at risk for many diseases including heart disease, cancer, and other smoking-related illnesses. The overall goal of this proposal is to study the P450s involved in nicotine and nitrosamine metabolism in human lung and their role in tobacco-mediated lung cancer. This project is using a variety of scientific techniques to determine which P450s in the lungs are involved in nicotine breakdown.

The cytochrome P450 (P450) are a multi-gene family of oxidative enzymes. The primary role of P450s is the biotransformation of a wide range of drugs and xenobiotics. These include the tobacco constituents nicotine and nitrosamines. In order for nitrosamines to cause cancer, they must be activated by P450 enzymes. P450 enzymes in the liver are known to metabolize and activate nicotine and nitrosamines to procarcinogens, but the role of P450s in the lungs are not as well understood.

Nicotine metabolites are generated in humans not only from tobacco smoke, but also from the use of nicotine replacement medications (i.e. patch, gum, inhalers, etc.) that are used to help people stop smoking. Thus, the treatment for smoking (the use of these medications) supplies people with nicotine which can also be converted to cancer causing nitrosamines. In other words, the treatment for tobacco addiction can also cause cancer.

In the first year of this grant, we have identified several P450s which are expressed in human lungs. These P450s are currently being studied further to determine if their levels are increased by exposure to nicotine and other constituents of tobacco smoke. Of particular interest is CYP2E1 which is also induced by ethanol consumption. To elucidate the molecular mechanism of CYP2E1 induction by nicotine, we have cloned 10kb of the upstream region of the CYP2E1 gene. Subfragments of this region are being cloned into a reporter vector to be used in transient transfections to identify the enhancer region of CYP2E1 involved in nicotine induction. This will also facilitate the identification of the orphan nuclear receptors involved in nicotine induction of CYP2E1. We have also cultured a human lung cell line that we can use in the laboratory as a model of human lung cells that are exposed to tobacco smoke. This will allow us to study, in the laboratory, the effects of tobacco smoke on specific P450s in the human lung.

A better understanding of P450s, the enzymes that breakdown nicotine, can lead to a better understanding of tobacco-mediated lung cancer. Furthermore, this research can establish a basis for improved and novel therapeutic approaches to the treatment of tobacco addiction and other tobacco-related diseases. A better understanding of nicotine and tobacco-related carcinogens would provide a means for determining the mechanisms by which tobacco use causes disease.

T91

Local deposition pattern of sidestream tobacco smoke in idealized human tracheobronchial airways

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Numerous investigators over past decades have noted lung cancer that is attributed to tobacco smoke, preferentially occurs at bifurcations in large bronchial airways. The paradox is that based upon the particle diameter of sidestream tobacco smoke (ETS ~0.2-0.6 microns), deposition is predicted using clinically verified dosimetry codes to preferentially occur in bronchioles and the pulmonary region of the lung. Unfortunately, even the most sophisticated dosimetry codes are not able to predict where a particle will deposit within a lung airway. Recently, computational fluid dynamics (CFD) has been used to predict local deposition within idealized lung airway geometries. Initial local deposition predictions using CFD lead to the hypothesis that the focal nature of tobacco smoke deposition is due to two major factors: local airflow patterns and particle size specific carcinogen content.

These CFD predications in several idealized human airway geometries confirmed what others had seen experimentally for particles larger than 1 micron, that areas at the bifurcation receive larger doses than adjacent areas. One report extended these predictions to particles in the size range of sidestream tobacco smoke. This theoretical prediction indicates that up to 100 times the number of particles (or dose) occur at bifurcations compared to surrounding areas in an airway. The surprising aspect of this theoretical prediction was that the enhancement factor was relatively independent of particle size. Based upon this theoretical prediction, 0.2-0.6 micron sidestream tobacco smoke particulate matter should exhibit an enhancement factor of up to 100 times at airway bifurcations. This theoretical prediction was tested experimentally in an idealized human airway model. The hollow human airway model was constructed using an identical airway geometry to that used in the theoretical prediction. This geometry was a 3-generation hollow tracheobronchial model based on the Weibel (1963) symmetric morphometry for airway lengths and diameters for airway generations 3-5. Monodisperse fluorescent polystyrene latex spherical particles (1.0 and 0.4 microns nominal diameter) were deposited in these models at a steady inspiratory flow of 7.5 l/min (equivalent to heavy exertion with a tracheal flow of 60 l/min). The models were opened and the locations of deposited particles were mapped using fluorescent microscopy.

Enhancement of deposition at bifurcations was found for both the 1.0 and 0.4 micron monodisperse particles. These experimental results agreed with the theoretical predictions, confirming that substantial deposition enhancement occurs at bifurcations for submicron particulate matter. The CFD predictions indicate that this is due to the local airflow patterns at the airway bifurcations. Future work includes repeating these experiments at other physiologically realistic flowrates and incorporating the results into sophisticated dosimetry codes. The dosimetry codes will be combined with time-activity patterns for children and adults to provide better estimates of local deposition, thereby improving risk assessment for individuals (adults and children) exposed to sidestream tobacco smoke.

T92

CT imaging to assist diagnosis of solitary pulmonary nodules

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Spiral Computed Tomography (CT) is used in many applications related to lung cancer. In addition to using CT for the early identification of possible lung cancers, an important task is to develop methods to discriminate between those findings that will turn out to be benign from those that will turn out to be malignant. One aspect of this problem is that the CT imaging exam often discovers solitary pulmonary nodules (SPNs). However, solitary nodules are not always cancerous; with benign and malignant nodules almost equally likely. In different studies, estimates range from 40-90% of all SPNs observed on CT to be benign. Unfortunately, there are few agreed upon image findings to distinguish benign from malignant nodules.

The goal of this research is to develop techniques that will assist in the accurate diagnosis of lung cancers using a combination of CT imaging and computer analysis methods. If differences between cancerous and non-cancerous SPNs can be determined accurately, then more invasive and more expensive tests – such as fine needle biopsy or surgical resection - can be avoided.

The specific aim of this project is to collect the necessary data so that image processing and computer analyses can take place, which are being funded from another project. The data that is being collected is CT image data and corresponding medical record data that will confirm the benign or malignant diagnosis. Once these data are collected, we will apply our image processing tools to measure properties of size, shape, intensity, texture and, when available, measures of contrast enhancement. These measures will be analyzed using pattern classification techniques to determine if we can accurately predict the medical diagnosis based on the parameters extracted from the image data.

We have completed the process of establishing the medical diagnosis in approximately 80 patients. We have established the radiologist's outlines of nodule boundaries in 35 of these cases to date. From these efforts, we expect to have 80 patients who have at least a single image for us to analyze; 30 of those patients will have had a volumetric imaging protocol performed with an intravenous contrast injection. These latter cases will be used to analyze combinations of measures of three-dimensional size, shape and contrast enhancement as well as two dimensional measures of shape, intensity and texture. All cases that have at least one image will be analyzed to determine of the two dimensional measures (2D shape, intensity and texture) can adequately predict diagnosis. As the data collection phase is being completed, we are initiating preliminary analyses. The results of these preliminary analyses will be presented.

As lung cancer screening programs get under way, manufacturers and other medical imaging equipment manufacturers are keenly aware of the need to develop tools to distinguish benign from malignant nodules. We are investigating this approach to create an accurate and noninvasive test so that we can reduce the need for additional, more invasive tests when investigating lung cancer.

T93

Environmental and genetic determinants of tobacco use: A multidisciplinary, longitudinal family-based design

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SRI International
In collaboration with D. Carmelli[1], K. Hemberger[1], T. Khroyan[1], H. Ring[1], L. Cheng[2], H. Hops[3], J. Andrews[3], E. Tildesley[3], D. McBride[3], K. Hudmon[4], K. Wilhelmsen[4], N. Benowitz[4], B. Koenig[5], L. Caron[5], J. Illes[5]
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Numerous studies of related and unrelated individuals support the hypothesis that cigarette smoking is influenced by both genetic and environmental factors. Few studies of smoking in families have described the pattern of transmission (genetic or otherwise) from parents to children. This study is designed to fill these research needs. The work presented in this poster is from the TRDRP-sponsored Integrated Research Project (IRP), entitled *Genetic and environmental influences on tobacco use in adolescents*.

The theoretical and methodological integration of the genetic and environmental perspectives on the initiation and regular use of tobacco remains elusive. Here we describe the ongoing effort of six research teams spanning the disciplines of health psychology, pharmacology, genetic epidemiology, molecular genetics, and biomedical ethics to operationalize and execute an integrative approach to the study of tobacco dependence. At the core of the project is a pre-existing, longitudinal investigation of social and behavioral risk factors for the adoption of substance use including tobacco in individuals (referred to as the "target") who were, on average, 13 years of age at intake (current average age is 29) and their families. Because the core study is longitudinal in nature, smoking behaviors extending from early adolescence to young adulthood have already been characterized through approximately annual assessments over 16 years. To complement these existing data, we have added ascertainment and data collection strategies to facilitate the concurrent investigation of tobacco use and nicotine metabolism phenotypes, along with genetic sources of variation in these phenotypes, for the target individuals and their biological families. In addition, our study aims to estimate the relative predictive power of genetic and environmental characteristics that might act as independent and/or interacting risk factors for tobacco use and dependence. We describe the conceptual framework for our integrative approach, including our methods employed to: (1) recruit participants to the study, (2) assess relevant tobacco-related phenotypes including nicotine metabolism parameters, (3) assess the quality of the DNA samples collected from the study participants, and (4) ensure compliance with local and federal guidelines for ethical and legal investigations of genotypic associations with tobacco-related phenotypes in families. Future directions for this work include the confirmation of any observed phenotype-genotype associations in ethnically-diverse samples to determine the generalizability of the findings. The identification of gene-environment interactions could enhance our understanding of risk factors associated with progression to regular tobacco use. A comprehensive view of susceptibility to tobacco use could then lead to targeted interventions to prevent, reduce, or stop regular and chronic tobacco consumption.

T94

Genetic and environmental influences on multiple tobacco-related phenotypes in families

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Numerous studies of twins support the hypothesis that cigarette smoking is influenced by both genetic and environmental factors. A growing number of studies based on the case-control design have identified a higher prevalence of certain variants of candidate genetic markers in smokers than in nonsmokers. While findings from both the twin and candidate gene literature are supportive of the hypothesis that smoking is in part genetically determined, few studies of smoking in families have described the pattern of transmission (genetic or otherwise) from parents to children. This study is designed to fill this research need. The work presented in this poster is from a component of the TRDRP-sponsored Integrated Research Project (IRP), entitled *Genetic and environmental influences on tobacco use in adolescents*. It is being conducted in collaboration with investigators from SRI International (G. Swan, D. Carmelli, H. Ring), City of Hope National Medical Center (L. Cheng), Oregon Research Institute (H. Hops, J. Andrews, L. Tildesley), UC San Francisco (K. Hudmon, N. Benowitz, K. Wilhelmsen), and Stanford University (B. Koenig, L. Caron, J. Illes).

Questionnaire data from 128 nuclear families ascertained as part of a longitudinal investigation of social and behavioral risk factors for substance use were used to estimate the heritability (H) of various smoking-related phenotypes. A classical variance component approach was used that assumes a polygenic model in which the smoking-related traits are determined by the additive effects of multiple unmeasured genes of small effect (polygenes), unmeasured individual specific environmental effects, and the effects of measured covariates. Gender effects were modeled as linear fixed effects through the mean and the variance of the smoking traits. The total phenotypic variance was partitioned into an additive genetic variance component and an individual specific environmental variance component, conditional on the gender effect, if gender was significant. Using the Sequential Oligogenic Linkage Analysis Routine (SOLAR) program, a maximum-likelihood approach with the likelihood ratio test was used to test for the significance of heritability and gender. The phenotypes demonstrating significant heritability included time to first cigarette upon wakening. ($H=0.44, p=0.00004$), FTND total score ($H=0.30, p=0.0044$), withdrawal symptom experience (sum of all possible symptoms; $H=0.25, p=0.005$), CAGE total score ($H=0.22, p=0.00175$), and two measures of smoking motivations: Stimulation ($H=0.28, p=0.0022$), and Sedation ($H=0.20, p=0.011$). These findings suggest that a biological pathway contributing to tobacco dependence and nicotine metabolism has a genetic component. Future directions include the confirmation of any observed phenotype-genotype associations in ethnically-diverse samples to determine the generalizability of the findings. The identification of gene-environment interactions could enhance our understanding of risk factors for the progression to regular tobacco. A comprehensive view of susceptibility to tobacco use could then lead to targeted interventions to prevent, reduce, or stop regular and chronic tobacco consumption.

T95

Agreement between proband and family members' report of smoking behavior

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Numerous studies of related and unrelated individuals support the hypothesis that cigarette smoking is influenced by both genetic and environmental factors. Few studies of smoking in families have described the pattern of transmission (genetic or otherwise) from parents to children. This study is designed to fill these research needs. The work presented in this poster is from the TRDRP-sponsored Integrated Research Project (IRP), entitled *Genetic and environmental influences on tobacco use in adolescents*. It is being conducted in collaboration with investigators from SRI International (G. Swan, D. Carmelli, H. Ring), the Oregon Research Institute (H. Hops, J. Andrews, L. Tildesley), City of Hope National Medical Center (L. Cheng), UC San Francisco (K. Hudmon, N. Benowitz, K. Wilhelmsen), and Stanford University (B. Koenig, L. Caron, J. Illes).

Before examining the data for evidence of familial aggregation, we determined which of the possible phenotypes appear to be valid as defined by agreement of the proband's report with the report from other family members. For the Family History Interview (FHI), a total of 481 probands provided the following information for themselves, their parents and their siblings: gender, age, smoking status (ever smoked more than 100 cigarettes in lifetime), and if a smoker, age started smoking, cigarettes smoked per day, and quitting status. Limiting the data to only probands and their biological parents and full siblings resulted in a total of 1,927 individuals. Members of families with two or more ever smokers were invited to complete the Smoking History Questionnaire (SHQ). A total of 867 (45%) questionnaires were received, of which 378 were from probands, 166 from mothers, 127 from fathers, and 196 from siblings. Data from the FHI and the SHQ were matched by individual and the proband's report was compared to the family member's self-report on ever smoking, ever quit, age started smoking, and amount smoked per day. Analysis of agreement suggest that the ever-smoking phenotype can be reported with good agreement (all kappa values $\geq .70$) while the ever-quit phenotype appears to be less valid (all kappa values $\leq .50$). Correlational analyses of continuous phenotypes indicate that age started daily smoking for self, mother, and sibling can be reported reliably by probands (all $p \leq 0.0001$) but less well for fathers ($p \leq .03$). Proband's report of number of cigarettes smoked per day was correlated modestly but significantly with the self-report of mother, father, and sibling (all $p \leq 0.01$). These results provide important knowledge as to which of the phenotypes derived from the FHI should be examined further for familial aggregation. Future directions for this work include the confirmation of any observed phenotype-genotype associations in ethnically-diverse samples to determine the generalizability of the findings. The identification of gene-environment interactions could enhance our understanding of why, following initial exposure, some people progress to regular tobacco use while others do not. A comprehensive view of susceptibility to tobacco use could then lead to targeted interventions to prevent, reduce, or stop regular and chronic tobacco consumption.

T96

Evidence that tobacco use trajectories in adolescents are associated with smoking among first-degree relatives

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Numerous studies of twins support the hypothesis that cigarette smoking is influenced by both genetic and environmental factors. A growing number of studies based on the case-control design have identified a higher prevalence of certain variants of candidate genetic markers in smokers than in non-smokers. While findings from both the twin and candidate gene literature are supportive of the hypothesis that smoking is in part genetically determined, few studies of smoking in families have described the pattern of transmission (genetic or otherwise) from parents to children. This study is designed to fill these research needs. The work presented in this poster is from the TRDRP-sponsored Integrated Research Project (IRP), entitled *Genetic and environmental influences on tobacco use in adolescents*. It is being conducted in collaboration with investigators from SRI International (G. Swan, D. Carmelli, H. Ring), City of Hope National Medical Center (L. Cheng), Oregon Research Institute (H. Hops, J. Andrews, L. Tildesley), UC San Francisco (K. Hudmon, N. Benowitz, K. Wilhelmsen), and Stanford University (B. Koenig, L. Caron, J. Illes).

Developmental trajectories of adolescent tobacco use may reflect genetic susceptibility to become a regular smoker. This analysis constructed developmental smoking prototypes for 276 adolescents from the "SMOFAM" study, a longitudinal family cohort study. These adolescents (67% female) averaged 13.1 ± 1.5 years of age at baseline, had complete smoking data for the first 10 years of annual assessments, and completed a family smoking history interview at the 1999-2000 assessment. Eight features characterizing trends in smoking behavior over the adolescent/young adult years were defined and standardized. 138 non-smokers formed one cluster, and the standardized feature scores for the remaining 138 ever-smoking participants were subjected to k-means cluster analysis revealing six distinct clusters. These six clusters accounted for a substantial proportion of overall feature variability (pseudo F statistic = 99.5). The percentage of first-degree relatives who were ever smokers was significantly different between the cluster groups, $F = 19.6$, $p < 0.0001$. Individuals from no or low-smoking clusters reported the lowest percentage of first-degree relatives who ever smoked, 32% and 46%, respectively. Individuals who started to smoke regularly at an early age and maintained a high level of consumption had the highest percentage of ever-smoking first-degree relatives (84%). These results indicate a relationship between prevalence of smoking among first-degree relatives and developmental tobacco use phenotypes among adolescents. Future directions include the confirmation of any observed phenotype-genotype associations in ethnically-diverse samples to determine the generalizability of the findings. The identification of gene-environment interactions could enhance our understanding of risk factors for progression to regular tobacco use. A comprehensive view of susceptibility to tobacco use could then lead to targeted interventions to prevent, reduce, or stop regular and chronic tobacco consumption.

T97

A computer simulation model for the surveillance of California Tobacco Control Policies

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Debates over state and national tobacco legislation and the use of state funds demonstrate that there is a need for information on the likely effects of state level tobacco control policies. Well developed, dynamic computer simulation models that are based on empirical evidence and that account for the variety of influences on tobacco use can be useful tools for informing policymakers. They can identify the effects of different policies on all smokers and on specific demographic subsets of smokers. In so doing, the model can be used to convey the importance of comprehensive policy approaches to tobacco control and to improve the focus of tobacco control policies.

The SimSmoke tobacco control policy simulation model may be used to track smoking and to evaluate tobacco control policies. The model tracks cohorts of smokers by age, gender, and racial/ethnic group over time, and predicts trends in smoking and tobacco attributable deaths. Specific modules analyze how public policies, such as taxes, mass media, clean air laws, cessation treatment and youth access policies, affect smoking rates and smoking related mortality. Modules also show how the effects depend on the manner in which a policy is implemented.

SimSmoke was originally a national model. Because of its rich data sources, pioneering research and policies in the field of tobacco control, California has been chosen as the first state in which to implement a state level model. SimSmoke is particularly well suited to examining these effects in a state with the diverse and changing demographics of California. The model examines the effect of past state level policies in California and develops predictions on the effect of future policies.

Policy makers will be able to monitor the value of each policy, and to see the effectiveness of each. They could then use the model to shape future policies. They could discern which age groups and racial groups are currently being affected by tobacco control policies in the state of California, and determine which polices to implement to improve the health of these groups. This computer model will help leaders understand the effectiveness of past policies and make better decisions about future policies. It can also help leaders to understand the importance of how policies are implemented and the interaction of policies with other policies already in place.