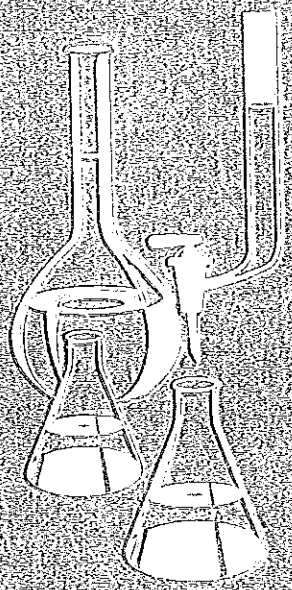
# Arizona Disease Control Research Commission



## 2000 – 2001
## Annual Report

January 2002

# ARIZONA DISEASE CONTROL RESEARCH COMMISSION

## ANNUAL REPORT
## 2000 – 2001

Jane Dee Hull, Governor

Henry Reeves, Ph.D., Chairman
Orme Lewis, Jr., Co-chairman

COMMISSION MEMBERS

*General Public*
C. Eileen Bond, J.D.
Orme Lewis, Jr.
Joseph A. Mislove, J.D.

*Medical Community*
Betty J. Gale, D.N.Sc.
Eladio Pereira, M.D.
Stuart F. Quan, M.D.

*Scientific Research Community*
T. Lon Owen, Ph.D.
Henry C. Reeves, Ph.D.
Walter H. Williams, Ph.D., M.D.

*Staff*
Executive Director: Dawn C. Schroeder, D.D.S., M.A.
Executive Staff Assistant: Damika D. Brock
Accountant II: Debra S. Young
Fiscal Service Specialist II: Daniel J. Powell
Administrative Assistant II: Ismene Quintanilla

1616 West Adams Street, Suite B-25
Phoenix, Arizona 85007
Telephone: 602.542.1028
Fax: 602.542.6380

*January 2002*

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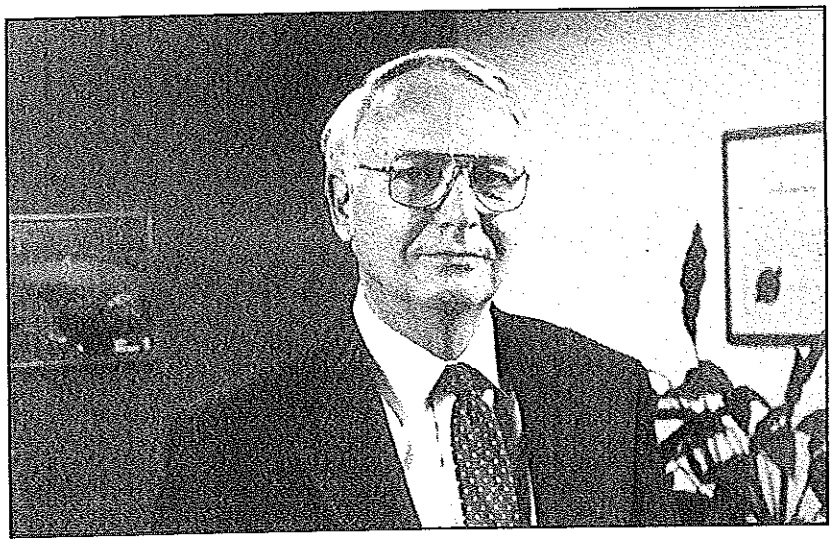


## *Message from the Chairman*

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 2001 Annual Report. Established by the Arizona Legislature in 1984, Commission activities contribute to improving the health of Arizonans through scientific research. Funding comes through the Tobacco Tax Initiative, passed by the voters in 1994. The ADCRC receives five percent of the revenues collected from the tax to fund tobacco-related disease research. The Commission was also named in the Healthy Arizona Initiative passed in November, 2000 and received an additional $2,000,000 in Tobacco Settlement Revenues. These funds were assigned by the Legislature to Brain Research including $1,000,000 for Alzheimer's Disease Research to augment a program in the Department of Health Services, $800,000 for Parkinson's Disease Research and $200,000 for other brain related diseases and disorders. The Legislature also removed the tobacco-related disease restriction from the Commission funding sources to allow a wider range of medical research programs to be funded. The final two-year cycle of funding under the Anticancer Drug Discovery Program has been awarded and the Commission looks forward to reviewing the proposals under the new Brain Research program.

In FY 2001 the Commission is reporting on the tobacco-related disease research program and the anticancer drug discovery program. New proposals beginning in FY 2002 are unrestricted. The researchers have made progress in many areas as outlined in their individual project abstracts.

The Commission continues to be involved in technology transfer and the patenting and licensing of discoveries funded with ADCRC monies. This year the Commission worked with representatives of the Arizona Board of Regents and other interested parties to develop a joint technology transfer blueprint for research supported with ADCRC and other State funds.

The Annual Report is prepared and submitted in January of each year to the Governor, the President of the Senate and the Speaker of the House of Representatives. We appreciate their continued support.

## *The Commission Members*

Nine Commissioners guide the work of the Arizona Disease Control Research Commission. They are appointed by the Governor and confirmed by the Senate. The Commission is divided into three communities – General Public, Medical and Scientific Research. Each community is represented by three Commissioners appointed for three-year terms. Generally, the terms of three members expire each year; Commissioners may be reappointed. The Chairman and Commissioners who served during 2000 – 2001 are presented below.

### *Henry Reeves, Ph.D., Chairman*

Professor Emeritus
Arizona State University

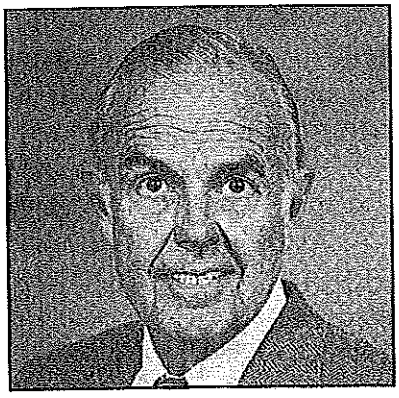
Commissioner Reeves is a member of the Scientific Community. He was a Professor of Microbiology at Arizona State University from 1969 to 1993. During that time he also served as chair of the Department of Microbiology from 1970 to 1973 and as Vice President for Research from 1985 to 1991. On leave from Arizona State University, he served as director of the Division of Physiology, Cellular and Molecular Biology at the National Science Foundation from 1976 to 1979. Commissioner Reeves received his B.S. from Franklin and Marshall College and his M.A. and Ph.D. from Vanderbilt University. He was first appointed to the Commission by Governor Symington to complete the term of Commissioner James Bloedel in 1995. He was reappointed in 1996. Governor Hull appointed him to his second full term in 1999. His term will expire in May 2002.

## General Public

*C. Eileen Bond, J.D.* — Prescott
Private Practice Specializing in Child Welfare Law

Commissioner Bond received her B.A. in History (Far Eastern Studies) and Master of Library Science from U.C.L.A. She received her J.D. from Arizona State University in 1971. Commissioner Bond retired from the Arizona Attorney General's Office in 1996 and is in private practice in Prescott, Arizona, where she specializes in the area of child welfare law. She serves on the Board of Directors of Child Haven, the Yavapai County Child Crisis Center, and as an advisor to the Yavapai County Family Drug Court. Commissioner Bond serves as a Disciplinary Hearing Officer for the Arizona State Bar Association and as a due process hearing officer for the Arizona Department of Education. Commissioner Bond was appointed by Governor Hull in May, 2000. Her term expires in May 2003.

*Orme Lewis, Jr., Co-chairman* — Phoenix
Managing Director, Select Investments, L.L.C.



Commissioner Lewis oversees commercial real estate interests and participates in environmentally sensitive businesses. He currently serves on the governing boards of the Arizona Historical Foundation, Arizona State University Foundation, Phoenix Children's Hospital, Boyce Thompson Southwestern Arboretum, and the Polycystic Kidney Research Foundation. He is a former member of the Governor's Regulatory Review Council and the U.S. Advisory Committee on Mining and Mineral Research. Commissioner Lewis was elected to the 23rd and 24th Arizona State Legislatures. He received a B.S. in Economics in 1958 from the University of Arizona. Commissioner Lewis was appointed to the Commission by Governor Symington in 1995 and reappointed by Governor Hull in 1998. His term expires in May 2001.

## General Public

*Joseph A. Mislove, J.D.* — Phoenix
Coppersmith, Gordon, Schermer, Owens & Nelson, P. L. C.

Commissioner Mislove has practiced health care law in Arizona since 1989, in both private practice and as in-house counsel for a managed-care organization with more than 350,000 members. He advises clients on legal issues concerning licensure, certification, and accreditation; arrangements that implicate physician self-referral, anti-kickback, and other fraud and abuse laws; EMTALA and general compliance programs; payor and medical service contracts; Medicare, Arizona Health Care Cost Containment System, and other public programs; and corporate matters. Commissioner Mislove received B.S. and M.B.A. degrees from Arizona State University in 1981 and 1986, and his J.D. degree from the University of Arizona in 1986. He is a member of the American Health Lawyers Association and the Arizona Association of Health Care Lawyers. Commissioner Mislove served as President of the Arizona Association in 1996-97. He was appointed to the Commission by Governor Hull in August 1999. His term expires in 2002.

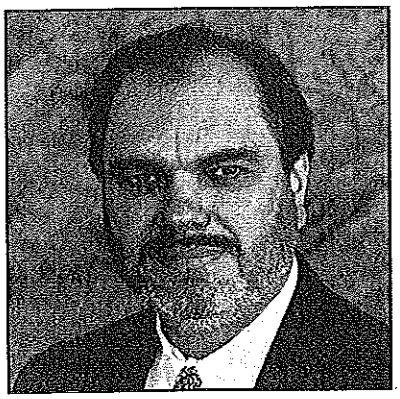
## Medical Community

*Betty J. Gale, D.N.Sc.* — Tempe
Professor and Chair, Division of Community Health Nursing, Arizona State University

Commissioner Gale has her D.N.Sc. from the University of San Diego and her M.S. in Community Health Nursing from Arizona State University. Commissioner Gale is Division Chair and Professor at the Arizona State University, College of Nursing. She is also a Clinical Associate Professor at the University of Arizona. Her practice and research focus on health promotion programs with low income older adults. Commissioner Gale recently completed a study on the sociocultural influences on functional health in older Anglo and Hispanic Women that was funded by the National Institutes of Health. Current projects, in collaboration with other partners, are Community-oriented Primary Care and Muscular Fitness in Older Adults. She is the Nurse Administrator of the Escalante Health Partnerships. Appointed to the Commission in August, 1999 by Governor Jane Hull, her term expires in May 2001.

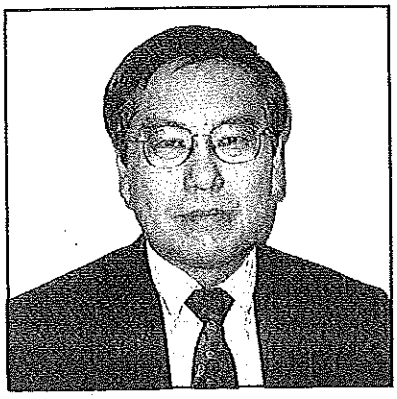
## Medical Community

*Eladio Pereira, M.D., F.A.C.P.* — Nogales
Chief, Internal Medicine,
Mariposa Community Health Center



Commissioner Pereira received his B.S. in Chemistry from Georgia Tech in 1979. He graduated *Magna Cum Laude* from Emory University School of Medicine in 1983. After completing his Internal Medicine Residency at the same institution, he joined the staff of Mariposa Community Health Center as a Scholar of the National Health Service. He returned to Emory University from 1990-1992 as Assistant Professor of Medicine and Director of the Intensive Care Unit, Grady Memorial Hospital, Atlanta. He has been a Fellow of the American College of Physicians since 1993. In February 1998, he was named Chief of the Medical Staff and Clinical Services at Mariposa, overseeing an 11-physician group that provides 60 percent of the medical care in Santa Cruz County. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales whose term expired in May 1996. He was reappointed in 1996. Governor Hull appointed him to a second full term in 1999. His term expires in May 2002.

*Stuart F. Quan, M.D.* — Tucson
Professor of Medicine and Anesthesiology
University of Arizona College of Medicine



Commissioner Stuart Quan received his A.B. in Psychology from the University of California, Berkeley, in 1970 and an M.D. from the University of California, School of Medicine, San Francisco, in 1974. He completed an internship and residency at the University of Wisconsin, Madison, and then did three Fellowships: Emergency Medicine, Critical Care, and Pulmonary Medicine. He is currently Professor of Medicine and Anesthesiology and Chief of the Pulmonary/Critical Care Section at the University of Arizona College of Medicine. Commissioner Quan is the founder of the Sleep Laboratory at University Medical Center and served as president of the American Academy of Sleep Medicine in 1999-2000. He is the principal investigator for two sleep research studies funded by the National Institutes of Health. Commissioner Quan was appointed by Governor Jane Hull in May, 2000 and his term expires in 2003.

## Scientific Research Community

*T. Lon Owen, Ph.D.* — Flagstaff

Professor of Medical Anatomy and Physiology,
Northern Arizona University

Commissioner Owen received his B.A. in Zoology from the University of California, Davis; a master's degree in Biology from California State University, Sacramento; and his Ph.D. in Physiology from U. C. Davis. He was a National Institutes of Health Postdoctoral Fellow at Michigan State University and Visiting Associate Professor in the Pharmacology Department of the University of Arizona College of Medicine. He has chaired the Research Committees of the American Heart Association at both the Arizona Affiliate and Southwestern Regional levels. His publications are in the areas of cardiovascular, aging and environmental physiology. Commissioner Owen was appointed to the Commission in June 1998 by Governor Hull and his term expires in May 2001.

*Walter Williams, Ph.D., M.D.* — Tucson

Associate Professor, Department of Radiology,
University of Arizona

Commissioner Williams received his B.S. with majors in Chemistry and Physics from the University of Missouri in 1963, his Ph.D. in Physical Chemistry from Purdue University in 1968 and his M.D. from Yale University in 1980. Commissioner Williams was a member of the Science Team for the Voyager Spacecraft Missions to Jupiter and Saturn and was a Senior Scientist at the Jet Propulsion Laboratory, California Institute of Technology prior to returning to school to study medicine. From 1985 to 1987, he was a clinical instructor in the Joint Program for Radiology and Nuclear Medicine at Harvard. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and reappointed in 1997. Commissioner Williams was appointed to a third term by Governor Hull in May 2000 and his term expires in 2003.

# Summary of 2000-2001 Commission Activities

The Commission had 71 contracts in two programs, tobacco-related research and anticancer drug discovery, with medical and health researchers in Arizona as of July 2000. The section headings list the program and whether the project is in its first, second or third year of funding. Research abstracts outlining the progress made during the year are contained in Sections A-D. Citations for scientific publications and abstracts arising out of the research are also listed.

Lay summaries for new anticancer drug discovery research projects made in 2000 can be found in Section E. Lay summaries for new unrestricted medical research projects are found in Section F. These projects begin in July, FY 2001. The summaries provide an overview of the new research.

Approximately 915 Requests for Proposals (RFPs) for 2000-2001 awards were mailed to potential applicants in September 2000. The amount available for new anticancer drug discovery research contract awards was approximately $1,700,000. The amount available for new unrestricted medical research was approximately $1,800,000. In response to the RFPs, the Commission received 14 anticancer drug discovery proposals and 96 unrestricted medical research proposals. Sections G and H list the research proposals received in response to each RFP.

In November and December the 96 medical research proposals were sent to a panel of national scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more than 100 out-of-state peer reviewers. Three reviews were sought for each proposal. The proposals and evaluations were distributed to the Commissioners who were formed into three, three-person subcommittees to facilitate the final review process. In the spring and summer of 2001 the Commission selected 28 proposals for funding. Anticancer drug discovery proposals were evaluated by a four-person panel of experts who made their recommendations to the Commission after interviewing each of the 18 principal investigators. The Commissioners selected 9 anticancer drug discovery proposals to receive contract awards. The number of cooperative projects funded increased this year resulting a smaller number of contracts selected with increased funding going to individual projects. During 2001-2002 the ADCRC will be managing 63 contracts.

# SECTION A

## CONTRACTS

## TOBACCO-RELATED RESEARCH

## YEAR ONE

David J. Bearss, Ph.D.

University of Arizona
Award Amount FY 2001: $49,007

## Development of a Transgenic Mouse Model of Pancreas Cancer

Pancreas cancer is the fifth leading cause of cancer death among adults in the United States. Close to 90% of patients diagnosed with pancreas cancer will die within the first year following diagnosis. The deadliness of this disease has encouraged a search for risk factors to identify high-risk individuals. Over the past several years a great deal has been learned regarding the factors that influence the onset and progression of many types of cancer. The most consistent risk factor that has been identified for pancreas cancer is cigarette smoking, with heavy smokers having over a ten-fold increase in incidence. At the molecular level, it is thought that the accumulation of defects in specific genes that contribute to the growth and development of normal tissue are responsible for the progression of cancer. Understanding the effects of genetic lesions that are common in the development of pancreas cancer will no doubt lead to new and more effective ways to diagnose, treat, and prevent this devastating disease. The goal of this proposal is to develop and characterize transgenic mouse models of adenocarcinoma of the pancreas that appropriately reproduce the human disease condition. In the first year of funding, we have generated transgenic mice that express the K-ras oncogene in the pancreas. The result of the expression of this oncogene is that these mice display pre-cancerous lesions in their pancreas. We are encouraged by these findings and are working to introduce additional genetic alterations in these mice that will result in the development of adenocarcinoma of the pancreas. Using this model, we hope to better understand this disease and develop strategies to prevent or cure pancreatic cancer.

Paul F. Consroe, Ph.D.

University of Arizona
Award Amount FY 2001: $49,522

Molecular Mechanism of Cannabinoid Action at the Human CB1 Receptor

Smoking has been implicated in several forms of cancer including the lung. This has major health consequences for the residents of Arizona since about 25% of the state's population smoke. Lung cancer takes a terrible toll on Arizonans. Treatment of cancer often involves the use of radiation and chemotherapy. These treatments often induce severe nausea, weight loss and pain in patients. Cannabinoids such as nabilone and dronabinol have been approved as antinausea agents and to relieve pain.

We have been studying the effects of cannabinoids on nerve cell surfaces known as the cannabinoid CB1 receptor. These receptors interact with G-protein to produce their effects. Using antisense technology, we have demonstrated that a specific G-protein ($G\gamma_2$) appears to be involved in cannabinoid antinociception.

Eugene W. Gerner, Ph.D.

University of Arizona
Award Amount FY 2001: $150,000

Interdisciplinary Basic Science Program in Colon Carcinogenesis

Colon cancer is associated with tobacco use and is the second leading cause of cancer deaths in Arizona and the United States. The hypothesis to be tested in this research is that tobacco-specific carcinogens interact with specific genetic risk factors to increase the likelihood of colon cancer incidence. This interaction could occur in colon epithelia or stroma. Specific aims are to determine if tobacco-specific carcinogens interact with genetic risk factors to influence colon cancer development in rodent models. Investigators will document the effects of tobacco-specific carcinogens on cell turnover and expression of genes involved in cancer development in colonic mucosa of genetically altered rodent models of colon cancer. Studies will also address the effects of tobacco-specific carcinogens on processes affecting blood flow in normal and neoplastic colonic tissues in genetically altered rodent models of colon cancer.

Publications:

Gerner EW, Broome-Powell M, Erdman SH, Ignatenko NA, Besselsen D. Tumorigenesis in two genetically altered rodent models of gastrointestinal carcinogenesis involves deregulation of pulyamine synthesis. COST 917: Biogenically Active Amines in Food Volume III, 2001.

Martinez JD, Taylor Parker M, Fultz KE, Ignatenko NA, Gerner EW. The molecular biology of cancer. Burger's Medicinal Chem and Drug Discovery (6th ed), 2001.

Lawrence H. Hurley, Ph.D.

University of Arizona
Award Amount FY 2001: $143,194

## Drug Targeting of G-Quadruplexes as a Way to Reestablish the Normal Death Program in Cancer Cells

During the first year of this award we have demonstrated that a small drug molecule is able to down-regulate an important oncogene named c-myc, which is found to be over expressed in more than 60% of human cancers. We have also carried out experiments that provide considerable insight into how this down-regulation of c-myc is produced. This mechanism involved direct interaction in the promoter region of the c-myc gene, which results in turning off this gene. Since c-myc is a very important cancer gene in a variety of human cancers including colorectal, breast, prostate, and pancreatic, this is an important discovery that could have a direct effect on treatment of cancer in Arizona citizens.

Publications:

Grand CL, Bearss DJ, Munoz RM, Han H, Von Hoff DD, Hurley LH. Specific inhibition of c-MYC expression by the cationic porphyrin TMPyP4 results in downregulation of hTERT expression and reduced telomerase activity. Preceedings of the Amer Assoc for Cancer Res 42: 1123, 2001.

William R. Roeske, M.D.

University of Arizona
Award Amount FY 2000: $49, 500

## Regulation of the Human Delta Opioid Receptor

Smoking has been implicated in lung cancer, which is responsible for 44 deaths per 100,000 people in the period of 1986-90 in Arizona. Treatment of lung cancer has only a 13% five year survival. Lung cancer pain is generally controlled using opioid drugs. Effective opioid drugs have a variety of undesirable side effects which include respiratory depression, constipation, nausea, addiction and withdrawal. A new class of opioid drugs known as the δ-opioid receptor drugs have recently been developed and studied in animal models. These drugs are effective in pain relief and have fewer side effects. However, these new drugs also show some evidence of drug tolerance in animal models. Our recent studies provide novel differences between δ-opioid receptor drugs such as SNC80 or DRDPE in the treatment of lung cancer pain without the induction of tolerance. Results indicate that important differences exist between the two leading δ-opioid agonists, DPDPE and SNC80.

Jean M. Schmidt, Ph.D.

Arizona State University
Award Amount FY 2001: $118,204

*In Vivo* Efficacy Evaluation of New Anticancer Drugs

Five new anticancer drugs, discovered by the ASU Cancer Research Institute, have been tested in an *in vivo* scid murine system to determine maximum tolerated dosage in preparation for evaluating these drugs against human tumor xenografts. The xenografts are prepared using human tumor cell lines that derived from tocacco-related human tumors. A human tumor lung line NCI H460 is being used to evaluate auristatin PYE and auristatin 15 PE (new synthetic anticancer drugs related to the dolastatins) in scid xenografts. In the first two experiments with auristatin PYE, a reduction in tumor size was observed with 3 and 7 dosage treatments. Xenograft studies are continuing with these and other of the CRI anticancer drugs to aid in their preparation for clinical trials. Cell cycle inhibition studies using flow cytometry and DNA array analyses have been done with three of the CRI drugs to compare their modes of action.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 2001: $106,949

Development of New Antitumor Agents

The goal of research carried out in this laboratory is to design drugs that are specific for cancer tissue compared to normal tissues. Such cancer drugs will show low toxicity with minimal nausea, vomiting, and loss of hair. After one year of work of this project, drugs targeting histological cancer types afflicting many Arizonans have been developed, namely melanoma and non-small-cell lung cancer. Our approach to developing these drugs has been to exploit levels of the reducing enzyme DT-diaphorase in our drug designs. Cancer cells can possess high or low concentrations of this enzyme, which can be employed in the design of specific antitumor agents as follows. A cancer possessing high levels of DT-diaphorase can be targeted by a drug activated by this enzyme. On the other hand, a cancer possessing a low level of DT-diaphorase can be targeted by a drug inactivated by this enzyme.

Publications:

Xing C, Skibo EB, Dorr RT. Aziridinyl quinone antitumor agents based on indoles and cyclopent[b]indoles: Structure activity relationships for cytotoxicity and antitumor activity. J Med Chem 44, accepted, 2001.

Skibo EB, Xing C, Groy T. Recognition and cleavage at the DNA major groove. Bioorg Med Chem 9: 2445-2459 2001.

Robert A. Berg, M.D.

University of Arizona
Award Amount FY 2001: $50,000

## Optimal Treatment of Prolonged Ventricular Fibrillation: CPR First *vs.* Defibrillation First

Immediate defibrillation is clearly the treatment of choice for typical ventricular defibrillation cardiac arrest, but the optimal treatment for prolonged ventricular defibrillation is not well established. It was our objective to determine whether a brief period of cardiopulmonary resuscitation prior to defibrillation would improve readiness of the myocardium for defibrillation and thereby improve outcome. Two studies were performed in the first year of this project, one using standard defibrillators and the other using the increasingly available automatic external defibrillators, those that are typically found in airports and other public access places. Provision of precountershock CPR improved the readiness for defibrillation in both studies but did not improve ultimate outcomes in either study. Defibrillation outcomes were far superior for standard defibrillators when compared with an automatic external defibrillator.

Oin Mary Chen, Ph.D.

University of Arizona
Award Amount FY 2001: $50,000

## Molecular Mechanisms of Oxidant and Nicotine Induced Cardiac Toxicity

Cigarette smoke contains nicotine and can generate oxidants. Oxidants cause enlargement of cardiomyocytes, which can alter certain genes and affect the contractile function of the heart. These genes include atrial natriuretic factor, beta myosin heavy chain, and skeletal alpha actin. We have obtained materials and optimized the technique to measure changes in these genes. These changes can be regulated by early signaling events. Oxidants activate three branches of MAP kinases: ERKs, p38 and SAPKs. We have established methods to measure the activation of these kinases by nicotine and to determine whether nicotine enhances the effect of oxidants in cardiomyocytes. Calcineurin and NF-AT3 have been recently reported to play a role in cardiac hypertrophy. Oxidants may activate NF-AT3. We will test whether nicotine enhances this response. In conclusion, we have obtained all the gears and tools to study whether nicotine and oxidants harm cardiomyocytes synergistically at the molecular level.

Abstract:

Tu VC, Chen QM. Activation of NF-AT3 transcription factor in oxidant induced cariomyocyte hypertrophy. Toxicologist 60: Abs 214, 2001.

Janet L. Funk, M.D.

University of Arizona
Award Amount FY 2001: $110,567

## Investigation of a Novel Neuroprotective Agent in Stroke

Stroke is a major cause of death in Arizona. In recent decades little progress has been made in preventing the neurological defects caused by strokes. However, as research begins to unravel the step-by-step processes leading to nerve death in stroke, it has become apparent that these destructive processes might be interrupted by harnessing the body's own defense systems. Our laboratories have now identified a new member of the family of substances produced by the brain in response to stroke. This new substance, which is produced by blood vessels in injured brain, is able to increase the size of blood vessels and, thus, may limit brain injury by allowing more blood to flow into injured areas during stroke. Future experiments are planned to find out whether giving additional amounts of this new substance intravenously can prevent further brain injury and thus be used as a new treatment of stroke.

Elaine Jones, Ph.D.,R.N.

University of Arizona
Award Amount FY 2001: $49,500

## Heart Health for Arizona's Deaf Communities

The vision which guided this project was that multi-ethnic deaf communities across the nation would have the capacity for sustained heart health promotion programs which addressed the physical, socio-cultural and ethnic dimensions of deaf adults and their families. A multidisciplinary team participated in this phase. We 1) surveyed 111 multi-ethnic deaf adults in sign language about risks for heart disease; 2) custom designed a heart health classroom curriculum for deaf adults on heart healthy nutrition, physical activity, stress management, and smoking cessation; 3) developed and conducted a 16 week program to train a deaf man as a lay heart health teacher; 4) developed a study design and evaluation plan for pilot testing a heart health intervention with classroom, home and community activities; and 5) applied to the National Institutes of Health for funding to pilot test the new multilevel Deaf Heart Health Intervention in Arizona.

Ronald J. Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY 2001: $373,051

## Stroke, the Blood Brain Barrier, Nicotine and Nicotinic Acetylcholine Receptors

The broad goals of this program of four separate research projects are: 1) to establish effects of tobacco use and nicotine exposure on incidence, severity, progression, and treatment of stroke, and on recovery from stroke; 2) to establish effects of nicotine on the blood brain barrier (BBB), which is a system of small capillaries in the brain that maintains and protects the brain and its unique fluid environment, the cerebrospinal fluid (CSF) by restricting exchange from the blood to the brain and CSF; 3) to establish whether nicotinic acetylcholine receptors (nAChR), which are targets of tobacco nicotine action and critically involved in natural chemical signaling throughout the body, are on microvessels or other components of the BBB; and 4) to establish some features of nAChR that are involved in BBB function and are likely to contribute to effects of stroke. The broad thesis of the research program is that tobacco use and nicotine exposure affects the structure and transport characteristics of the BBB, and that these effects have deleterious consequences during stroke.

Studies completed during the first year of this project indicate that nAChR are found on blood vessels that constitute the BBB and affect BBB function. Building blocks for nAChR are expressed as messenger RNA in human microvascular endothelial cells, which line brain blood vessels and create part of the BBB. Acute and chronic nicotine exposures have effects on BBB function *in vivo* and in culture models. Immunostaining techniques localize nAChR building blocks to the brain vessels. A good deal of work has involved establishing experimental models and developing experimental tools necessary for the projects. Progress in the clinical study has been slow due to unexpectedly low enrollment of subjects, precluding conclusions about effects of tobacco use on stroke. However, data obtained to date is consistent with the project's central hypothesis that effects on the BBB occur with nicotine exposure, offering promise that continuing work in the project will define mechanisms involved in effects of nicotine and tobacco use on stroke and on the BBB. Aside from facilitating design of new strategies to prevent and treat stroke, insight might also be gained into how to regulate function of the BBB, particularly to facilitate therapeutic drug access to the brain.

John W. Regan, Ph.D.

University of Arizona
Award Amount FY 2001: $49,015

## FP Prostanoid Receptor Isoforms in Human Heart Disease

The goal of this project is to identify the prostaglandin receptor isoforms present in the human heart that are activated by $PGF_{2\alpha}$ and determine what second messenger pathways they stimulate. Prostaglandin receptors that are activated by $PGF_{2\alpha}$ are called "FP receptors" and recently we have discovered a new variant in the sheep ovary that we named the $FP_B$ isoform to distinguish it from the previously known isoform that we call $FP_A$. Prostaglandins, such as $PGF_{2\alpha}$, are linked to the cardiac hypertrophy and cell death that occurs during hypertension. Therefore, to understand the role of $PGF_{2\alpha}$ in this process, it is important to know the FP receptor isoforms that are present in the heart. During the period of this progress report, we have been successful in the identification of a FP prostanoid receptor isoform from human heart tissue, but it is not the $FP_B$ isoform predicted above, Instead it is a new isoform that we named $FP_S$. This isoform has not been previously described in the literature and may help us to understand the actions of $PGF_{2\alpha}$ on the heart.

### Publication:

Thompson EJ, Gupta A, Vielhauer GA, Regan JW, Bowden GT. The growth of malignant keratinocytes depends on signaling through $PGE_2$ Receptor $EP_1$. **Neoplasia** 3:402-410, 2001.

David L. Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY 2001: $126,000

## The Effect of Nicotine in an Animal Model of Both Cardiovascular and Alzheimer Disease

Our first studies were aimed at confirming the sex differences in the effect of cholesterol on production on AD-like neuropathology. We confirmed that females (rabbits) are less effected by dietary cholesterol, and have shown that this is likely due to natural estrogen cycles. We have also assessed the effect of nicotine (non-smoke) on AD-like neuropathology in an alternate model. Using a transgenic mouse model of AD (known to develop beta-amyloid plaques in the brain) we have been able to show in pilot data that chronic injections of nicotine do reduce the levels of AD-like neuropathology. We have just completed the 10-week experiments (feeding of rabbits) for histology and are in the process of completing those immunohistochemical studies.

We have also found that the water administered to the male animals has a major effect on the body-wide pathologic nidus of cholesterol. Pilot data suggest that tap water may augment the pathologic effect of cholesterol in the spleen and liver. The effect in the brain is currently being investigated. These findings may have great importance to the people of Arizona, in that nicotine, without other agents found in cigarette smoke, may have benefit in combating the influence cholesterol may have in the Alzheimer's and heart disease.

Edward D. French, Ph.D.

University of Arizona
Award Amount FY 2001: $128,093

## Nicotine Dependence and Dopamine Neurons: Electrophysiological and Molecular Studies

During this past year we began a systematic examination of the effects of nicotine on nerve cells in the brain's reward center of the rat and mouse. Nicotine acts on nicotinic acetylcholine receptors (nAChR) to produce changes in cellular excitability. Since there are several subtypes of nAChR, it is important for the future development of therapeutic treatments for nicotine addiction to identify the specific receptor subtype that mediates nicotine's rewarding effects. Our approach is to combine electrophysiological, molecular biological and behavioral techniques to assess the effects of acute and long-term nicotine exposure on dopamine and non-dopamine neurons in the brain's pleasure/reward center. Although our initial experiments were carried out in the rat, we soon realized that the potential availability of transgenic/gene knockout mice would provide a more powerful tool by which to study the nAChR subtypes mediating nicotine neuronal effects. The selectivity of these genetic mutants for different nAChR surpasses current pharmacological agents.

During the funding period we developed the electrophysiological techniques needed to record from single nerve cells in the mouse. Our results show that, unlike the rat, dopamine neurons in the mouse are predominantly inhibited by nicotine injections. However, the effects of additional nicotine injections are smaller suggesting that dopamine neurons desensitize to nicotine's effects within a few minutes. Radioligand binding in mouse brain sections indicates that these animals have a unique profile for the distribution of nAChR subunit messages. By understanding the cellular responses by which nicotine becomes an addictive substance we may be able to devise new therapies to treat nicotine dependence which affects 25% of the population of Arizona.

Dean Coonrod, M.D., PMH

Maricopa Medical Foundation
Award Amount FY 2000: $48, 609

## No Mas Cigarros - Smoking Cessation for Latinos

Smoking cessation is an important part of tobacco control. Efforts that enable smokers to quit may help diminish the substantial burden of smoking-related diseases. These illnesses account for at least one third of all deaths in women aged 35 to 69 in the U.S. Each year 1,600 women in Arizona die of lung or cervical cancer, both of which are related to smoking. The burden of these diseases is disproportionately borne by minority women. For example, cervical cancer is more common in Latino women and when it occurs, is more deadly because it is identified at a more advanced stage. Currently, between 8 and 15% of Latino women smoke. However, it is anticipated that, as these women become more acculturated, an increasing percentage will smoke, thus leading to more smoking-related deaths. In light of the sizable population of Latino women in Arizona, it is critical that we identify a means of assisting their efforts to stop smoking.

For this reason we designed a study with three phases: I) Collect baseline data about the study population centered around Maricopa Medical Center. II) Design a culturally competent smoking cessation program from the American Lung Association's Freedom from Smoking program and create as a comparison group, a literal translation of the program. III) Test the efficacy of the program.

Phase I was accomplished with interviews of 126 Latino smoking women from the target population. Most (57%) had no insurance or Medicaid/Medicare, and median income was $10, 000-19,999. 68 respondents (54%) were born in Mexico, 53 (42%) in the US, and 5 (4%) declined to answer. The median length of US residency for those born in Mexico was 6-10 years. Sixty respondents (49%) reported that they were not intending to quit smoking within the next 6 months; 25 (21%) were thinking about quitting within the next 30 days. Interestingly, stage of quitting was substantially correlated with scales that measured 1) the degree to which important others opposed smoking, 2) the extent to which smoking behavior was externally cued, 3) perception of the negative impact of smoking, 4) perception of positive outcomes of quitting, and 5) perceived attributions of smokers. In this generally unacculturated group, variables which tended to influence stage of quitting did not differ across levels of acculturation. These and other findings were used to assist in the design of the smoking cessation programs.

Phase II was completed by obtaining the Freedom From Smoking curriculum, hiring a bilingual smoking cessation specialist who was trained in the Freedom From Smoking curriculum. The materials and handouts were translated for our comparison curriculum. This individual along with a trained clinical psychologist, who as also bilingual and is Mexican American, modified the

curriculum to make it more relevant to the population to be served by the project. Specific issues were also raised with three consultants with expertise in Hispanic culture to assist in the process of creating the curriculum. Once the two curricula were created, the consultants rated the curricula as to cultural competence using a scale derived from published scales of cultural competence. The raters found that, overall, our goal of creating a culturally competent curriculum was accomplished. It should be noted that one important difference from the Freedom From Smoking program was the provision of individual classes as outlined in the protocol to facilitate a rigorous evaluation using a randomized trial design. Another important deviation was the withdrawal of funding from the pharmaceutical manufacturer of bupropion due to the longer than anticipated time to produce the curriculum.

Phase III was initiated. Enrollment in total was limited to 26 subjects. In addition, completion of the program was not high due to numerous barriers in this population, transportation, home issues and the individual as opposed to a group format for the classes. Exhaustive efforts at recruitment were attempted with no large rates of enrollment. For this reason in consultation with ADCRC staff, we focused our efforts to disseminating our program to other organizations in the state and with the American Lung Association. An analysis was done on twenty-six women asked to participate in the *No Mas Cigarros* tobacco cessation program. Of those women, ten completed questionnaires regarding their satisfaction with the program and six were found to have moved. Ten participants could not be located. In general, most women believe that the classes helped them to find ways to deal with stress, presented the information in a manner that reflected an understanding of the Hispanic culture, and reflected an understanding to the lifestyles associated with smoking. Most women found the classes to be helpful, enjoyed the emphasis of family relationships on cessation success, believed the classes were easily understood and found them to be inoffensive. Parts of the program found to be most helpful included the flexibility with the class schedules, the knowledge level of the instructor, the methods of coping with stress, and the ideas to help avoid smoking when cravings are present. No part of the program was found to be unhelpful by a majority of the participants.

Hugh Miller, M.D.

University of Arizona
Award Amount FY 2001: $123,600

## The Effectiveness of Counseling and Bupropion Hydrochloride in Prevention of Postpartum Smoking Recidivism

The success of smoking cessation in pregnancy is fleeting in that postpartum relapse continues to be rampant having a dramatic impact on Arizona's women, children and families. We have spent the last year overcoming some unexpected obstacles in the execution fo the funded clinical trial. The trail called for the randomization of postpartum women to standard of care, relapse prevention counseling with a placebo or bupropion SR. We had anticipated being able obtain the drug/placebo as clinical trial material from Glaxo Wellcome, but were not able to convince their research committee to support this trial. As a consequence, we were forced to consider alternate strategies to preserve the blinding required of the study. Ultimately, with the help of our investigational pharmacy, we were able to overcome this obstacle and successfully launched the study. Although this process delayed the initiation of the study by several months, in spite of the delay we have completed the first year, achieved our enrollment goals and anticipate completing this trial on schedule.

James F. Collins, Ph.D.

University of Arizona
Award Amount FY 2001: $49,500

## Characterization of the Effect of Nicotine on the Lung Sodium-Phosphate Transporter (NaPi-IIb)

Smoking is well known to have adverse effects on surfactant production (which is a protective, mucous coating that lines the lungs). Surfactant producing cells in the lung show high expression of a phosphate transporting protein, which allows these cells to intake the Pi necessary for surfactant production. Our recent data have demonstrated that nicotine and cigarette smoke exposure decrease expression of this key gene in isolated lung cells and in rats. We surmise that this reduction in gene expression may have correlations with the smoking-induced reduction in surfactant production. Further experiments will be aimed at understanding the relationship between this gene and surfactant production and will involve isolated cell and whole animal studies. These studies are highly relevant to the State of Arizona as a large percent of the population is known to be smokers here, and pulmonary related diseases directly related to smoking cost the taxpayers millions of dollars.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 2001: $50,000

## Nicotine Effects on Human Stem Cell Differentiation *in Vitro*

Little work has been done to determine the potential effect of nicotine on the developing immune system of the fetus. A clear understanding of how tobacco products cause the loss of immune function would go a long way towards developing effective counter-measures to prevent immune system dysfunction. Indeed, since the active addicting component of tobacco is nicotine, and new drugs currently being developed for the treatment of depression and pain are derived from substances that interact with the same cellular components that react with nicotine, information derived from a study that targets nicotine action on the immune system will be crucial to assure that the drugs do not cause inhibition of immune function. In the past year we have shown that the stem cell system that we intend to use to measure the effects of nicotine on stem cell development does, indeed, measure stem cell activity. We have also found in a parallel study of human stem cell development that the ability of stem cells isolated from infants of mothers who have been exposed to nicotine are severely limited in their ability to develop into immune cells. These two studies should provide insight as to how nicotine exposure can alter the function of blood cells before birth and how these effects can be prevented.

Adele M. Turzillo, Ph.D.

University of Arizona
Award Amount FY 2001: $ 50,000

## Steroid Production and the Oxidative Stress Response in Ovarian Follicular Cells: Effect of Nicotine and Cotinine

The goal of this research is to determine the effects of constituents of cigarette smoke on ovarian function. Thus far, we have determined that nicotine influences production of an important ovarian hormone, androstenedione. Since androstenedione is an essential precursor for estrogen synthesis, inhibition of androstenedione production by nicotine may contribute to lower estrogen production in women who smoke. We hypothesize that nicotine exerts negative effects on ovarian function by a mechanism called oxidative stress. We have characterized several parameters of oxidative stress in healthy and unhealthy (atretic) ovarian follicles. In the next year we plan to test whether nicotine can induce oxidative stress in healthy follicles and cause them to undergo atresia. Continued progress on these studies will enhance our understanding of the mechanisms underlying the negative effects of smoking on fertility in women.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 2001: $150,000

## Analysis of Autoantibodies to T-Cell Receptors in Rheumatoid Arthritis

Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) are classic autoimmune diseases prevalent in Arizona. RA has an abnormally high incidence of approximately 5%. In previous ADCRC-supported research, we reported that individuals with RA had elevated levels of immune macroglobulins (IgM Class) directed against variable domains of human T-cell receptors. Since T cells are involved in inflammatory destruction of joints in RA, we hypothesized that the autoantibodies might exert a regulatory or protective function. To determine the properties of these molecules and to assess their functions, we produced monoclonal autoantibodies. Thus far, we have generated and characterized seven RA-derived IgM monoclonal anti-TCR autoantibodies in terms of recognition properties and sequence of the genes used to provide the specificity in the variable domains. In addition, we have generated and partially characterized an additional eight monoclonals, including four IgG molecules from an SLE patient to assess differences between this class of molecules and the IgM group in structure and disease-related function. In *in vitro* tests on antigen-activated T cells, we found that the production of the inflammation-causing cytokine II-2 was substantially decreased by treatment of the cells with the anti-TCR antibodies. Furthermore, the antibodies themselves did not cause programmed death (apoptosis) of the cells. Thus, we have initial evidence that the human monoclonals may function to suppress the inflammatory response characteristic of destructive antoimmune diseases. Additional studies showed that the anti-TCR autoantibodies were functionally and genetically distinct from the so-called Rheumatoid Factors that are characteristic markers of RA.

**Publications:**

Marchalonis JJ, Adelman MK, Robey IF, Schluter SF, Edmundson AB. Exquisite specificity and peptide epitope recognition promiscuity properities shared by antibodies form sharks to humans. J Mol Recog 14:110-121, 2001.

# SECTION B

## CONTRACTS

## TOBACCO-RELATED RESEARCH

## YEAR TWO

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 2001: $147,501

## Effects of Antioxidant Nutrients and Smoking on Type-Specific HPV Persistence

This past year laboratory analyses of HPV types and nutrient concentrations were completed and statistical analyses performed. In this cohort, 42% had an HPV infection at baseline and 54% had a least one type of HPV infection during the study period. Same type oncogenic HPV infections were more likely to persist than non-oncogenic infections with the highest persistence rate associated with type 16. The most common HPV infections were with types 53, 39, 16, 52, and MM8. The rate of new infection was 2.8% per month. Dietary consumption of vitamin E, lutein, and vegetable, as well as circulating *cis*-lycopene and vitamin B12 concentrations, may reduce HPV persistence. Findings from this study suggest that consuming diets rich in vitamin E (*i.e.*, vegetable oil), lutein (*i.e.*, spinach), and vegetable may reduce risk of HPV persistence. Collectively, the results from this study suggest that improved nutritional status may reduce HPV persistence.

Iman Hakim, M.D.

University of Arizona
Award Amount FY 2001: $136,091

## The Role of High Tea Consumption in the Modulations on DNA Oxidative Damage in Smokers

The role of tea drinking as a potential inhibitor of carcinogenesis merits careful evaluation. The study seeks to randomize 135 smokers to one of the 3 groups (45 smokers per group) of a 4-month tea drinking intervention trial. The main aim of the study is to compare high consumers of green or black tea to the control group for the effect of tea consumption of oxidative DNA damage, as measured through urinary 8-OhdG. Adherence and biologic response to the high tea consumption were measured in all randomized participants. Preliminary adherence data show that consumption of 4 cups of tea per day is a feasible approach. Furthermore, preliminary analysis of the first group of smokers (n=50) show a potential decrease in the urinary levels of 8-OhdG among the green tea group. Detailed adjusted analyses will be done upon completion of all subjects to clarify the relationship between tea intake and DNA damage.

Bertram L. Jacobs, Ph.D.

Arizona State University
Award Amount FY 2001: $150,000

## Specific Induction of dsRNA-mediated Suicide in Lung Cancer Cells

Most anti-cancer agents work by making cancer cells commit suicide. We have been investigating induction of suicide in cancer cells by a novel potential anti-cancer agent, dsRNA. dsRNA induces suicide in a very different manner from other known anti-cancer agents. dsRNA appears to induce suicide even in cancer cells that have become resistant to treatment with traditional anti-cancer agents. The problem with using dsRNA is that it causes both cancerous and normal cells to commit suicide, leading to unacceptable side-effects. We are proposing to develop a method to specifically make dsRNA only in cancer cells, thus allowing specific killing of cancer cells while minimizing injury to normal cells in the body. We plan to do this by engineering an otherwise harmless virus that will make dsRNA only in cancer cells. Tumors could then be infected with this virus which would specifically kill the cancer cells without harming surrounding normal cells.

Jesse Martinez, Ph.D.

University of Arizona
Award Amount FY 2001: $49,990

## p53-Dependent Apoptosis in Lung Cancer

Lung cancer is the number one cancer killer in the United States with 150,000 new cases diagnosed every year. Its epidemiology and predisposing factors have been intensively studied, and tobacco consumption has clearly emerged as the single most important risk factor for the development of lung cancer. As with other cancers, the development of lung cancer is a multi-step process during which the tumor progressively evolves more of the characteristics of a metastatic tumor. Frequently, these tumors exhibit defects in those processes that regulate cell growth. Some of the molecules that regulate cell growth are the 14-3-3 proteins.

We have examined the expression of 14-3-3 proteins in bioposies of human lung tumors using a variety of techniques and found that they are overexpressed. Importantly, our latest research results suggest that 14-3-3 proteins can regulate the tumor cell's sensitivity to radiation-induced cell death. Hence, we believe that these proteins may play a role in lung cancer development and that by manipulating either the activity of the 14-3-3 proteins or their abundance in tumor cells, we may be able to modify the sensitivity of lung tumors to treatment by conventional radiotherapy. Furthermore, because it appears that 14-3-3 expression occurs at specific stages during tumor development, these proteins may be useful as markers for diagnosis.

Claire M. Payne, M.D.

University of Arizona
Award Amount FY 2001: $150,000

## Aberrant Expression of Redox-Associated Proteins NF-kb(p65) Thioredoxin and Inducible Nitric Oxide Synthase as Biomakers of Colon Cancer Risk

We have made considerable progress in the evaluation of the proteins NF-κB, a factor that enhances gene expression; thioredoxin, a secreted factor that protects against cell death; and NOS2, an enzyme that produces nitric oxide and protects against cell death as biomarkers of colon cancer risk. The level of expression of these interrelated proteins in the colon was compared to the results of a live cell bioassay that measures induced cellular death in the colonic tissue from patients obtained during colonoscopy or from the operating room, and also to the level of dedifferentiation (*i.e.* cellular immaturity). We determined that aberrant staining of these 3 proteins was highly correlated with death resistance and dedifferentiation, two processes associated with tumor progression. However, in 6 of the samples from 4 different patients with colon cancer, aberrant staining with either NOS2, NF-κB or thioredoxin staining was the only predictor of colon cancer risk.

Publications:

Bernstein C, Payne CM, Bernstein H, Garewal H. Activation of the metallothionein IIA promoter and other key stress response elements by ursodeoxycholate in HepG2 cells: Relevance to the cytoprotective function of ursodeoxycholate. **Pharmacology** (in press).

Payne CM, Bernstein H, Bernstein C , Kunke K, Garewal H. The specific NOS2 inhibitor, 1400W, sensitizes HepG2 cells to genotoxic, oxidative, xenobiotic and ER stresses. **Antioxidants & Redox Signaling** (in press).

Washo-Stultz D, Crowley-Weber CL, Dvorkova K, Bernstein C, Bernstein H, Kunke K, Waltmire CN, Garewal H, Payne CM. Role of mitochondrial complexes I & II, reactive oxygen species and arachidonic acid metabolism in deoxyccholate-induced apoptosis. **Cancer Letters** (accepted with revision).

Crowley-Weber CL, Payne CM, Gleason-Guzman M, Watts GS, Futscher B, Bernstein C, Garewal H, Bernstein H. Development and molecular characterization of colon cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. **Carcinogenesis** (accepted with revision).

Donato Romagnolo, Ph.D.

University of Arizona
Award Amount FY 2001: $49,415

## Transcriptional Repression of the Breast Cancer Gene BRCA-1 by Tobacco Polycyclic Aromatic Hydrocarbons

To date, no mutations in the BRCA-1 gene have been identified in sporadic breast cancers, whereas the expression levels of BRCA-1 in breast tumors are lower than those observed in normal mammary tissue. The knowledge gained through these studies offers evidence that exposure to AhR-ligands may be a risk factor in environmental carcinogenesis of the breast. Because BRCA-1 is involved in DNA repair, loss of BRCA-1 protein may favor the accumulation of DNA damage and the onset of sporadic breast cancer. This event may increase in women exposed to these environmental pollutants the susceptibility to developing mammary neoplasia. Finally, a significant implication of the findings reported here is that basal expression of BRCA-1 may be positively regulated by low-levels of exposure to ligands of the aromatic hydrocarbon receptor. This may represent a mechanism of protection against low-dose exposure to polycyclic aromatic hydrocarbons and may assign to the BRCA-1 gene a role of sentinel marker against low-dose/chronic exposure to this class of environmental xenobiotics.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY 2001: $50,000

## Enzyme Active Site Tailored Anticancer Drugs

Cancer cells carry out biochemical processes that maintain the cancerous state of the cell and promote the unrestrained cell division characteristic of cancer. One of those processes requires the action of a particular enzyme in the cell. We are preparing and testing chemical compounds designed to block the action of this enzyme. The chemical compounds are tailored to fit into the enzyme and chemically react with it, thereby destroying its activity. Compounds in four different chemical classes were designed and prepared, to offer a variety in chemical reactivity and to potentially offer different toxicity profiles. Compounds were tested in cell culture for effectiveness at inhibiting growth of human lung, colon, prostate, and bladder cancer cells, as well as leukemia and fibrosarcoma cells. Several compounds were found to potently inhibit cancer cell growth. This work may identify new anticancer agents for the benefit of Arizona residents.

Publication:

Okolotowicz KJ, Lee WJ, Hartman RF, Kim AY, Ottersberg SR, Robinson Jr. DE, Lefler SR, Rose SD. Inactivation of Protein Farnesyltransferase by Active-Site-Trageted Dicarbonyl Compounds. Arch Pharm Med Chem 334:194-202, 2001.

Danny L. Brower, Ph.D.

University of Arizona
Award Amount FY 2001: $45,593

## Genetic Probes for the Study of Integrin Structure-Function Relationships

Heart attacks are caused by the cross-linking of aggregates of blood platelets into an occluding clot; the platelets are linked to one another and to the artery wall, by proteins that are members of the integrin family of cell surface receptors. Our studies are aimed at generating and characterizing a set of mutations in integrin genes. These mutations will provide tools for subsequent studies designed to understand how the structures of integrins relate to integrin function. This basic information can then be used to assist in the intelligent design of agents for the therapeutic inhibition of integrin function. Using a genetic screen in the model organism Drosophila, we have to date identified close to 60 new integrin mutations and have determined the molecular defect in most of these. We continue to create and characterize more potentially informative mutations in the gene encoding the βPS integrin protein.

Mary C. Davis, Ph.D.

Arizona State University
Award Amount FY 2001: $47,156

## The Effects of Smoking and Menopause on Physiological Stress Responses in Middle-aged Women

This project examines the potentially damaging physiological consequences of smoking when it coincides with menopause and stress. To date, approximately 230 women have been contacted to determine their eligibility for participation in the project. Of these, 16 pre- and post-menopausal women have successfully completed the laboratory stress session, during which cardiovascular and stress hormone measures are collected. Most women screened out of participation were ineligible because they were 1) not clearly either pre- or post-menopausal, or 2) taking medications that affect physiological stress responses. The sample recruited so far is comprised of both smokers and nonsmokers, Caucasians and women of color, and appears to be representative of mid-life women in Arizona. As expected, preliminary evaluation of physiologic responses during the laboratory session suggests that the protocol is eliciting a strong reaction in women, which should allow for powerful comparisons between the stress responses of pre- and post-menopausal smoking groups.

Eugene Morkin, M.D.

University of Arizona
Award Amount FY 2001: $150,000

### Grafting of Stem Cell-Derived Cardiomyocytes to Repair Myocardial Infarction

Coronary artery disease causes heart attacks (myocardial infarctions), which may result in death or disability from congestive heart failure. This is a major tobacco-related public health problem affecting more than 4 million individuals within the United States. After myocardial infarction the heart is unable to repair itself because heart cells in adults have lost their ability to divide. The goal of this proposal is to develop a strategy for repair of myocardial infarction using cardiomyocytes grafting. A major problem with this approach has been poor survival of engrafted cells. To overcome this limitation, we have studied the use of a naturally modified extracellular matrix (collagen-1) as scaffolding for engrafting heart cells. Three weeks after grafting, the collagen scaffold was completely integrated with the injured myocardium. The scaffold prevented cardiac dilation and improved function by shifting ventricular pressure-volume curve toward normal. Furthermore, where angio-neogenesis was present within the graft, mature small arteries were connecting to native coronary vasculature. These preliminary results suggest collagen-1 scaffolding grafted onto injured myocardium integrates with the tissue, induces mature vessel formation within the graft, and results in improved cardiac diastolic function by preventing cardiac dilation.

Alexander M. Simon, Ph.D.

University of Arizona
Award Amount FY 2001: $50,000

### Role of Gap Junction-Mediated Communication in Preventing Endothelial Dysfunction

There is good evidence that smoking contributes to endothelial dysfunction, a key part of the atherosclerotic process. The mechanism by which smoking leads to diminished blood vessel responses is not well understood. One possibility is that smoking could lead to a deficiency of gap-junction mediated communication in vascular cells. Gap junctions are aggregations of intercellular channels that connect adjacent cells. They are composed of a family of related proteins called connexins. The objective of this project is to examine the effects of disrupting intercellular communication in the blood vessel wall, using mice which lack specific connexins. We found that eliminations of connexin40 dramatically reduces endothelial communication and that ablation of both connexin40 and connexon37 abolishes communication. Elimination of connexin40 was found to cause a striking decrease in the levels of connexin37 protein. These results suggest either that connexin40 and connexin37 interact in endothelial cells or that their synthesis is co-regulated.

Marvin J. Slepian, M.D.

University of Arizona
Award Amount FY 2001: $50,000

## Microtubule-Dependent Ingergrin Function: Role in Atherosclerosis and Restenosis

The migration of smooth muscle cells (SMCs), cells of the arterial wall, into the lumen of the artery leads to narrowing. This is a vital mechanism in the development of atherosclerosis. The interactions of SMCs with their surrounding extracellular matrix, through adhesion receptors known as "integrins" are vital in cell migration. This study examines the role of microtubules on SMC integrin function. Over the past year we demonstrated that the microtubule inhibitory agent Combretastatin A4 (CA4) limits SMC adhesion and spreading and induces programmed cell death (apoptosis) in an integrin-specific fashion. CA-4 effects appear to be mediated via "inside-out signaling" impacting on $\beta 3$ integrin function. *In vivo* local delivery of CA-4 was shown to limit arterial re-narrowing following balloon injury. These finding support our working hypothesis for novel activity of CA-4 involving selective disruption of microtubule-dependent $\beta 3$ integrin functions. Defining the mechanisms regulating integrin function will open the door for Arizona citizens to new anti-atherosclerotic therapies.

Ronald J. Lukas, Ph.D.

St. Joseph's Hospital
Award Amount FY 2001: $149,713

## Molecular Bases for Nicotine Dependence

Nicotine dependence is thought to drive the habitual use of tobacco products by an estimated one million individuals in the State of Arizona alone at enormous economic and personal costs. Nicotine's powerful and multi-faceted effects on the brain and body must begin with its actions at its principal targets, the diverse group of chemical signaling molecules called nicotinic acetylcholine receptors (nAChR). This project seeks to establish how extended nicotine exposure affects individual forms of nAChR.

Work completed in the first two years of this renewed project supports our hypothesis that extended exposure to nicotine induces long-lasting changes in numbers and function of nAChR. Doses and time-dependencies of these effects differ across nAChR subtypes. We have found that different nicotine-like drugs mimic or block effects of nicotine at specific forms of nAChR. We also have made the surprising finding that the loss in nAChR function is progressively deeper as nicotine treatment times are lengthened. Electrophysiological studies have been extended to examination of the $\alpha3\beta4^*$-nAChR subtypes found in neurons, and chemical studies have investigated genetically engineered nAChR like those that predominate in the brain ($\alpha4\beta2$-nAChR) or that may be located in brain areas critical for mood and cognitive functions ($\alpha4\beta4$-nAChR). Of the nAChR subtypes examined, the most abundant in the brain, the $\alpha4\beta2$-nAChR subtype, shows the highest sensitivity to block effects induced by chronic nicotine exposure. It also is the most sensitive to block by the drug mecamylamine, which has been successfully used in smoking cessation.

Our studies are identifying nAChR forms that are most powerfully affected by chronic nicotine exposure, and our work is uncovering drugs that mimic or block those effects. This information is of potential use in the design of strategies and therapies to block nicotine dependence and relieve unpleasant effects of nicotine withdrawal, thereby promoting cessation of tobacco use and control of tobacco-related disease. Our findings prompt new hypotheses that long-term losses in nAChR function contribute to collateral changes in brain chemistry and function, thereby contributing to nicotine dependence.

Ronald M. Lynch, Ph.D.

University of Arizona
Award Amount FY 2001: $48,640

## Effect of Nicotine on Hypothalamic Glucose-Responsive Neurons

A primary reason smokers do not stop smoking is weight gain following cessation of nicotine dosing. Specialized neurons within the brain detect changes in nutrients and hormones to regulate body weight. We propose to study the effects of nicotine on these neurons in rodents, an accepted model for nicotine exposure and metabolic homeostasis. Initially, we identified markers for nutrient-sensitive neurons and analyzed their distribution in the brain. However, a primary limitation to our studies is that these cells are few in number and, therefore, difficult to identify and isolate. We developed to transgenic mouse line in which nutrient-responsive cells express a protein called Green Fluorescent Protein (GFP) that emits light when excited. Using GFP fluorescence, we will isolate these rare cells and characterize the components allowing them to sense nutrient levels. These studies may ultimately provide a better understanding of the physiological basis of an important aspect of nicotine addiction.

Publication:

Lynch RM, Tompkins LS, Brooks HL, Dunn-Meynell AA, Levin BE. Localization of glucokinase gene expression in the rat brain. Diabetes 49 (5):693-700, 2000.

Ian D. Bier, N.D., Ph.D., L.A

IB Scientific, LLC
Award Amount FY 2001: $149,846

## Auricular Acupuncture, Education and Smoking Cessation: A Randomized, Sham-Controlled Trial

Treatment was completed for two groups, however recruitment numbers were below expectations. The second year of the project was extended for six-months to complete the required patient recruitment. Another group is being recruited for an October 15, 2001 start.

Objectives of the study are as follows:

1. Determine the effects of sham acupuncture as compared to true acupuncture given alone and in combination with a smoking cessation educational program on the following dependent variables:
   (a) number of cigarettes smoked during treatment and 3, 6, 9, 12, 15, and 18 months later;
   (b) level of cigarette craving during treatment and 3, 6, 9, 12, 15, and 18 months later;
   (c) participant's health and psychological status at 3, 6, 9, 12, 15, and 18 months following treatment;
   (d) participant drop-out rate from treatment.
2. Determine the efficacy of acupuncture alone, versus in combination with a smoking cessation educational program.
3. Identify predictor variables to determine who might or might not respond to acupuncture and educational intervention based on psychological and health status characteristics predictive of outcome in conventional treatment (*i.e.*, anxiety, depression).
4. Identify possible adverse effects of acupuncture treatment.

Data analysis will begin in year three.

Paul A. St. John, Ph.D.

University of Arizona
Award Amount FY 2001: $45,493

## Effect of Nicotine on Neuronal and Glial Development: Interaction with Ethanol

Many prenatal infants are exposed to two common drugs, nicotine via smoking and alcohol. Each year in Arizona, 8500 pregnant women who smoke will deliver infants three times more likely to be premature, with increased mortality and morbidity. Our previous studies showed that low concentrations of nicotine slow the growth of nerve cell processes and significantly reduce the number of glial cells, the supporting cells in the nervous system responsible for neuronal development and function. In the past year, we have begun to determine which types of nicotinic acetylcholine receptors, the biological substrates for nicotine in the body, are present in neurons and glial cells, as a prerequisite to understanding the mechanism(s) by which nicotine exerts its deleterious effects on these cells. Our studies have shown that sustained exposure to nicotine can cause a dramatic loss of cell surface nicotinic receptors via stimulated internalization of receptors into the cell.

### Publications:

St. John PA, Gordon H. Agonist cause endocytosis of nicotinic acetylcholine receptors on cultured myotubes. J Neurobiol (in press).

Thomas G. Beach, M.D.

Sun Health Research Institute
Award Amount FY 2001: $36,025

### Smoking as a Disease Modifier in Alzheimer's Disease: An Autopsy Study

This project has examined the relationship of cigarette smoking to Alzhemier's disease (AD). Several studies have suggested that AD is less common in people who smoke, while other studies have suggested the opposite. The weakness of these studies is that they were done on living people, since an accurate diagnosis of AD cannot be obtained during life. Only microscopic examination of the brain after death can establish the diagnosis with certainty. This study has compared the severity of autopsy-diagnosed AD in smokers and non-smokers and has found that the disease starts 4.4 years earlier in smokers and affects the brain more severely, in terms of the microscopic lesions of the disease. These results indicate that reducing cigarette smoking in Arizona will substantially decrease the attack of Alzheimer's disease as well as the severity of disease in those who contract it.

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY 2001: $50,000

### Nicotine-Induced Effects on Immune Functions: Neural-Immune Mechanisms

Rheumatoid arthritis (RA) represents a significant health problem for Arizona residents. Nicotine in tobacco smoke may adversely affect RA by changing sympathetic and hypothalamic outflow to alter release of norepinephrine/epinephrine and cortisol levels. These nervous system pathways modulate immune functions that may contribute to disease pathology. Our studies examine whether nicotine increases arthritis severity by altering nervous system pathways that modulate immune functions. We observed an increase in disease severity following chronic nicotine treatment. We found nicotine treatment alters macrophage cytokine profiles in a manner that promotes strong cell mediated immune responses and inflammation, and thus increases disease severity. We also propose that chronic nicotine treatment will shift of T helper cell cytokine production towards a Th1 cytokine profile that would be expected to promote inflammation in arthritic joints, as well. These data suggest that chronic nicotine exposure as a result of smoking or treatments to quit smoking exacerbate arthritis.

# SECTION C

## CONTRACTS

## TOBACCO-RELATED RESEARCH

## YEAR THREE

Anne E. Cress, Ph.D.

University of Arizona
Award Amount FY 2001: $48,900

## Targeting of Lung Cancer Cell with Anti-Adhesive Agents

Our project was to test if anti-adhesive agents (peptides) could be developed to prevent human lung tumor cells from adhesion to a protein called laminin. We have obtained pure peptide reagents in mg amounts. We have discovered that the peptides are biologically active as determined by cell adhesion assay. The peptides can inhibit human tumor cell adhesion to laminin. The results of this work have been published in Cancer Research and were selected as cover art for the journal. These peptide reagents will now be tested in other model systems to determined their ability to biologically alter the invasive and migratory phenotype of human tumor cells. In addition, the techniques developed in this project are being used to screen for other small molecule inhibitors of tumor cell binding to laminin. The results from the project taken collectively have added significantly to our understanding of the mechanism of cell adhesion as well as providing lead compounds for the alteration of tumor cell adhesion to laminin.

Publication:

DeRoock IB, Pennington ME, Sroka TC, Lam KS, Bowden GT, Bair EL, Cress AE. Synthetic peptides inhibit adhesion of human tumor cells to extracellular matrix proteins. **Cancer Research** 61:3308-3318, 2001.

A. Jay Gandolfi, Ph.D.

University of Arizona
Award Amount FY 2001: $49,520

## Role of Tobacco-Derived Cadmium in Prostate Disease

Prostate cancer incidence and mortality has been linked with cigarette smoking and cadmium. In 1995, mortality due to prostate cancer in Arizona was in the top 40% of the United States. As there is no acceptable animal model of prostate cancer, we have recently developed a tissue slice model for human prostate. Slices made from regions of the prostate that are targets for disease have been incubated for long periods and found to retain their specific characteristics. Treatment of these slices with cadmium produces a toxicity in the region in which cadmium would be expected to produce its cancer. Human prostate slices represent a unique and highly relevant model to investigate tobacco-derived cadmium-induced prostate cancer.

A.A. Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 2001: $47,515

## Mechanism-based Discovery of Novel Antitumor Agents for Plant

During the course of the third year of this project, 91 plants species were collected and extracted yielding 273 extracts. These extracts were screened in a panel of 3 cell lines representing lung, breast and central nervous system cancers and a mechanism-based assay for the ability to cleave DNA.

Of the 273 extracts tested, a total of 12 extracts showed potential anticancer activity. We have completed bioassay-guided fractionations of two of the most promising extracts discovered this year, namely those of *Bebbia juncea* and *Berlandiera lyatra* (chocolate daisy) and continued fractionation studies we initiated earlier on two plants, namely *Acourtia thurberi* and *Phoradendron juniperinum*. The structure of the novel isocedrane with moderate anticancer activity from *Acourtia* has been revised on spectroscopic data and the structure of the DNA-cleaving compound from *Phoradendron* has been identified as 5-caffeoyl-*epi*-quinic acid. The anticancer constituent in *Bebbia* has been identified as dehydrofalcarinol and those in *Berlandiera* have been identified as pumilin and 3α-epoxypumilin. Interestingly, dehydrofalcarinol was found to preferentially inhibit the growth of lung and CNS glioma cell lines compared with the breast cancer cell line. We plan to submit these compounds to the NCI 60 cell line panel with the hope of developing them as anticancer drugs. If these can be developed as drugs to treat solid tumors, our results will have an impact on the more elderly and/or tobacco-dependent portions of Arizona's population since these occur more often in our State.

### Publications:

Furbacher TR, Gunatilaka AAL. Catalytic Inhibition of Topoisomerase Iiα by Demethyl-zeylasterone, a 6-Osophenolic Triterpenoid from *Kokoona zeylancia*. Journal of Natural Products, (in press).

Evan M. Hersh, M.D.

University of Arizona
Award Amount FY 2001: $150,000

## Program Project to Develop Novel Gene Therapies for Tobacco-Related Cancers

The purpose of the project is to develop immuno-gene therapy for lung cancer *via* animal models, clinical trials and immunological studies. In the animal model component, the Lewis Lung Model (3LL) in the mouse was established. Transfection with HSP-65 or null vector totally inhibited tumor growth. Pre-vaccination with either resulted in modest tumor inhibition. Heat inducible IL-2 gene transfer was also minimally effective *in vivo*. It did not have a systemic immune effect.

In the clinical trial a total of 24 patients were screened for admission to the study. Only 7 were found to be eligible and 6 were treated. Five of the six patients showed tumor progression on the investigational therapy (consisting of weekly intratumoral HLA-B7 x 6, plus daily subcutaneous low dose IL-2 x 56 days). One patient initially manifested stable disease, but eventually progressed. All patients tolerated treatment well with minimal grade I toxicity, only to the Il-2. Thus, these studies have not lead to the development of novel effective approaches to the gene therapy of lung cancer. However, they have inspired several follow-on studies with additional new strategies. It is hoped that they will eventually lead to the development of gene therapy for lung cancer.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY 2001: $50,000

## Development of a Peptide Therapy of Small Cell Lung Cancer

Current therapy for lung cancer is inadequate, as most patients relapse and die from their disease. New agents are needed to diagnose and treat lung cancer. To address this need, we screened millions of peptides with lung cancer cell lines. The rationale is that one or more of the random peptides will bind specifically to a cell surface marker present on lung cancer but not present on normal cells. Lung cancer cells were co-incubated with combinatorial, bead-bound peptide libraries. Each head has a single peptide species protruding from it so that a receptor or other protein on a lung cancer cell may bind it. After screening libraries with lung cancer cells, the cells rosetted around individual beads suggesting a specific peptide-cell surface receptor interaction. Over 200 beads that were rosetted by lung cancer cells were selected from the library. Specificity was not confirmed, as colon cancer cells rosetted the same beads that the lung cancer beads rosetted. However, we developed to new technology to overcome binding limitations. By incubating peptide-beads with adherent cells overnight or longer, we observed growth of the cells on peptide-beads, indicating true binders.

Robin K. Pettit, Ph.D.

University of Arizona
Award Amount FY 2001: $150,00

## Biological Research for Advancing New Anti-Infective Drugs to Clinical Trials

Infectious disease is the third leading cause of death in the United States and the leading cause worldwide. Antibiotic resistance and emerging infectious agents have complicated this public health problem. With ADCRC funding, we have expanded the Cancer Research Institute's (CRI) drug discovery program to include antimicrobials - a program very relevant to cancer patients who frequently die of infectious disease. This year, we continued to screen CRI natural products and semisynthetic compounds for antibacterials and antifungal activities. The antimicrobial activities of various pancratistatins, terpene lactones, combretastatins, resveratrol and cribrostatin 6 were reported. The mechanism of antifungal action of auristatin PHE, spongistatin 1 and trimethocide was determined to be tubulin destruction and inhibition of cell division. Spongistatin 1 protected mice from systemic fungal infection, and anprocide was effective against topical bacterial infection in mice. *In vivo* toxicity and efficacy studies are in progress for cribrostatin 6, and *in vivo* efficacy studies continue with auristatin PHE. With ADCRC support, these promising antibiotics will proceed much more rapidly from the laboratory to thousands of Arizona patients with tobacco-related cancers and associated infectious disease.

Publications:

Wyoke T, Pettit GR, Winkelmann G, Pettit RK. *In vitro* spectrum and postantifungal effects of the potent dolastatin 10 structural modification auristatin PHE. Antimicrobial Agents and Chemotherapy (inpress).

Pettit RK, Hamel E, Verdier-Pinard R, Roberson RW, Hazen KC, Pettit GR, Crews LC. Antifungal and cancer cell growth inhibitory activities of 1 -(3', 4', 5' - trimethoxyphenyl)-2-nitro-ethylene, Mycoses (in press).

Joy J. Winzerling, Ph.D., R.D.

University of Arizona
Award Amount FY 2001: $50,000

## The Effect of Chemicals Found in Cigarette Smoke on Iron Metabolism of Lung Cancer Cells

Airborne iron particles can enter the lung carried in the acid aerosol of smoke or on dust particles from areas with high iron deposits. We evaluated proteins involved in iron metabolism in A549 human lung cancer cells. Low concentrations of iron that, could be delivered by airborne particles, induced changes in the levels and activities of these proteins. These changes reflected exposure time as well. Our results indicate that these cells can adapt to a range of iron concentration, and that once the initial response occurs, they can tolerate an increasing and moderate range of iron concentration. High iron concentration or prolonged exposure can provoke additional changes. These results suggest that a biological window exists that allows cells to adapt to chronic iron exposure as necessary to meet iron requirements. We found the 40-60 cc of smoke as could be obtained from a long drag on a cigarette can change the levels and activities of these proteins. Finally, we found that iron can regulate an important early or intermediate protein that controls the synthesis of several other proteins. This finding could shed light on the role of iron in cell proliferation.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY 2001: $49,500

## Adenylyl Cyclase Superactivation After Chronic Opioid Receptor Stimulation

Opiate drugs such as morphine and Demerol® are the primary choice in the management of pain from various forms of cancer, including lung cancer resulting from smoking. Lung cancer was responsible for 440 deaths per one million population in the period 1986-1990 in Arizona. We and others have found that chronic use of opiates causes tolerance and second messenger alterations such as adenylyl cyclase (AC) superactivation. Adenylyl cyclase superactivation has been proposed to play an important role in opiate tolerance, dependence and withdrawal.

The construction of recombinant cell lines stably expressing the human delta opioid receptor provides a useful tool to study the molecular mechanisms of adenylyl cyclase superactivation. We have shown that Chinese Hamster Ovary (CHO) cells expressing the human delta opioid receptor exhibit AC superactivation and cAMP overshoot in response to chronic SNC 80, a delta opioid agonist. Co-expression of the alpha-subunit of transducin-1 and the human delta opioid receptor attenuates overshoot. The expression of alphatransducin-1 scavenges free beta-gamma subunits of the G-proteins($G\beta\gamma$). We hypothesize that $G\beta\gamma$ subunits play an important role in opioid-mediated AC superactivation in CHO cells expressing the human delta opioid receptors.

Paul F. McDonagh, Ph.D.

University of Arizona
Award Amount FY 2001: $49,366

## The Effects of Cigarette Smoke Exposure on the Leukocyte Contribution to Ischemia-Reperfusion Injury in the Heart

This research project investigates the effects of side-stream cigarette smoke (SSCS, second-hand smoke) exposure on the leukocyte contribution to myocardial ischemia-reperfusion injury. In the third year, we tested the hypothesis, in rats, that prior exposure to SSCS (exposure determined from Year 1 and 2 studies) would exacerbate the reactivity of the white blood cells to an ischemic stimulus and increase the severity of myocardial ischemic injury (heart attack). We found that prior SSCS exposure did cause a significant increase in neutrophil (PMN) activation and an increase in platelet-PMN conjugate formation. Further, we found a striking relationship between SSCS exposure and the mortality associated with myocardial ischemia-reperfusion injury. That is, we found that those animals exposed to SSCS, for only three hours/ day for one week, prior to being subjected to the model heart attack, tended to die. Those animals not exposed to SSCS, tended to survive. This remarkable finding has several important implications. First, SSCS, caused a priming of the white cells, making them hypersensitive to an ischemic stimulus. Second, the increased activation fo the white cells likely increased the severity of the ischemic injury and the mortality in the SSCS group. Third, this finding helps explain the mechanism behind the observation that the mortality associated with heart attacks and strokes increases dramatically in major metropolitan areas, such as Phoenix, on 'bad air' days.

### Publications:

Mendoza S, Gorman G, Gonzales R, Cohen Z, McDonagh P. Exposure to side stream cigarette smoke increases circulating neutrophil-platelet interactions early in reperfusion following myocardial ischemia. **FASEB J** 15(4): A35, 2001.

Raymond B. Nagle, M.D.

University of Arizona
Award Amount FY 2001: $118,423

## Improved Extracellular Matrix for Treatment of Tobacco Related Occlusive Vascular Disease

Aging and smoking remain major risk factors for the development of disease of the blood vessels. Surgical treatments for damaged blood vessels remain limited to the use of devices that bypass the area of disease. Unfortunately these devices do not perform optimally primarily due to the production of these devices from materials that are not living. The results of the research performed as part of this research program provide evidence that a new type of artificial blood vessel may soon be available. Using a cell based treatment, the new vascular replacement device shows the ability to stimulate the formation of new blood vessels. Basic science research and preclinical animal studies have shown that these new devices exhibit improved function and may be able to replace diseased blood vessels. In the near future this research may provide an artificial blood vessel that stimulates the formation of a new living blood vessel in a patient which will remain functional for decades.

Publications:

Kidd KR, Nagle RB, Williams SK. Angiogenesis and neovascularization associated with extracellular matrix-modified porous implants. J Biomed Mater Res, 2001.

Michael Berens, Ph.D.

Barrow Neurological Institute
Award Amount FY 2001: $49,228

## Human Recombinant Receptors for Nicotine

Nicotine is the substance thought to drive the use of tobacco products by about 25% of Arizonan and American adults. Nicotinic acetylcholine receptors (nAChR) are the molecular targets of nicotine action but also play important roles throughout the brain and body in health and disease via their responses to the natural chemical messenger, acetylcholine. Thus, it is important to understand nAChR if we are to better understand brain and body function in disease and in health and how to deal with tobacco use.

We have used genetic engineering techniques to create human cell lines that express different building blocks that assemble to form human nAChR. The $\alpha4\beta2$-nAChR we have created in one cell line and made a $\alpha4$ and $\beta2$ building blocks, or subunits, are the most abundant nAChR forms in brain. Our characterization of these nAChR includes confirmation that they have exceptionally strong interactions with nicotine. The $\alpha4\beta2$-nAChR we have created in another cell line also have strong interactions with nicotine, but other drugs discriminate between these two kinds of nAChR, suggesting approaches to design nAChR subtype-specific drugs for therapeutic purposes. There are some nAChR subunits that act as wild-cards, able to integrate with other subunits to form additional novel forms of nAChR, and we have generated some containing $\alpha4$, $\beta2$ and $\alpha5$ wild card subunits. $\alpha4\beta2$-nAChR that they form have diminished affinity for nicotine and many of its analogs. We have now cloned virtually every of the known human nAChR subunits, and we have new cell lines that hold promise as sources for nAChR containing $\alpha2$, $\alpha3$ and $\alpha6$ subunits instead of $\alpha4$ subunits. These cell lines also promise to be valuable tools because nAChR composed of these subunits may mediate influences of nicotine on pleasure/reward, mood, and learning/memory. Many of our techniques for studying nAChR have been adapted to high throughput analysis, meaning that the time to gather useful data is shorter. Overall, funding for this work has enabled us to create valuable models for studies of acute or chronic action, particularly with relevance to development of nicotine dependence, that have world-wide distribution and are used as novel research tools by both acadmeic and industrial communities.

Allan L. Bieber, Ph.D.

Arizona State University
Award Amount FY 2001: $148,608

## Composition of A Unique Receptor for Nicotine

Nicotine dependence and habitual use to tobacco products are initially based on actions of nicotine at the diverse family of chemical signaling molecules called nicotinic acetylcholine receptors (nAChR). However, our understanding is deficient about the precise composition of several important nAChR subtypes and proteins that associate with them, compromising knowledge about how nAChR are affected by long-term nicotine exposure and consequences for nervous system function. To address this deficiency, sophisticated techniques involving matrix-assisted laser desorptin/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) used successfully to analyze simpler proteins have been adapted for use to analyze complex, cell membrane-associated proteins using nAChR as the models. Refinement of sample preparation has been achieved to facilitate MALDI-TOF-MS analyses of nAChR. In parallel, molecular biological studies have been used to engineer other nAChR subtypes in full-length and truncated forms for future MALDI-TOF-MS analyses. This was accomplished by expression in transfected cell lines or from transcription/translation expression systems to generate purer protein. The latter was augmented by inclusion of pancreatic microsomes to allow translation into a membrane compartment and at least some degree of post-translational modification, which is a process that has been studied using MALDI-TOF-MS to establish the composition of less abundant, but important, nAChR subtypes found in the brain and their assembly partners, as well as to characterize subtle changes in structure of those molecules.

**Publications:**

Peng JH, Lucero L, Fryer J, Herl J, Leonard SS, Lukas RJ. Inducible, heterologous expression of human α7-neuronal nicotinic acetylcholine receptors in a native nicotinic receptor-null human clonal line. **Brain Res** 825:172-179, 1999.

Lukas RJ, Lucero L, Buission B, Galzi JL, Puchacz E, Fryer JD, Changeux JP. Neurotoxicity of channel mutations in heterologously expressed α7-nicotinic acetylcholine receptors. **Eur J Neurosci** 13:1849-1860, 2001.

Joseph Grandpre, Ph.D.

University of Arizona
Award Amount FY 2001: 48,447

### Diverted Youth: Testing a Tobacco Cessation Intervention for Adolescents

The final year of this research project designed to assess the relative efficacy of theoretically-based tobacco cessation counseling interventions for adolescents has been productive and completes the study. Adult and peer counselors have initiated counseling sessions with 259 clients (187 of whom have completed the program). As in prior years, contacts with community resources such as schools, juvenile court officials, and probation officers have been maintained and new contacts have been established. Previous efforts to develop contacts in more remote areas of the state continued with some measure of success. Program expansion and collaboration efforts also continue to be discussed with the Arizona Smokers' Helpline and the Tobacco Education and Prevention Program. At this time it appears that a version of this program will be implemented on a permanent basis by the aforementioned entities.

Daniel E. Shapiro, Ph.D.

University of Arizona
Award Amount FY 2001: $48,080

### Physician-Patient Communication with Heavy Smokers: Comparing Motivational Interviewing with the Perspective Approach

In Arizona there are approximately 550,000 smokers with our state suffering 5,700 smoking related deaths annually. Of those smokers, it is likely that physicians have contact with as many as 412,000. This study compared smoking rates over six months when heavy smokers (resistant to quitting) were counseled with either single session motivational or prescriptive counseling by a health professional. Results suggested that neither approach was superior with males, but females were significantly more likely to respond to the motivational condition by making brief (24 hour) quit attempts. This is important as prior research has suggested that when smokers do successfully quit, most have made practice attempts in the past. It is notable that roughly 33% of the sample of 218 participants made one 24-hour quit attempt during the 6 months they were followed for the trial. We conclude that even if smokers appear resistant to quitting, they should be encouraged to quit. In future work we hope to test these findings with pregnant smokers.

Neil M. Ampel, M.D.

University of Arizona
Award Amount FY 2001: $49,995

## The Effect of Cigarette Smoking on Immune Function and Outcome in Male Patients with Active Coccidioidomycosis in Arizona

During this third year of studying differences in outcome and immune response to Valley Fever (coccidioidomycocis), we have followed a total of 113 subjects. Seventy-three presented with Valley Fever lung disease while 40 had infection beyond the lungs (dissemination). African-Americans were more likely than whites to have dissemination. We developed a laboratory technique that measures the protective immune response to Valley Fever. It requires a small volume of blood (less than 1 teaspoon) and results are available in about 24 hours. Among 18 donors with disseminated Valley Fever, the severity of illness was inversely proportional to their response in the immune test. This relationship was not seen among 16 subjects with Valley Fever involving only the lungs. With the loss of availability of the Valley Fever skin test, the use of this new blood test may be very useful in assessing the response of persons with Valley Fever.

Publications:

Ampel NM, Kramer LA, Kerekes KM, Johnson SM, Pappagianis D. Assessment of the human immune response to T27K, a coccidioidal antigen preparation, by flow cytometry of whole blood. Med Mycol (in press).

R. Clark Lantz, Ph.D.

University of Arizona
Award Amount FY 2001: $50,000

## Environmental Tobacco Smoke Exposure in Developing Lung Effects on Surfactant Metabolism

This application is investigating the effects of environmental tobacco smoke (ETS) on production of surfactant in developing postnatal lungs. Surfactant is a complex mixture of phospholipids and proteins that lines the lungs and is essential for appropriate lung function. This year we have continued to investigate mediators (transforming growth factor beta (TGF-$\beta$)) that can affect the production of surfactant in neonatal lungs. We have determined the normal expression of the receptors for TGF-$\beta$ and have been investigating the effects of ETS on their expression. We have identified the location and temporal expression of the three different forms of TGF-$\beta$ receptors in neonatal lung. Since ETS exposure of young children can lead to severe lung problem, this research is important for the children of Arizona.

Thomas P. Davis, Ph.D.

University of Arizona
Award Amount FY 2001: $49,610

## Nicotine Effects on Blood-Brain Barrier Integrity Function and Permeability

The blood-brain barrier (BBB) is a system of capillaries that regulate the passage of molecules to and from the brain. Disruption of BBB is a critical event in the progression of stroke, a leading cause of death and disability among Arizona residents. Despite the fact that cigarette smoking is a well-established risk factor for stroke, little is known about the effects of nicotine on the BBB.

Acute administration of nicotine lead to an opening of the BBB *in vivo* , shown by an increase in [$^{14}$ C]-sucrose permeability measured in the rat (Appendix Figure 1). This treatment also alters the transport of $^{86}Rb^{+}$ across the BBB (Appendix Figure 2), indicating that nicotine may disrupt ion transport mechanisms at the BBB. These changes in BBB function appear to occur independently of nicotine-induced changes in cerebral blood flow (Appendix Figure 3). These data indicate that nicotine alters the permeability and function of the BBB in the whole animal, supporting our previous *in vitro* work.

Abstract:

Hawkins BT, Egleton RD, Huber JD, Campos CR, Davis TP. Acute and chronic effects of nicotine on $^{86}Rb^{+}$ uptake across the *in situ* blood-brain barrier of the rat. Soc Neurosci Annual Meeting, November 1-5, 2000.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY 2001: $132,661

## Effects of Nicotine on the Development of Human T Cells

Although there have been some reports indicating that nicotine has deleterious effects on the immune system, little work has been done to determine the mechanisms of smoking-induced loss of immune function. During the first year of this project, we found that the addition of nicotine to an organ culture system that we devised to study T cell development, causes profound changes in mouse thymus-derived T cell production. We also found that nicotinic receptors are present on developing T cells, suggesting that nicotine binds to the cells and delivers signals to the cells that alters their development. In the second year of this study, we have characterized the nicotine receptors found in mouse and human thymus cells, as well as when they are expressed during development. We also found that nicotine can activate genes that are involved in the death of T cells, including T cells derived from the cord blood of infants born to women who were smokers. During the third and final year of the project, we solidified these results and submitted them for publication. We also found that T cell precursors in the cord blood of infants born to women exposed to nicotine during pregnancy do not develop the normal complement of T cells in our organ culture system. This latter results suggest that exposure to nicotine before birth may have profound effects on the developing immune system of infants.

Joseph L. Graves, Jr., Ph.D.

Arizona State University West
Award Amount FY 2001: $50,000

## The Impact of Nicotine on the Genetics of Aging: Exposure and Selection for Resistance

We now have conclusive evidence that nicotine resistance evolves at a cost to Darwinian fitness. Our data shows a pronounced trade-off between resistance to nicotine; physiological performance; and aspects of larval fitness, development time and viability. We also illustrate that genetic background affected the response to selection for nicotine resistance. For example, in generation 4, MLB Stocks, patterns of larval emergence by day were not significantly different between the controls and the nicotine-selected treatments, while in the MLO stocks a significant response had occurred with the total number of flies emerging favoring the nicotine-selected. Both stocks showed a trade-off in the emergence profile, with the control treatments having significantly higher number of flies emerging early, but the nicotine-selected treatments emerging later. In generation 10 adult fitness characters were assayed. Survival of 0.04% nicotine & yeast medium was significantly greater for all nicotine-selected treatments (in both genetic backgrounds, MLB female Control v. selected $t = 3.35$, 9=0.02; MLO female Control v. selected, $t = 6.95$, $p = 0.002$; D.F. = 4, for all tests; MLB male Control v. selected $t= 3.06$, $p = 0.03$; MLO male Control v. selected, $t = 20.65$, $p = 0.0003$; D.F. = 4, for all tests.) Antagonistic pleiotropy was indicated by the depressed early fecundity exhibited by the nicotine-selected stocks (MLB Control v. selected, 7-day fecundity, $p = 0.0001$; MLO Control v Selected, 7-day fecundity, $p = 0.0001$).

In generation 14, physiological performance and larval characters were further evaluated. Desiccation resistance was not significantly different between selected and control populations. Ethanol tolerance showed differentiation in MLB but not MLO stocks, and starvation resistance was significantly different ($p<0.001$) for both. Development times of MLB and MLO stock assayed in 0%, 0.04, &0.1% nicotine showed that selected stocks developed more slowly than controls in all concentrations. However, both selected-stocks responded by having higher viabilities in nicotine concentrations, as compared to 0% nicotine food. These results taken together indicate further examples of antagonistic pleiotropy between genes that confer nicotine resistance and general fitness in non-nicotine environments.

This study has implications for the evolution of resistance to a xenobiotic compounds. This occurs at a cost to general fitness. In this study, this was seen by both general fecundity and also development time. These results together would suggest that nicotine may be even more toxic then originally proposed, since traditional studies have only focused on within generation, somatic effects. Thus the long-term genetic effects of nicotine resistance may need to be included in any calcualtion of its toxicity.

Mark L. Witten, Ph.D.

University of Arizona
Award Amount FY 2001: $131,618

## Dietary Vitamin E Supplementation and its Effect on Sidestream Cigarette Smoke-Induced Lung Injury

We have learned that sidestream cigarette smoke (the major component of environmental tobacco smoke) can induce lung injury as evidenced by changes in pulmonary function and lung permeability. However, we found that dietary supplementation with vitamin E up to 75 times higher than recommended levels of vitamin E consumption can protect lung function and lung immune system function by decreasing the changes in the cytokines interleukin-1 and tumor necrosis factor and lipid peroxidation. The cytokines interleukin-1 and tumor necrosis factor promote inflammation in the lungs. Lipid peroxidation causes the destruction of cell membranes. Consequently, dietary vitamin E supplementation up to 75 times over normal recommended dietary intake can be beneficial to Arizona residents against the most significant indoor air pollutant, environmental tobacco smoke.

Mark Brown, M.D.

University of Arizona
Award Amount FY 2001: $97, 240

## The Effect of Tobacco and Its Constituents on Placental Development Differentiation and Immunologic Function

By harvesting tissue from the placentas of smoking and non-smoking mothers, we have been able to show that smoking is associated with changes in the immunologic function of the placenta. These changes, if reflected in the developing baby's intrauterine environment, would make it more likely for the child to develop asthma and allergies. Some of these changes are more pronounced in women who both smoke and have allergies themselves. We are currently trying to determine which of the thousands of compounds in tobacco smoke might be responsible. By better understanding these changes, in the future we may be able to develop treatments either during pregnancy or in early infancy to reduce the risk of allergy development in the young child. This is especially important in Arizona, which has one of the highest rates of allergy and asthma problems in the country.

Michael Burgoon, Ph.D.

University of Arizona
Award Amount FY 2001: $129,102

## The Smoke Free Work Place: Overcoming Resistance and Implementing Changes in Smoking Behavior

This year's effort on the Smoke Free Work Place project have, so far, largely consisted of tracking employee health behavior, compiling and maintaining a database to facilitate continuing analysis of longitudinal data collected from the first two project years and making preparations for the final survey associated with the project. A number of structural changes at the work site have created challenges for this project but work continues. Analysis of all project data, upon completion of the final work place survey, will contribute much needed information to the literature on work place tobacco cessation interventions. At this stage, it appears that such interventions hold some promise in promoting employee health, but given the rather limited organizational support available in times of economic fluctuation in the health care industry, it is unlikely that such interventions will live up to that promise.

# SECTION D

## CONTRACTS

## ANTICANCER DRUG DISCOVERY

## YEAR TWO

## FY 2001

A.A. Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 2001: $205,440

## Discovery of Novel Anticancer Drugs from Rhizosphere Microflor of Desert Plants An Unexploited Source of Bioactive Natural Products

During the course of the second year of this project, roots of 22 species of desert plants representing 22 genera and 15 families have been sampled. Of these, 267 fungi have been cultured and their extracts prepared for screening in a panel of 2 cell lines representing breast, lung, and central nervous system cancers. Extracts derived from 6 fungi which were found to inhibit the growth of at least one cancer cell line by >90% were selected for detailed investigation. Bioassay-guided fractionation of these fungal extracts was initiated and from *Aspergillus terreus* we have isolated and characterized four known metabolites and one new microbial metabolite; from *Aspergillus niveus* viriditoxin; from *Aspergillus flavipes* one new and three known aspochalasins; from *Parasphaeria quadriseptata* we have isolated monocilin, and from an unidentified fungus, monocillin. All these compounds exhibited significant anticancer activity and are currently being tested for their mechanisms of anticancer action. Based on the results obtained these compounds will be submitted to NCI for evaluation in their 60 cell line panel. If these compounds turn out to be active against solid tumors such as breast, prostate, colon and lung cancers, our results will have an impact on the more elderly and/or tobacco-dependent portions of Arizona's population since these cancers are prevalent in our state.

Bertram L. Jacobs, Ph.D.

Arizona State University
Award Amount FY 2001: $250,000

## Viral Vectors for Treatment of Brain Cancer

With the funding provided by this grant we have begun a concerted effort to develop viruses that can specifically kill brain cancer cells. There are three compelling reasons to launch a concerted interdisciplinary brain cancer research effort in Arizona: 1) the incidence of primary brain cancer is on the rise, with disproportionate increases in the aging population; 2) survival rates for patients diagnosed with the most common forms of primary brain cancer have not improved in the past 25 years; and 3) strong basic and clinical science programs have matured in the state to the degree that interdisciplinary projects merging these efforts stand to make a unique contribution to the field. We believe that progress along the lines of the studies outlined in this proposal will serve to advance treatment opportunities for brain cancer patients and to raise the awareness nationally of the outstanding cancer research in Arizona.

Emmanuel Katsanis, M.D.

University of Arizona
Award Amount FY 2001: $201,467

## Improvement of Anticancer Immune Responses Generated by Chaperone Protein Associated Tumor Peptides

In recent years biologic approaches to cancer treatment have shown promise. Tumor-derived heat shock proteins (HSPs) can function as unique anti-cancer vaccines. We and others have reported that vaccination of mice with tumor-derived purified HSPs elicit tumor specific protective immunity. We have developed a relatively simple, rapid and efficient procedure utilizing a free-solution-isoelectric focusing (FS-IEF) technique to obtain fractions rich in multiple immunogenic HSPs from lysed tumor cells. We have found that FS-IEF derived HSPs (termed FS-IEFcc) when used to immunize mice provide significant protection against tumor growth. Enrichment for HSPs using FS-IEF has enabled us to overcome the lack of tumor material that may often be encountered by purifying HSPs in the clinical setting. Moreover, we have demonstrated that of FS-IEFcc vaccines can be improved further by loading them onto dendritic cells (Dcs). In fact, FS-IEF cc pulsed Dcs are significantly superior anti-tumor vaccines than Dcs pulsed with purified HSPs or lysed tumor cells. We have also reported that tumor cells undergoing apoptosis do not elicit an immune response unless endogenous or exogenous HSPs are induced/provided at site of immunization. FS-IEFcc isolated from normal livers were more effective adjuvants than liver derived purified HSPs, in enhancing immunogenicity of apoptotic leukemia cells. This approach shows promise as a vaccine and adjuvant against human cancers.

Eugene A. Mash Jr., Ph.D.

University of Arizona
Award Amount FY 2001: $249,874

## Rational Design of Thioredoxin Active Anticancer Drugs

We are studying a protein called *thioredoxin* that is overproduced in human tumors. Inhibition of thioredoxin reduces and sometimes eliminated the cancer-causing effects. Thioredoxin is regarded as a promising target for anticancer drug therapy. We are developing chemical inhibitors of thrioredoxin for possible use as anticancer drugs. Compounds related to a lead compound from the National Cancer Institute database were synthesized and tested for thioredoxin inhibitory activity. Two promising drug candidates emerged. These compounds were more active and less toxic than the lead compound in initial screening and were subsequently tested in a living mouse model system against human lung cancer and leukemia. One or both of our drug candidates proved to be active against these cancers. We have studied the mechanism of action of the lead compound at the molecular level so that we may produce even more selective and potent drug candidates. We believe the active compounds crosslink thioredoxin. In effect, they work like superglue to permanently stick parts of the protein together.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2001: $749,980

## Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs

Overall goals and objectives of this project are focused on accelerating the progression from the discovery of new anti-cancer drugs to the initiation of Phase I clinical trials. ADCRC support has allowed us to reduce the overall process following discovery and has encouraged the U.S. National Cancer Institute to enter the developmental process at critical points to advance a new drug to the next stage.

The ADCRC and NCI resources are now being focused on several of our anti-cancer drug candidates including pancratistatin prodrug, phenstatin prodrug, dolastatin 15, auristatin 15-PE, auristatin PYE, cephalostatin 1, and bryostatin 5 in a manner that is decreasing the time required for preclinical development. Preclinical development requires completion of a multi-step process that includes both *in vitro* and *in vivo* testing of each compound. CRI is also developing new and better methods to synthesize them in sufficient quantities for testing. With adequate resources, we can considerably shorten the preclinical development phases of our anti-cancer drugs.

Publications:

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Pettit GR, Ducki S, Orr B. Antineoplastic agents 453. Synthesis of pancratistatin prodrugs. **Anticancer Drug Design** 15:389-396, 2000.

Pettit GR, Lippert JW III, Taylor SR, Tan R, Williams MD. Synthesis of phakellistatin 11: A miconesia (Chuuk) marine sponge cyclooctapeptide. **J Nat Prod** 64:883-891, 2001.

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Pettit GR, Melody N, Herald DL. Antineoplastic agents 450. Synthesis of (+) -pancratistatin from (+) -narciclasine as relay. **J Nat Prod** 66:2583-2587, 2001.

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McNulty J, Mao J, Gibe R, Mo R, Wolf S, Pettit GR, Herald DL, Boyd MR. Studies directed towards the refinement of the pancratistatin pharmacophore. **BioOrg Med Chem Lett** 11:169-172, 2001.

Clark H, Ginanni N, Laughlin KV, Smith JB, Pettit GR, Herald DL, Boyd MR. The transient increase of tight junction permeability induced by bryostatin 1 correlates with rapid downregulation of protein Kinase C-α. **Exptl Cell Res** 261:239-249, 2000.

Chen Z, Mocharla VP, Farmer JM, Pettit GR, Verdier-Pinard P, Hamel E, Pinney KG. Preparation of new anti-tubulin ligands through a dual-mode, addition-elimination reaction to a bromo-substituted α,β- unsaturated sulfoxide. **J Org Chem** 65:8811-8815, 2000.

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Pettit GR, Grealish MP. Antineoplastic agents 475. A cobalt-phosphine complex directed reformatsky approach to a stereospecific synthesis of the dolastatin 10 unit dolaproine (Dap). **J Org Chem** (in press).

Pettit GR, Melody N, Pettit WE, Pettit RK. Synthesis of 10b-(R) and 10b-S-hydroxy-pancratistatin, 10b-(s)-hydroxy-isopancratistatin and related isocarbostyrils. **Heterocycles**, (in press).

Pettit GR, Grealish MP, Hamel E, Pettit RK. Antineoplastic agents 465. Structural modification of resveratrol: sodium resversatatin phosphate. **J Med Chem** (in press).

Pettit GR, Davenport RJ, Coffman T. The dolastatins 27. Stereospecific synthesis of dolaisoleuine employing a new cobalt complex-directed reformatsky reaction. J Org Chem (in press).

Pettit GR, Moser BR, Boyd MR, Scmidt JM, Pettit RK, Chapuis JC. Antineoplastic agents 460. Synthsis of combretastatin A-2 prodrugs. Anticancer Drug Design (in press).

Nogawa T, Kamano Y, Yamashita A, Pettit GR. Isolation and structure of six new cancer cell growth inhibitory bufadienolides from the chinese traditional drug Ch'an Su. J Nat Prod (in press).

Edward B Skibo, Ph.D.

Arizona State University
Award Amount FY 2001: $141,145

## Preclinical Development of the PBI's

In the previous project year, we were able to develop computer models that greatly assisted in anticancer drug design. In addition, simple chemical and biochemical tests were developed that could predict the efficacy of a drug even before animal studies were carried out. In the present project year the computer models and the simple assays were used to identify new leads as well as develop further the PBI antitumor agents. The histological types of cancers targeted by the compounds developed in this laboratory include melanoma and ovarian cancers. Both of these cancer types are common in residents of Arizona.

Specific accomplishments include the DT-diaphorase active site computer model (this enzyme is over expressed in cancer cells and is a useful target), development of the PBI major groove model, preparation of new PBI analogues, and the identification of new lead compounds.

### Publication:

Suleman A, Skibo EB. Insights into the mechanism and substrate specificity of human DT-diaphorase through molecular modeling. Biochemistry, (in press).

Luke Whitesell, M.D.

University of Arizona
Award Amount FY 2001: $194,880

## Development of Antisense Oligonucleoptides as Chemotherapeutic Agents for Intratumoral Administration

Excessive activity of the growth factor receptor encoded by the *IGR-IR* gene allows cancer cells to escape death and grow out of control. During the first year of ADCRC support we attempted to inhibit the production of IGF-IR using small pieces of DNA called antisense oligos. This approach proved ineffective despite the use of a variety of oligo sequences, modification and delivery techniques. As a result, we adopted a new strategy this past year of ADCRC support. We examined compounds that do not decrease the level of receptor, but instead, its ability to function in cells. After demonstrating significant antitumor activity for a previously reported compound (AG1024), we examined the activity of six new potential IGF-IR inhibitors provided by the biotechnology firm *Telik, Inc.* One of these compounds, 3B6, was able to inhibit receptor activity better than AG1024 in intact tumor cells. We hope to use 3B6 as a lead compound for the development of other more potent and selective anti-IGF-IR drugs with which to treat cancer patients.

Publications:

Beliakoff J, Katsanis E, Whitesell L. Tyrphostin AG1024-mediated inhibition of cellular proliferation and induction of apoptosis in neuroblastoma cells. Proc Am Assoc Cancer Res 42: 853, 2001.

# SECTION E

## NEW CONTRACT AWARDS

## ANTICANCER DRUG DISCOVERY

## BEGINNING FY 2002

Ebbinghaus, Ph.D., Scot E.

University of Arizona
Award Amount FY 2002: $50,000

## Triplex DNA Based Gene Therapy of Lung Cancer

Lung cancer will account for an estimated 2,600 deaths in the state of Arizona and 2,800 new cases will be diagnosed. In comparison with other common tumor types, lung cancer will account for more deaths than breast cancer, colon cancer and prostate combined, both in Arizona and nationwide. Although significant progress has been made in the treatment of non-small cell lung cancer (NSCLC), 85% of patients who are diagnosed with lung cancer will eventually die of lung cancer. Recent progress in the understanding of the molecular events that lead to the development and progression of lung cancer have provided attractive therapeutic targets, including the Her-2/neu oncogene. HER-2/neu is over-expressed in approximately 25% of lung adenocarcinomas, and the gene product is a growth factor receptor that sends a signal to a lung cancer cell for unrestricted growth and spread to distant organs. Not surprisingly, the limited progress that has been made in the clinical development of Herceptin, a monoclonal antibody of proven but limited efficacy in breast cancer that targets the HER-2/neu protein, is being evaluated in NSCLC. The ability to inhibit Her-2/neu expression at the genetic level would provide a great advance in the therapeutic potential of the strategy to inhibit Her-2/neu expression for the treatment of NSCLC.

The overall goal of this research proposal is to develop a strategy to prevent the expression of the HER-2/neu oncogene. The proposed research is based on the remarkable ability of specifically designed piece of synthesized DNA to bind very specifically to the DNA of the HER-2/neu oncogene through the phenomenon of DNA triple helix formation. In this proposal, we will develop a very novel molecular biology technology that allows for the production of a single stranded DNA inside of living cells. We propose to use this novel technology to produce a single stranded DNA (ssDNA) to inhibit the expression of HER-2/neu in NSCLC cells that over-express this oncogene. In this proposal, we will evaluate the ability of our expressed ssDNA to prevent Her-2/neu expression by triple helix formation, and we will design and synthesize an adenovirus gene therapy vector that will be capable of producing this ssDNA in NSCLC cells in culture. We will evaluate the inhibition of HER-2/neu expression by the ssDNA for its ability to kill NSCLC cells (cytotoxic effect), prevent the proliferation of NSCLC cells (cytostatic effect), and reverse the invasiveness and metastatic potential of NSCLC cells in culture. Future work beyond the scope of this proposal will evaluate the effectiveness of this strategy in the treatment of animal models of NSCLC. This adenoviral gene therapy vector will provide a model for the further development of a clinically effective gene therapy for the treatment of NSCLC by the inhibition of HER-2/neu.

Guerriero, Ph.D., Vince

University of Arizona
Award Amount FY 2002: $49,801

## The Stress Protein Hsp70 as a Target for Anticancer Therapy

The purpose of this proposal is to provide preliminary information on a novel method to treat certain types of cancer. Cancers are a major health problem for the people of the state of Arizona and new methods to treat such diseases must be developed. It is well known that the stimulation of cell death in cancer cells is a target for the development of new therapies. The completion of sequencing of the human genome has recently been announced and thus opens up the possibility of analyzing thousands of new gene products with unknown functions. No doubt some of these newly described gene products will have application in medical treatment of diseases such as cancer. This laboratory has recently identified a novel human gene that codes for a protein called HspBP1 that could be used to stimulate cell death in cancer cells while having a minimal effect on normal cells. The purpose of the research described here is to further investigate the utility of this novel protein in the treatment on cancer.

The hypothesis to be tested by this proposal is that treatment of cancer cells with the protein called HspBP1 will cause cell death by blocking Hsp70 activity. The main problem with using proteins as intracellular drugs is getting them inside the cell. The method for increasing the intracellular HspBP1 levels will be to produce a protein with a protein transduction domain. The domain will facilitate transport of the protein into the cell. This method is preferred since it can also be used for whole animal studies and has the greatest potential for therapeutic use. The hypothesis will be tested using the following specific aims: 1) introduce HspBP1 into cells using a protein transduction domain and characterize the resulting cancer cell death, 2) determine the region of HspBP1 that causes the death of cancer cells by producing mutant proteins that lack specific regions, and 3) determine the mechanism by which HspBP1 causes cell death by identification of proteins that interact HspBP1 in normal and cancer cells.

Gunatilaka, Ph.D., Leslie

University of Arizona
Award Amount FY 02: $220,000

## Discovery, Evaluation and Development of Anticancer Drugs from Rhizosphere Microflora of Desert Plants

Cancer accounts for a vast number of human deaths and suffering. Each year 6.5 million people are diagnosed with cancer worldwide. In the U.S. more than 10 million people are living with a history of cancer, and in excess of one million new cancer cases develop annually. According to the Arizona Cancer Registry published by the Arizona Department of Health Services, in 1997 cancer was responsible for 8,429 deaths and 19,384 newly diagnosed cases in Arizona. Cancer incidence and deaths in our state are on the rise as many elderly continue to move here due to its desirable climate.

Cancer treatment currently relies on surgery, radiation and chemotherapy (treatment with anticancer drugs). Chemotherapy is the only effective way to treat a disseminated cancer. Unfortunately, there are no good anticancer drugs available to treat major solid tumors such as cancers of the lung, breast and prostate effectively without undesirable side effects. There is an urgent need for the discovery and development of new, effective and non-toxic anticancer drugs to treat these cancers.

The majority of anticancer drugs in use today are from natural sources such as plants and microorganisms. With initial two year funding from the ADCRC, we have shown microorganisms (bacteria and fungi) growing on the roots of desert plants (in a region called "rhizosphere") of Arizona to be a rich source of potential anticancer drugs. We have made significant progress by sampling rhizosphere microorganisms of 112 desert plants and having 18,653 bacteria and 1,937 fungi in our collection. Of these, so far we have investigated extracts derived from 660 fungi and 393 bacteria using cell lines derived from human cancers of the lung, breast and central nervous system, and 10 compounds with potential anticancer activity have been isolated. This expanded project will bring together investigators in natural products chemistry, microbiology, biochemistry (with expertise in angiogenesis), cancer biology, pharmacology and toxicology. Goals of this project are to further evaluate in mouse models anticancer activity of 2 of the above 10 compounds selected on the basis of their selective activity/novelty, and to evaluate over 1,000 fungal extracts available with us in two new target-oriented assays for the discovery of non-cytotoxic anticancer drugs. One new assay will identify compounds which will stop the growth of the blood supply (a process called angiogenesis) to solid tumors starving them of oxygen and nutrients, thus halting their growth. The other target-oriented assay will identify compounds that bind to heat shock proteins. These proteins provide good targets for anticancer drug discovery because they facilitate the development of cancer by increasing the stability ans activity of multiple regulatory proteins with in a cancer cell. Up to 3 compounds active in these 2 target-oriented assays will also be evaluated in B -16 melanoma mouse model. We are hopeful that

having access to this unique source of bioactive natural products, combined with the use of selective and improved anticancer bioassays, will lead to the discovery of effective new non-toxic drugs to treat solid tumors, increasing the survival and quality of life of victims of cancer not only in Arizona but worldwide.

Hersh, M.D., Evan M.

University of Arizona
Award Amount FY 02: $137,500

## Treatment of Brain Tumors with Glioblastoma Cell-Derived Antigens (Cell Products) Pulsed into Dendritic Cells or Dexosomes

Glioblastoma multiforme (GMB) is uniformly fatal malignancy associated with a greater than 90% mortality within 1 year a diagnosis. Of the 17,200 malignant brain and other nervous system tumors per year in the United States, 45%, or 7,740, are glioblastoma multiforme (GMB). Surgery, radiotherapy and chemotherapy are used to treat this disease and can induce remissions, but they have not impacted the long-term survival. The median survival time in the best centers is approximately 260 days from diagnosis. Anaplastic astrocytoma (AA) carries a similar prognosis and constitutes about 38% of brain and central nervous system tumors.

Recently, data has been developed to show that malignant brain tumors express tumor-associated antigens (proteins) on their cells which can be recognized and attacked by the immune system. Animal tumor model systems have shown that vaccination against malignant brain tumors is effective in reducing the tumor burden and prolonging survival compared to control animals. Most recently, a study was published that reported vaccination of a small number of GMB patients with dendritic cells pulsed with an extract of the patients own brain tumor. This treatment led to an immune response and prolonged survival. The newly developed dendritic cell technology uses white blood cells obtained from the peripheral blood of patients from which small stable vesicles (dexosomes) are derived as another basis for an anticancer vaccine. Dendritic cell therapy has already been shown to be effective in other cancers such as kidney cancer and malignant melanoma. Consequently, it is hoped that this project, based on the above described data and technology, will result in a new highly effective and virtually completely non-toxic treatment for patients with malignant brain tumors initially presenting as GMB. The research may also be extended to AA during the grant period.

The objective of this grant is to develop an immunotherapeutic pharmaceutical product from glioblastoma multiforme cells that can be used in conjunction with dendritic cells or dexosomes as a tumor vaccine. It may ultimately be used in combination with the immuno-stimulatory cytokine GM-CSF. GMB tumor cells will be obtained at the time of surgery in approximately 30 to 40 patients per year for 2 years through the Neurosurgery Service at the University Medical Center (Allan Hamilton, M.D., Co Investigator) and the Neurosurgery Service at Tucson Medical

Center and Northwest Hospital (Abhay Sanan, M.D., Co- Investigator). The patients will be followed and managed by the Radiation Oncology Department of the Arizona Cancer Center and the University Medical Center (Baldassarre Stea, M.D., Ph.D., Co-Investigator). GMB tumor cells will be obtained at the time of surgery for primary or recurrent disease. Specifically, the objectives are to: 1) establish a bank GMB tumor cells, tumor cell lines and specific products such as lysates and eluates; 2) characterize these at the immunological and molecular level; 3) pulse the specific products into dendritic cells and dexosomes and test their ability in the laboratory to stimulate the immune response of the white blood cells of normal subjects and patients with GMB; 4) extend the observation in GMB to AA if time and resources permit; 5) use the accumulated data as the basis for the development of dendritic cell or dexosome vaccines for the treatment of GMB that would be proposed for clinical trials in the future; and 6)select one of these reagents which can be standardized as a pharmaceutical product.

Hurley, Ph.D., Laurence H. — University of Arizona
Award Amount FY 02: $149,677

### Et 743-Duples DNA Adducts as Therapeutic Agents and Molecular Lures

Ecteinascidin 743 (Et 743) is an anticancer drug produced by a marine tunicate that is currently in phase II clinical trials in the United States and Europe. This drug has shown remarkable responses in patients with soft tissue sarcomas. Its clinical efficacy in the other cancer remains to be proven. In common with other cytotoxic agents, Et 743 shows dose-limiting toxicities that preclude giving larger doses, that would be expected to be more clinically effective. We have recently made two key observations that led to important insights into how we can minimize the nuclear damage while maintaining therapeutic effectiveness and, also make a major advance in determining the underlying structural biology of repair of DNA damage caused by cancer chemotherapeutic agents. The latter advance requires the application of the most advanced technology in proteomics research.

The long-range goals of this project are to develop a novel dosage form of Et 743 that will have improved therapeutic activity and characterize in detail the nucleotide excision repair (NER) complex involved in recognition and repair of drug lesions bound to DNA. The specific aims of the research project are: 1) construction of site-directed Et 743-DNA adducts in duplex oligomers and demonstration of their cellular uptake and stability in biological systems; 2) comparison of *in vitro* cellular toxicity of Et 743 with Et 743-DNA duplex adducts in cancer cell lines and normal bone marrow cells; and 3) isolation and mass spectral analysis of Et 743-DNA duplex adducts with associated repair protein complexes from NER-competent mammalian cell lines.

Lukas, Ph.D., Ronald L.

Barrow Neurological Institute
Award Amount FY 02: $27,500

## Novel Drug Treatment for Cerebellar Medulloblastoma and Small Cell Carcinoma of the Lung

Cerebellar medulloblastoma (CMED) is the most common brain tumor in children. CMED seems to occur as a tumor or "primitive neuronal stem cells." These stem cells normally give rise to all of the mature nerve cells of the brain. However, in CMED, signals to induce maturation of stem cells seem to be interrupted, causing the cells to continue dividing.

Small cel carcinoma of the lung (SCCL) is a deadly lung tumor. SCCL is thought to originate from neuroendocrine cells in the lung. SCCL or lung neuroendocrine cells have many features of nerve cells. Lung neuroendocrine cells are thought to help shape development of the lung and to help lungs adapt to environmental challenges or trauma throughout life. However, a loss of growth control in those cells, again probably due to interrupted signals to control cell division, is thought to be associated with development of SCCL tumors.

Nicotinic acetylcholine receptors (nAChR) are chemical signals receiving molecules that play important roles in the developing and in the mature brain and body. They also happen to be targets for nicotine from tobacco. There is evidence that nAChR play roles in mediating signals controlling division of neuronal stem cells and lung neuroendocrine cells.

Current therapies to treat CMED or SCCL remain imperfect. Development of effective drugs to treat these cancers, alone or in combination with other therapies, is needed.

The central hypothesis of the project is that abnormal chemical signaling through nAChR contributes to formation of CMED and/or SCCL. The major goal of this pilot project is to determine whether drug therapy targeting nAChR, alone or in combination with other tumor cell treatments, has utility in controlling CMED or SCCL. Very simply, one aim of the project is to determine whether changes in chemical signaling through nAChR can regulate division or maturation of CMED or SCCL cells. If so, and if progress in the project allows, then another aim will be to identify and characterize nAChR on cells from either CMED or SCCL.

To achieve these goals, proven techniques in cell culture and in identification and characterization of nAChR will be used. Human CMED or SCCL cell lines will be used as models. Critical experiments will ascertain effects on cell survival and rates of division after exposure to drugs that activate or block nAChR signaling. These drugs will be tested alone or in combination with other treatments. If these drugs are effective, then cell samples will be assessed for expression of nAChR using gene, drug binding, and protein analyses. Signaling through nAChR will be assessed using chemical ion and electrical assays and on abilities to activate or

inactivate signals that promote division of CMED or SCCL cells. The project is virtually certain 1) to help elucidate roles played by nAChR in lung and brain development, 2) to clarify relationships between CMED or SCCL and their normal cell origins, and 3) perhaps to provide insight into novel sources of neuron-like precursors that could be used to other therapeutic purposes. Most important, however, will be demonstrating whether drugs targeting nAChR can provide novel therapies to treat CMED and SCCL.

Mash, Ph.D., Eugene Jr.

University of Arizona
Award Amount FY 02: $200,000

## Rational Design and Production of Anticancer Drugs that Bind Cytosolic Akt

A defining feature of cancer cells is their ability to survive under adverse conditions where normal cells will die through a process of programmed cell death. This characteristic allows cancer cells to thrive where normal cells cannot and also makes cancer cells resistant to cell killing by chemotherapy. Inhibiting the signaling pathways that promote cancer cell survival offers a rational and attractive way of selectively inhibiting cancer growth. The protein Akt is a key player in this cell survival signaling pathway in cancer cells. It, therefore, is an attractive target for the development of drugs to promote death in cancer cells. We have adopted a novel approach to interfering with signaling by Akt and are designing, producing , and testing inhibitors of the function of this protein. The inhibitors will be developed as leads for new anticancer drugs.

The hypothesis upon which our work is based is that the PH domain of cytosolic Akt provides a novel target for the development of drugs to selectively inhibit cancer cell survival and to inhibit cancer growth. The goals for this research program are five-fold: 1) to use the available x-ray crystal structure data for the lipid binding PH domains of several proteins to model that of Akt and to conduct molecular docking studies to design compounds for selective inhibition of Akt translocation and activation; 2) to chemically synthesize potential inhibitors of Akt suggested by the modeling, and their analogues; 3) to test these inhibitors for activity and to use the data obtained to better understand binding at the PH domain of Akt and the role of Akt in normal and tumorigenic cells; 4) through an iterative process, to design, prepare, and test ever more potent and specific inhibitors of Akt; and 5) to conduct pre-clinical development of the most active compounds as anticancer drugs.

In proof-of-principle studies we have shown that 3-deoxy-phosphatidylinositols inhibit the binding of membrane phosphatidylinositol-3-phosphate in the PH domain of Akt, thus preventing translocation of Akt to the plasma membrane and its activation by phosphorylation. Although these compounds inhibit cancer cell growth in culture, they are not good drug candidates because they are broken down by lipases in the body. Thus, the objective of our study is to design, synthesize, and test the efficacy of novel small molecules that bind at the PH domain

of Akt. The specific aims of the proposal are to: 1) use molecular modeling to rationally design small molecule inhibitors of the binding of membrane phosphatidylinositol-3-phosphates to the PH domain of cytosolic Akt; 2) chemically synthesize the inhibitors and analogs designed in specific aim 1; 3) conduct *in vitro* enzymatic and cancer cell studies to characterize the biological activity and selectivity of the compounds synthesized in specific aim 2; 4) employ a process (design→synthesis→evaluate SAR→redesign) to develop chemical agents with potent pharmacological activity; and 5) conduct pre-clinical development studies of the most active agents. To achieve the stated goals, the specific aims will be addressed by an interdisciplinary research team with expertise in protein crystallography, computer-assisted protein modeling, chemical synthesis, enzyme biochemistry, and cancer biology. This team has previously worked together and is responsible for much of the current knowledge about the role of Akt in cancer.

Pettit, Ph.D., George R.

Arizona State University
Award Amount FY 02: $400,000

## Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs

In the coming year, some 600,000 people in the United States will die from devastating attacks by one or more types of human cancer. That most tragic death toll in the United States, and on a much larger scale internationally, will not be reduced until more generally effective and curative anticancer drugs are discovered and developed. Although a small number of anticancer drugs are now available that have greatly improved cancer treatment and provided various levels of curative treatments for about 20 types of human cancer, twelve of the major types of human cancer have continued to remain generally refractory to current anticancer drugs and urgently require discovery and development of generally curative anticancer drug treatments. Illustrative are diagnosis and death rates statistics available recently--with lung cancer 177,000 new cases diagnosed and 158, 700 deaths; colorectal cancer 133,500 diagnosed and 54,900 deaths; breast cancer 185,700 new cases diagnosed and 44,560 deaths; prostate cancer 317, 100 new cases diagnosed and 41,400 deaths; pancreatic cancer 26,300 new cases diagnosed and 2,780 deaths; non-Hodgkin's lymphoma 52,700 diagnosed and 23,300 deaths; leukemia 27,600 diagnosed and 21,000 deaths; and ovarian cancer 26,700 diagnosed and 14,800 deaths expected. With continuation of these tragic death rates for the overall 200+ types of human cancer, it is abundantly clear that a great acceleration in the discovery and development of new anticancer drugs in vitally important and urgently needed.

Five new anticancer drugs discovered in the ASU Cancer Research Institute and now at various levels of development have been selected for accelerated preclinical research leading to clinical trials. As these very promising anticancer drug candidates are moved toward the clinic, resources (as available) will also be devoted to the scale-up, procurement, and processing of other very

promising plant, marine organism, and microorganism anticancer constituents. The availability of these leads in larger quantities will greatly speed up actual isolation of the new anticancer drugs. In turn, that will ensure a steady stream of new anticancer drugs prospects moving toward eventual clinical trials. The five anticancer drug candidates selected for vigorous research directed at making them available as quickly as possible for clinical trails comprise structurally new, powerful and very potent anticancer substances isolated primarily from terrestrial plants and marine animals. They are combretastatin A-1 prodrug, pancratistatin prodrug, hydroxyphenstatin prodrug, tyrostatin phosphate prodrug, and fluorcomstatin prodrug. The rapid introduction of these new anticancer drugs into human cancer clinical trials should lead to a series of very important advances in improving human cancer treatment.

Whitesell, Ph.D., Luke

University of Arizona
Award Amount FY 02: $181,599

## Heat Shock Proteins as Targets for Drug Discovery

Human cancers result from the accumulation of specific alterations (mutations) in genes within the cells of a particular organ such as the breast, nervous system or bowel. Most of these mutations result in the production of abnormal versions of proteins that are involved in the control of cell growth and survival. Because they are unique to the tumor, these mutant proteins provide a wide variety of potential targets for the development of new, more selective anticancer drugs. A particularly interesting approach to inhibiting the function of these proteins in order to treat cancer could be provided by drugs that alter the function of a helper protein in cells called heat shock protein 90 (Hsp90). This protein acts as a guide or "chaperone" for other proteins, especially damaged or mutated ones. We think it could provide an unusually good target for anticancer drugs because it facilitates the development of cancers by increasing the stability and activity of multiple mutant proteins within a cancer cell, not just one . In addition, Hsp90 itself does not appear to become mutated in cancer and so it provides a stable, reliable target. Over the past five years, we and others have identified several classes of drugs that bind to Hsp90 and inhibit its function in tumor cells causing them to die. One of these compounds called 17-N-allylamino-17-demethoxygeldanamycin (17AAG) has recently entered the first phase of clinical trials in cancer patients, but this drug and all the other Hsp90-binding compounds studied so far suffer from serious limitations to their clinical applications due to problems with either solubility, toxicity or poor potency. In order to develop a better drug with which to attack Hsp90 function, we are proposing a collaborative effort between investigators with expertise in medicinal chemistry, cancer biology, experimental therapeutics, and clinical oncology. Using a molecularly-based screening approach, we plan to develop a semi-synthetic Hsp 90-binding drug that can alter Hsp90 function in cancer cells and cause them to die as a result of disrupting the abnormal regulatory pathways that caused them to become malignant in the first place.

To achieve the goal of exploiting Hsp90 as a new anticancer drug target, a great need exists to find better agents with which to alter its complex functions both in cells and in whole animals. Very recently, a new class of drugs based on the readily available antibiotic novobiocin has been identified that binds to Hsp90-active drugs that we have worked on in the past. Unfortunately, novobiocin is not very potent and high concentrations must be used to see effects on Hsp90 cellular function. We now hypothesize that a more potent and specific derivative can be synthesized, both to treat cancer in a unique way and to help us understand more about how Hsp90 works in tumor cells. To test this hypothesis we have developed a plan designed to accomplish the following objectives: 1) to generate a library of at least 100 novobiocin derivatives by chemically modifying specific sites on the molecule that affect its binding to Hsp90; 2) to identify the 5 most potent and selective derivatives by using a series of call-based and biochemical assays 3) to characterize the activity of the best derivatives at a molecular level against human breast cancer cells that carry different patterns of well defined genetic defects; and 4) to define the antitumor activity of the best derivatives against the same human breast cancer cells growing as established tumors in mice. The proposed research will provide important new information about the best way to target Hsp90 in tumor cells. If our underlying hypothesis proves true, it will also identify specific new compounds for clinical trials in cancer patients.

# SECTION F

## NEW CONTRACT AWARDS

## MEDICAL RESEARCH

## BEGINNING IN FY 2002

Baldwin, Ph.D., Thomas O.

University of Arizona
Award Amount FY02: $50,000

## Disruption of Cell-Cell Communications to Block Bacterial Pathogenicity

Pathogenic bacteria are formidable foes. They rapidly evolve resistance to our best antibiotics. Now it turns out that they are not merely the single-celled organisms that they appear; rather, they signal each other and, when they have reached a certain population level (establishing a quorum), they act coordinately to overcome our disease defenses. Disruption of these quorum sensing signals can prevent the development of disease. These signaling molecules (SM) are usually non-toxic and act at extremely low concentrations (1/1000 or less than the concentration of a typical antibiotic treatment). Targeting the SM is an attractive approach to therapeutic disease control as confusing the bacteria does not supply the selective pressure that killing them does. Consequently, development of resistance to SM therapy should not develop as rapidly as resistance to antibiotic therapy. During the last several years, there has been a lot of research to identify quorum sensing systems in bacteria. We now know that quorum sensing is the rule as opposed to the exception. Now is the time to study individual quorum sensing systems and develop treatments to trick the bacteria into thinking that they are below critical threshold levels to cause disease. Successful completion of this research will have significance to persons who have compromised immune system, *e.g.*, late stages of tobacco-induced cancer, and will directly benefit the citizens of Arizona through better disease control therapies.

We have chosen four bacteria to focus on in these studies: *Bordetella pertussis* (whooping cough); *Helicobacter pylori* (ulcers and cancers), *Escherichia coli* (food poisoning and diarrhea), and *Xenorhabdus luminescens* (generally not considered a human pathogen but may have SM that interfere with other virulent pathogens). There are indications that quorum sensing is present in each of these bacteria; however, the quorum sensing systems have not been well defined. The first objective is to identify quorum-sensing components in these bacteria. Then we will identify SM antagonists, in order to confuse the bacteria. Finally, we will evaluate *in vitro* delivery systems to determine the best way to treat cultures of these bacteria to inhibit their quorum sensing responses. Many SM are ephemeral compounds and can be easily destroyed if not handled properly. These objectives will be achieved by cloning promoters of virulence factors and coupling these promoters to bacterial luciferase genes to monitor expression of light in the presence of various SM and SM analogs with and without cloned genes for quorum-sensing transcription factors. We will reconstruct the quorum sensing systems from these pathogenic bacteria in laboratory strains of *E. coli* so that experimental conditions can be controlled and hazards to the researchers will be minimized.

Bernstein, Ph.D., Harris

University of Arizona
Award Amount FY02: $300,000

## Interactive Biologic Effects of Smoking Components (Benzo(a)pyrene, Nicotine) and Dietary Factors (Bile Acids) as Early Indicators of Progression Towards Gastrointestinal Malignancy

Each year in Arizona about 900 people die from colon cancer (the second leading cause of death in the state) and about 200 people die from esophageal cancer (the ninth leading cause). On the basis of these current mortality rates, about 78,000 of Arizona's current residents (about 2%) will die of cancer of the colon and esophagus unless there are significant improvements in prevention and treatment. Most cancers of the colon and esophagus are due to lifestyle factors including diet and smoking. Numerous studies indicate that smoking is an important risk factor for adenocarcinoma of the colon and esophagus. We hypothesize that smoking in combination with certain dietary factors contribute to a increased risk of gastrointestinal malignancy by decreasing the ability to repair DNA damage and selecting for populations of cells that are apoptosis resistant. These two pathobiologic conditions can then lead them to genomic instability, increased mutations and cancer. The experiments proposed here are designed to: 1) identify molecular events, *e.g.*, alterations in gene expression, and cellular events *e.g.*, resistance to apoptosis, decreased ability of cells to die, that occur in the early phases of progression to adenocarcinoma of the colon and esophagus; 2) determine the interactive effect of cigarette smoke components benzo(a)pyrene, nicotine and bile acids on specific DNA repair proteins in cells of the colonic epithelium and Barrett's esophagus, and the effects of loss of expression on cellular events, *e.g.*, cell cycle changes, apoptosis and proliferation, leading to cancer; 3) determine the ability of epithelial cells of the human esophagus and colon to repair DNA damage in the progression to adenocarcinoma; and 4) develop a practical method for identifying fields of colonic cells predisposed to development of cancer that can be performed on standard tissue preparations.

The hypothesis to be tested is that deeper understanding of the early molecular and cellular events information of adenocarcinoma of the colon and esophagus can lead to development of mechanistically-based biomarkers for assessing cancer risk. The ultimate goal of this proposal is to develop practical biomarkers for assessing risk of these cancers that are hypothesis-driven and take into consideration both environmental and dietary factors that are major contributors to cancer of both the esophagus and colon. The availability of such biomarkers would be invaluable for guiding individual patients towards appropriate dietary and smoking behavior modification, and for monitoring progress during cancer chemoprevention treatment.

M. Bonner Denton, Ph.D.

University of Arizona
Award Amount FY 02: $66,338

## A Unique Mass Spectrometer for Biomedical Studies

Modern analytical methods, especially mass spectrometry, play a large role in drug discovery and development. However, in many instances the procedures are slow because of the time needed to develop a sample preparation scheme prior to analysis or because of the need for time consuming chromatographic separation of the components of a biological extract. The hypothesis to be tested is the unique $H_2$ laser TOF mass spectrometer developed in our laboratories possesses superior capabilities to other instruments. These superior capabilities include 1) no need for complex and time consuming sample preparation schemes, 2) the elimination of chromatographic separation of the analyte from extraneous compounds in a biological extract, 3) simplicity of analysis, 4) the ability to selectively detect formation of reaction products of the compound of interest with macromolecules without the need for extensive mass spectral analysis, and 5) improved sensitivity. The mass spectrometer needs to be upgraded and the capabilities of this instrument could greatly facilitate the discovery and development of drugs used in the treatment of lung cancer, and other diseases caused by tobacco use.

The goal of this project is to confirm the hypothesis that the $H_2$ laser TOF mass spectrometer has significant advantages over other existing mass spectrometers for solving biomedical problems. This goal will be achieved by 1) coupling the $H_2$ laser to a newer time-of-flight mass analyzer with improved sensitivity, resolution and ease of use; 2) development of a simple, general sample preparation method applicable to all compounds of interest; 3) eliminating the need for separation of complex mixtures using chromatography; 4) expanding the investigation of the compound classes which can be ionized by the $H_2$ laser; 5) investigating inlet systems and sample derivation to define the limits of volatility needed by a sample for analysis using the $H_2$ laser TOF instruments; 6) determining the detection limits for the analysis of biological samples using the $H_2$ laser TOF mass spectrometer and comparing the results with published procedures using other instruments; and 7) testing the idea that reaction of a photoreactive compound with non-photoreactive compound produces a product which is visible to the $H_2$ laser.

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY 02: $49,673

## Mechanisms of Cell Cycle Control: Axolotl Heart Regeneration

The major goal of this proposal is to identify genes that stimulate reentry into the cardiomyocyte cell cycle. This strategy will be used as a gene therapeutic approach for repair of myocardial infarction. The damaged heart is unable to provide an adequate output of blood and heart failure ensues. In the United States, about half a million individuals are diagnosed with heart failure and over four million persons are currently affected. Similar proportions of the Arizona populations are affected. The relationship of tobacco smoking to heart disease is well established. The damaged heart is severely compromised because the adult heart cannot repair itself by making new cells. At present, there is no means of reversing cardiac cell death due to smoking, short transplantation of a new heart. Unfortunately, the limited availability of donor hearts raises a strong need for an alternative therapy. It would be a major advance if the signals that stimulate cell division were known so that heart cells around an infarction might be induced to divide and repair the damaged region. To attain this goal, we are using a heart regenerative model in amphibians to identify regulators that are able to restart the cell division cycle. Amphibians are unique because they have the inherent ability to repair the damaged myocardium after injury. Our aim is to identify and characterized the gene(s) in amphibians that re-induces mitosis. This information can then be transferred to repair the human myocardium.

The long-term goal of this proposal is to determine the biochemical block that inhibits DNA replication and cytokinesis in mammalian cardiomyocytes. Separate signals may be required to overcome the block that initiates DNA synthesis during S-phase, and cytokinesis that begin during G2/M. The short-term goal is to discover signals that could be manipulated, *in vivo*, to restart cell division. Four aims will be examined using a heart regenerative model in amphibians: 1) determine the zone of replicating cardiomyocytes in the amputated ventricular apex by labeling DNA with a synthetic DNA marker, BrdU, and immunostaining with anti-BrdU antibodies; 2) identify signals that could potentially be used in restarting the cell cycle, (Amputated hearts will be tested for changes in myogenic genes by *in situ* hybridization.); 3) growth factors also will be examined for their influence on cell division in the heart repair zone; and 4) using subtractive hybridization, novel genes that are up-regulated after ventricular wounding will be identified. Information gained from this study will be potentially useful for understanding the basis of remodeling the damaged myocardium during human cardiovascular disease. In future studies, this work will be used to develop a transgenic model of heart repair.

Bernard W. Futscher, Ph.D

University of Arizona
Award Amount FY 02: $46,200

## Transcriptional Repression as Mechanism of Maspin Gene Inactivation in Breast Cancer

Breast cancer is the most common cancer that afflicts American women and is the second leading cause of cancer death. It is estimated that 1 in 8 American women who live to 5 years of age will develop this disease. Thus, breast cancer is a significant health issue in the United States. The delineation of the molecular mechanisms that are responsible for the conversion of a normal breast cell into a malignant breast cancer cell will likely play a significant role in the diagnosis, monitoring and treatment of this disease. One mechanism that participates in this conversion is the inappropriate silencing of the tumor suppressor gene named Maspin. When the Maspin gene is inappropriately silenced, breast cells acquire malignant characteristics such as increased mobility, increased invasion and metastatic properties, and the ability to stimulate the growth of new blood vessels.

Studies performed in our laboratory using breast cancer cell lines as models of the disease have identified a molecular mechanism that appears responsible for the silencing of the maspin gene. In addition, we have identified drug therapies that can reactivate Maspin gene expression in these malignant breast cancer cells. The goal of the proposed studies is to translate our basic laboratory findings to the analysis of clinical breast cancer specimens and determine the potential relevance of our findings to the clinical setting.

The goal of this study is to identify the molecular mechanisms responsible for transcriptional silencing of the tumor suppressor gene, Maspin, in human breast cancer. The hypothesis is that aberrant cytosine methylation of the Maspin gene promoter is a major mechanism responsible for Maspin's transcriptional repression in breast cancer. We base this hypothesis and its importance to human breast cancer on data collected from a variety of laboratories including our own.

Two specific objectives are designed to determine if aberrant cytosine methylation of the CpG island promoter of the Maspin tumor suppressor gene is a mechanism of Maspin gene inactivation in clinical breast cancer specimens. They are to determine: 1) if Maspin gene expression is lost or decreased in human breast cancer cells, and 2) if the Maspin gene promoter is methylated in breast cancer cells.

If our hypothesis is proven correct, then our work will identify a new mechanism of Maspin inactivation in clinical breast cancer. Furthermore, this information would provide a framework for future translational studies designed to determine if the identified changes in Maspin 5-methylcytosine patterns will be useful as a detector of disease, a prognostic indicator, and as a target of therapeutic intervention.

Arthur F. Gmitro, Ph.D

University of Arizona
Award Amount FY 02: $50,000

## Development and Clinical Evaluation of a Confocal Microendoscope

The diagnosis of lung cancer and other pulmonary disease is difficult, especially at the early stages when therapeutic interventions are most effective and the potential for cure is highest. Bronchoscopy is an effective tool for diagnosis of disease in the upper portions of the trachea and lung but is not very sensitive in detecting early changes. We have developed a specialized instrument for *in vivo* microscopic imaging of tissue, called a confocal microendoscope, which could be used as an adjunct to conventional bronchoscopy to improve the accuracy of diagnosis. Another major problem in the diagnosis of lung disease is that abnormalities are often detected deeper in the lung on a chest x-ray or CT exam, but there is usually no way to make a definitive diagnosis of the disease without extracting a sample of lung tissue. There are major difficulties with these biopsy procedures in obtaining adequate tissue samples for diagnosis. The confocal microendoscope also has the potential to assist with the diagnosis of disease deep in the lung. Clearly, most diseases of the lung are strongly linked to smoking and so improved diagnosis of lung disorders is critically important to the subpopulation of smokers. Early and more accurate diagnosis of lung disease, which is the goal of this work, will help save lives and reduce the overall cost of health care to the citizens of the state of Arizona.

This research project is aimed at development and evaluation of the confocal microendoscope for imaging the lung. The ultimate goal is to demonstrate that the instrument can improve the accuracy of diagnosis of lung disease. Objectives of the proposed work are to complete development of the instrumentation, evaluate the quality and characteristics of images of lung tissue that can be obtained with the instrument, and to show the feasibility of using the instrument for *in vivo* lung imaging. Three different modes of operation for the instrument will be investigated. One mode uses the instrument as an added microscopic imaging capability for a clinical bronchoscope. The second uses it as an imaging catheter that can be routed deep into the lung. The third mode uses if for imaging tissue during needle biopsy. We will determine which of these modes of operation is practical and evaluate the clinical relevance of the information that the instrument is capable of providing.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 02: $195,664

## Discovery, Optimization of Production and Evaluation of Novel Anticancer Drugs from Rhizosphere Microflora of Desert Plants

Cancer accounts for a vast number of human deaths and suffering. Each year 6.5 million people are diagnosed with cancer worldwide. In the U.S. more than 10 million people are living with a history of cancer, and in excess of one million new cancer cases develop annually. More than 30% of all these cancer deaths can be attributed to tobacco smoke. According to the Arizona Cancer Registry published by the Arizona Department of Health Services, in 1997 cancer was responsible for 8,429 deaths and 19,384 newly diagnosed cases in Arizona. The majority of these deaths are due to lung cancer caused by tobacco smoke.

Cancer treatment currently relies on surgery, radiation and chemotherapy (treatment with anticancer drugs). Chemotherapy is the only effective way to treat a disseminated cancer. Unfortunately, there are no good anticancer drugs available to treat major solid tumors such as cancer of the lung, breast and prostate effectively without undesirable side effects. There is thus an urgent need for the discovery and development of new, effective and non-toxic anticancer drugs to treat these cancer.

The majority of anticancer drugs in use today are from natural sources such as plants and microorganisms. With initial two year funding from Arizona Disease Control Research Commission we have shown microorganisms (bacteria and fungi) growing on the roots of desert plants (in a region called "rhizosphere") to be a rich source a potential anticancer drugs. We have made significant progress by sampling rhizosphere microorganisms of 99 desert plants and having 18,418 bacteria and 1769 fungi in our collection. Of these, so far we have screened 487 fungi and 360 bacteria for potential anticancer agents using cell lines derived from cancers of the lung, breast and central nervous system. Extracts prepared from 35 fungi and 4 bacteria were found to be toxic to these cancer cell lines and several of the extracts were chemically investigated. Four compounds with anticancer activity have been identified. The goal of this project is to continue our investigation of this novel and unexploited natural source for clinically useful anticancer drugs by sampling plants growing under very harsh conditions, and by including several new methods to search for novel compounds with potential anticancer activity. We also plan to use molecular biologic techniques to identify microorganisms and manipulate culture conditions to optimize production of promising anticancer compounds. We are hopeful that having access to this unique source of bioactive natural products, combined with the use of selective and improved anticancer bioassays, will lead to the discovery of effective new drugs to treat currently untreatable forms of cancer, thus increasing the survival and quality of life of victims of cancer, not only in Arizona but worldwide.

Karl B. Kern, M.D.

University of Arizona
Award Amount FY 02: $50,000

## The Mechanism of Post Resuscitation Myocardial Dysfunction: Potential Role of Inducible Nitric Oxide (iNOS)

Tobacco use, especially cigarette smoking, is a major cause of heart disease. Generally manifested as hardening of the heart arteries, or atherosclerosis, tobacco-related cardiac disease results in premature heart attacks and often sudden cardiac arrest. Recently released statistics from the American Heart Association (2000 Heart and Smoke Statistical Update) report that Cardiovascular Disease (CVD) is still the leading cause of death for both men and women. It is estimated that close to 3,000 Americans die every day of CVD. Nearly one-third of these deaths are sudden from cardiac arrest. According to the American Heart Association, about 1 in 5 cardiovascular deaths are attributed to smoking. Extrapolating these data to our own state suggest that 20,000 Arizonans die each year from heart disease and that between 4,000-5,000 of these deaths are directly attributed to tobacco use. The only viable treatment for those experiencing sudden cardiac arrest is cardiopulmonary resuscitation or CPR. When CPR is started early and followed by rapid defibrillation, successful reversal of cardiac arrest can occur. Unfortunately, even in those people with successful resuscitation, many will not survive long enough to leave the hospital and return home. The stress of cardiac arrest on the heart's pumping function, after it resumes beating, is now recognized as a major contributor to the poor long-term survival. Understanding the mechanism of the heart's failure after successful resuscitation is a key to achieving improved long-term survival for thousands of Arizonans suffering sudden cardiac death each year.

The goal of this proposal is to improve long-term survival for victims of sudden cardiac death, often associated with tobacco use, both acute and chronic. The primary objective is to evaluate a potential mechanism of post-resuscitation heart dysfunction. Preliminary data suggest one possible mechanism could be related to the level of nitric oxide within the myocardium. We hypothesize that cardiac arrest stimulates the production of extraordinarily high levels of myocardial nitric oxide, which suppress normal heart pumping function. Specific aims will include the measurements of pro-inflammatory cytokines (proteins that stimulate the production of very high levels of myocardial nitric oxide), nitric oxide metabolites in the myocardium, and the activity of nitric oxide synthase (iNOS), the enzyme responsible for increasing the nitric oxide levels in the heart muscle, as well as left ventricular systolic (squeeze) and diastolic (relaxation) function both before and after resuscitation from cardiac arrest. Correlation between post-resuscitation myocardial dysfunction and myocardial nitric oxide and iNOS induction will be evaluated. The effect of iNOS inhibition versus iNOS facilitation on post-resuscitation heart function will then be investigated in a randomized, blinded, placebo-controlled fashion.

Raymond B. Nagle, M.D., Ph.D.

University of Arizona
Award Amount FY 02; $131,520

## Establishment of a Arizona Cancer Tissue and Serum Bank for the Purposes of Improving Life for Men with Prostate Cancer

Prostate cancer is the most common form of non-skin cancer in men in the United States. This year alone it is estimated that 180,400 men will be diagnosed with prostate cancer and nearly 32,000 men will die of this disease. Because prostate cancer is associated with increasing age in males, it is a particularly important problem in the state of Arizona with its relatively large retired population. In Arizona, prostate cancer represents the most common invasive cancer type with a crude rate of 133.9 patients per 100,000 males. More than 3000 men in Arizona are diagnosed each year with prostate cancer. This year, 600 men in Arizona are expected to die from this disease. In addition to being a common problem, the morbidity caused by prostate cancer (terrible bone pain, urinary incontinence, and impotence) is devastating. While the etiology of prostate cancer is not known, several studies have shown a relationship between smoking and risk of prostate cancer, particularly fatal prostate cancer. Smokeless tobacco use also has been associated with risk of prostate cancer.

Currently, there are two major clinical problems in prostate cancer. First, prostate cancer is characterized by its wide biologic variability. This presents a difficult problem for disease management. While the disease remains organ confined in most men, in some individuals there is rapid progression of the cancer with widespread bony metastases and severe morbidity and mortality. There are no methods available to predict those men with prostate cancer who are going to have aggressive tumors and require radical therapy versus those men with prostate cancer who have slow growing tumors that may never become clinically apparent. Identification of relevant prognostic markers for prostate cancer provides the best chance for successful clinical management of this disease. The second problem is the need to identify new targets that are present in prostate cancer cells and not present in normal prostate cells. When these new targets are identified, new therapies can be designed to hit those targets.

The overall goal of this proposal is to create the Arizona Prostate Cancer Tissue and Serum Bank. This bank will assure that both tissue and serum are available to apply the latest molecular techniques to make a major impact against prostate cancer. We hypothesize that patients with prostate cancer can be more effectively treated if new targets are identified that separate aggressive from non-aggressive cases. Additionally, we hypothesize that the identification of new targets in specimens from the bank will enhance our ability to make an impact against prostate cancer through the development of new therapies for the treatment and prevention of this disease. Our objectives are to: 1) establish the Arizona Prostate Cancer Tissue and Serum Bank with attention to quality control of tissue acquisition, storage and follow-up information; 2) utilize the bank to examine prognostic factors (*e.g.* who will and will not have disease recurrence);

and 3) identify new prostate cancer therapeutic and prevention targets.

The Governor of the state of Arizona signed the legislation creating the Prostate Cancer Task Force. The concept for the Arizona Prostate Cancer Tissue and Serum Bank (APCTSB) arose from the work of this task force.

Mary Kay O'Rourke, Ph.D.

University of Arizona
Award Amount FY 02: $219,797

## Integrated Epidemiological Study of Valley Fever

Valley Fever (coccidioidomycosis) is caused by breathing the spores of the soil fungus *Coccidioides immitis*. Valley Fever poses a special problem for people with impaired host defenses, pregnant women, and people visiting regions where the fungus grows, particularly southern Arizona. Researchers recently found that elderly smokers have 3.7 times greater chance getting Valley Fever. Approximately 0.5% of infections spread outside of the lungs, 50-60% of these types of infections are fatal if untreated. A survey of two Tucson neighborhoods found that 32 people out of 1000 got Valley Fever over a 10-year period. For the past several years in Arizona the rate of people getting Valley Fever has more than doubled without explanation. The only practical preventive measure currently available against this disease is to avoid regions where the fungus grows. Valley Fever occurs in the southwestern United States where the climate is arid and warm. Dust storms are associated with increased number of infections, but little is known about the source of the fungal spores, or the specific ecologic conditions required for the soil fungus to grow. Because of this, research studies have not been able to evaluate risk of exposure to the pathogen beyond a regional level. Valley Fever fungus is considered a biological terrorist agent and requires Biocontainment Level-3 (BL3) handling, the same level as Anthrax. The resulting safety requirements needed to protect laboratory workers prevent the commercial analysis of potentially contaminated soils and add to the difficulty in studying the epidemiology of this disease.

We propose to improve the targeting and monitoring of public health interventions against Valley Fever and the evaluation of new cases of the disease. We will achieve this goal by: 1) determine the precise incidence of the disease at the neighborhood level; 2) evaluate environmental and behavioral risk factors, including smoking, that contribute to Valley Fever; 3) develop less cumbersome laboratory analysis techniques and soil sampling strategies that are more likely to be adopted by commercial laboratories; and 4) propose preventive measures to reduce the incidence of Valley Fever. Door-to-door household surveys are key in getting enough information about cases of the disease to accurately describe neighborhood rates of Valley Fever. We will also study dog cases of Valley Fever in neighborhoods because dogs are much more likely to get Valley Fever than humans. They are also generally less mobile and are potentially a better measure of local exposure to the fungus. We will also take air samples to monitor exposure in selected

neighborhoods before we survey households. By identifying and sampling hot spots, we can more efficiently evaluate the ecological requirements for fungal growth. Once we know the soil and vegetation characteristics supporting fungal growth, we can target prevention activities more cost effectively. Recent advances in DNA analysis now provide the opportunity to develop more cost effective and humane ways to identify the soil fungus that are currently available. Once these techniques are developed and proven, commercial laboratories can evaluate Valley Fever concentrations in soil.

Alyssa Panitch, Ph.D.

Arizona State University
Award Amount FY 02: $50,000

## Bio-Responsive Self-Assembling Dextran-Based Blood Substitutes for Trauma

Suspensions of the polysaccharide dextran in physiological saline solutions are sometimes used to replace vital fluids depleted during blood loss in trauma victims. Limitations of this treatment include: a) the control of bleeding is not treated because these substitutes do not contain factors that will help clot blood and stem bleeding; and b) tissue-damaging inflammatory responses are not suppressed–the body's natural wound response can be extensive in these situations causing further damage to the victim. The proposed research is intended to result in a new material that can be used as a fluid volume replacement that will provide the vital factors necessary to promote blood clotting and prevent severe inflammatory responses.

The specific aims of the project are to: 1) create a new modified dextran that will selectively and locally self-assemble into hemostatic clot-like plugs at sites of injured tissues by conjugating the dextran to peptide molecules that mimic the body's natural ability to clot; and 2) develop a dextran-peptide conjugate that selectively binds to injured tissue surfaces to form a protective barrier against trauma-induced inflammatory cell damage to surrounding healthy tissues by conjugating some of the dextran to peptides that will bind to the endothelium, the natural lining of blood vessels.

The overall objective is to improve care for trauma patients and to decrease the rate of intervening complications due to blood loss.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 02: $100,000

## Development of New Anticancer Drugs for Improving Treatment of Tobacco Related Human Cancer

Human cancer constitutes some 200 related diseases that continue to cause a catastrophic number of deaths accompanied by tremendous personal and economic diasters. Of the nearly 600,000 annual deaths from cancer in the United States, over 200,000 of these are related to tobacco use, and the overall total corresponds to about 21% of all mortality. The total medical and economic loss estimates this year from cancer are expected to be over 120 billion dollars and that indicates combined medical costs and economic loss in Arizona of well over one billion dollars per year! Only 48% of cancer patients can now be treated curatively, and that number will not increase until more effective and curative anticancer drugs are discovered and developed. Truly important advances in improving human cancer treatment are quite dependent upon discovery and development of new and curative anticancer drugs. The ADCRC Tobacco Tax Research Funding will be sharply focused on that objective.

The Arizona State University Cancer Research Institute (ASU-CRI) is completely committed to pursuing research directed at the discovery and development of new and effective anticancer drugs for improving human cancer treatment. Acceleration and expansion of this vigorous research program directed at discovery and development of new anticancer drugs for those types of cancer arising from tobacco use will be continued. Further development toward clinical trials of our most promising and advanced anticancer drugs, such as dolastatin 15, auristatin 15 -PE, auristatin M, dolastatin 16 and 17, combretastatin A-1, A-2, A-3, D-1 and D-2 prodrugs, auristatin C, cephalostatins 1 and 7 and the narcistatin prodrugs has been proposed for ADCRC contract support. In addition, we will continue to isolate and/or synthesize sufficient quantities of these exciting anticancer drugs to ensure adequate supplies for U.S. National Cancer Institute (NCI) preclinical research necessary to clinical decisions and further development beyond our Institute resources. The ADCRC research will also include continuing the synthesis of vitally important clinical supplies of our anticancer drugs for the NCI such as dolastatin 10.

Garth Powis, D. Phil

University of Arizona
Award Amount FY 02: $50,000

## Thioredoxin Peroxidase, A Novel Mechanism for Protection Against Lung Toxicity

The lung is continually exposed to reactive oxidant species generated from the air we breath and has developed several mechanisms to protect itself against oxidant damage. When the exposure to oxidant species is increased, for example by high oxygen tension or by oxidants in cigarette smoke, these protective mechanisms can be overwhelmed resulting in a loss of lung function. Understanding the mechanisms by which the lung protects itself against oxidant damage is important for our attempts to prevent and reverse cigarette smoke and other oxidant-induced lung damage. Most work has focused on the ability of antioxidant mechanisms in the lung such as the glutathione/glutathione peroxidase system to scavenge the oxidant species. However, by themselves these scavenging mechanisms are insufficient to account for the protection against oxidant lung injury. A newly discovered mechanism that specifically protects cells against oxidant damage to the proteins controlling gene expression by oxidant species is the thioredoxin/thioredoxin peroxidase system. The role of this system in protecting against lung injury has not been previously studied.

The objective of our work is to investigate the role of the thioredoxin/thioredoxin peroxidase system in protecting the lung against oxidant damage caused by high oxygen tension and by nitric oxide (NO) found in cigarette smoke. The hypothesis upon which our studies are based is that thioredoxin and thioredoxin peroxidase are up-regulated during exposure of the lung to oxidant species and protect the lung against oxidant stress by maintaining the activity of redox sensitive gene transcription. The long term goal of our work is to understand at the molecular level the redox mechanisms that protects cells against oxidant species, formed endogenously and present in the environment including cigarette smoke, so that we may design better protective and therapeutic strategies for oxidant-induced human diseases. The specific aims of our study are to: 1) investigate the effects of exposure of lung epithelial cells to oxidant stress caused by hyperoxia and nitric oxide on expression and on the redox state of thioredoxin; 2) investigate the effects of increased thioredoxin and thioredoxin peroxidase expression on lung epithelial cell transcription factor activity and monolayer permeability in response to oxidant stress as in 1 above; and 3) investigate the effect of oxidant stress caused by hyperoxia on transcription factor activity and lung function in an inducible thioredoxin transgenic mouse model.

Mary E. Reid, Ph.D.

University of Arizona
Award Amount FY 02: $128,620

## A Pilot Study of Lung Cancer Prevention with Selenium Supplementation

Lung cancer is the leading cause of cancer death among both men and women in the United States and is one of the world's leading causes of preventable death. There are a variety of factors that contribute to lung cancer risk; however, the strongest association with lung cancer is tobacco smoking, particularly cigarette use. Former smokers constitute a larger proportion of the population in the State of Arizona compared with five years ago due to the increase in the number of people who have quit smoking. Unfortunately, the risk of developing lung cancer in former smokers remains high for many years after smoking cessation and may not reach that of lifelong nonsmokers for more than 20 years. Until recently, there was little evidence to support the use of any specific agent or supplement to reduce the risk of developing lung cancer in former smokers. However, very promising results have emerged from a selenium supplementation trial carried out in individuals of relatively low selenium intake. Therefore, if we can contribute evidence that supports the use of a supplement that can reduce the risk of developing lung cancer in former smokers, we can significantly impact the lives of former smokers. Additionally, given the relatively low selenium status in the State of Arizona, it is important and far-sighted that we investigate the effectiveness of selenium supplementation in reducing the risk of lung cancer in the former smokers of Arizona.

We will randomize 200 male and female former smokers from the Tucson, Arizona, metropolitan area into two groups that will receive either a 200 mcg/day supplement of high selenium yeast or a matched yeast placebo. Prior to the onset of the trial, exposure to smoking and dietary patterns will be documented. Selenium and specific DNA measures for each participant will be collected throughout the 12 month follow-up period from blood, buccal cell and sputum samples. Changes in blood selenium concentration and the status of selected DNA measures will be assessed. The primary hypothesis of this chemoprevention study is that selenium supplementation of 200 mcg/day of high selenium yeast, in former smokers, will be associated with an increased blood selenium and a decrease in DNA markers of lung cancer risk.

Karl H. Schram, Ph.D.

University of Arizona
Award Amount FY 02: $93,067

## Biomarkers of Systemic Fungal Infections

Disseminated fungal infections are becoming a serious problem for immunocompromised patients, causing considerable morbidity and mortality. If the fungal infection can be detected at an early state, appropriate therapy can be initiated and the consequences are much less severe. Current methods of detection have a number of problems which limits their clinical usefulness.

A new method of detecting fungal infections is described in this proposal. The hypothesis to be tested is that each fungal specie produces unique molecules, i.e., biomarkers, which can be detected and quantified at very low levels using mass spectrometry with the following advantages over existing methods: 1) provide a method of early detection of a fungal infection, 2) identify the genus and species of the fungus causing the infection, 3) quantify the level of fungal load, and 4) provide a means to measure the patient's response to therapy. The mass spectrometric methods to be developed will not depend on the detection of antibodies or DNA and, if successful, would permit the detection of multiple fungal infections. The methods, after development, would be rapid, sensitive and specific and would be appropriate for a clinical setting.

The goal of this project is to test the hypothesis that cyclic peptides produced by fungi and yeast can be used as biomarkers of systemic infections in immunocompromised patients. Mass spectrometric methods will be developed which will 1) allow for the rapid identification of cyclic peptides in the crude extracts of yeast/fungal cultures, 2) allow tentative structures to be assigned to new cyclic peptides identified in this word, 3) be applied to the analysis of spiked to determine the detection limits of the techniques, and 4) be used for the analysis of patient blood samples to permit a) early detection of systemic yeast/fungal infections, b) to determine the genus and species of the fungus/yeast causing the infection, and c) determination of the fungal/yeast load in the patient, thus, indicating the patients response to therapy. Methods to be used include extractions of yeast/fungal, spiked blood and patient blood samples with subsequent analysis of the extracts using electrospray ionization mass spectrometry with an ion trap or time-of-flight mass analyzer. Low levels of the cyclic peptides will be monitored using matrix assisted laser desorption ionization.

If the hypothesis is true and if the methods to be developed are successful, a powerful tool would be available for the effective treatment of immunocompromised patients who encounter fungal/yeast infections.

Kemmons A. Tubbs, Ph.D

Intrinsic Bioprobes Inc.
Award Amount FY02: $136,684

## Proteomic Analysis of Nicotine Receptor Structure and Composition

An understanding of the basis for nicotine dependence and habitual use of tobacco products requires knowledge about the biological targets of nicotine action. These targets are complex proteins called nicotinic acetylcholine receptors (nAChR). nAChR are found throughout the nervous system and exist in several different forms reflecting their composition from different building block called subunits. Interactions between nAChR and either nicotine or the natural chemical signaling agent, acetylcholine, alter activity of nerve cells, thereby altering electrical circuit activity in the nervous system. These actions provide a basis for at least some forms of behavior. Prolonged and/or repeated exposure to nicotine has additional effect on nAChR. These effects must contribute to the habitual use of tobacco products (and related adverse health and economic consequences) by an estimated 25% of adult Arizonans. The precise subunit composition of several important forms of nAChR is not known. It is not known whether nAChR associates with other kinds of cell proteins, that could affect construction or localization of nAChR within the cell. Also lacking is knowledge about how nAChR subunits are changed during or after their synthesis, which could affect their assembly into intact nAChR, and localization and function of nAChR. The deficiencies in our knowledge compromise our understanding about how nAChR function is altered by exposure to nicotine.

The overall objective of this project is to address deficiencies in our understanding about structure and composition of diverse forms of human nAChR. Our previous work developed and refined sophisticated chemical techniques to characterize complex proteins like nAChR that reside in cell membranes. These techniques are largely based on a process of combined sensitivity, resolving power, and flexibility called mass spectometry (MS). MS analysis is a central component of a new technology for studies of proteins called proteomic analysis. We will use these proteomic techniques to characterize diverse forms of nAChR containing different subunits. MS and related techniques will be used first to identify and characterize subunits that assemble to create different forms of human nAChR generated by genetically engineered cells or found naturally in human tissue. MS techniques also will be used to identify and characterize other cellular components that interact with specific forms of nAChR, using nAChR subunits as bait to capture those interacting components. A third experimental objective is to determine how nAChR subunits are modified during or after their synthesis, using MS and related techniques to characterize nAChR subunits before and after they are modified. Hypotheses to be tested are: 1) specific nAChR forms contain one, two, three or more kinds of subunits; 2) other proteins have the ability to alter nAChR assembly, subcellular localization, coupling to other chemical signaling molecules, and function, associated with nAChR subunits; and 3) nAChR subunits are modified by addition of sugars, phosphates, and links to the cell membrane after they are synthesized. Collectively, these studies will provide new and important information about the architecture and composition of receptors for nicotine and natural mediators of chemical signaling.

Marlys Hearst Witte, M.D.

University of Arizona
Award Amount FY 02: $49,514

## Angiopoietin-2 and Lymphatic Development: Links to Lymphedema-Angiodysplasia Syndromes

The lymphatic vascular system parallels the blood vasculature and serves, as one of its key functions, to return fluid and large molecules including proteins that have leaked out the small blood vessels and entered the tissue spaces to the bloodstream. When the lymphatic circulation fails, whether from blockage due to cancer, infection or from a genetic defect in the formation of lymphatic vessels called angiodysplasia (AD), a brawny proteinaceous swelling termed lymphedema (LE) of the affected limb, body cavity, or other body part results. The estimated frequency is thousands in Arizona and hundreds of million worldwide. LE-AD leads to disfigurement, disability, and occasionally death even in utero before birth. More than 30 of the different primary LE-AD conditions are inherited. Many more that are apparent at birth or in early childhood are not inherited. LE-AD is not uncommonly associated with blood vessel or heart abnormalities.

During the past several years, two major families of protein growth factors, vascular endothelial growth factor (VEGF) and the angiopoeitins, have been linked to the early (vasculogenesis) and later development and regrowth (angiogenesis) of blood vessels. Recent genetic studies, including our own, have identified specific chromosome linkages and the specific gene abnormalities in two of the many familial LE syndromes, one form of Milroy leg lymphedema and lymphedema-distichiasis (double row of eyelashes). One gene mutation involves the lymphatic lining receptor (VEGFR3) for lymphatic vessel growth factor (VEGF-C), and the second gene (FOXC2) directs development of a variety of embryonic tissues. Just this past year, working with scientists at Regeneron Pharmaceuticals, we have documented arrested lymphatic embryonic development associated with LE accumulation, most notably milky lymph from the intestine, chyle, refluxed into the abdominal and chest cavity, in a strain of mouse genetically engineered to lack the gene for angiopoietin-2 (Ang2).

The present study designed to test hypothesis that Ang2 is required for the formation and linkage of the primitive unconnected lymph sacs that communicate with distended fluid-filled tissue spaces in the limbs and internal organs to form the continuous system of lymphatic channels and lymph glands that drains into the central venous system in the neck. We propose to: 1) further examine how the lack of Ang2 alters lymphatic development, both structurally and functionally, and promotes edema fluid accumulation in embryonic, neonatal, and adult life using high resolution micro- and macrolymphatic imaging techniques; 2) delineate the pattern of Ang2 expression in the developing lymphatic system; 3) determine whether selective Ang2 replacement in breeding experiments using an extra dose of Ang2 gene targeted to specific tissues such as skin or intestine can prevent or modify the lymphatic abnormalities in the Ang2 knockout mouse; 4)

evaluate whether and how isolated lymphatic lining (endothelial) cells in tissue culture (*in vitro*) and in a chick embryo preparation respond to added Ang2 and Ang2 inhibitor in terms of lymphatic growth and vessel formation; and in parallel clinical studies; and 5) continue to collect family pedigrees, DNA (genetic material), and lymphatic tissues/fluids from families and individuals with LE-AD syndromes resembling Ang2 knockout mice to evaluate the possible role of Ang2 related gene mutations and/or dysfunction in these conditions.

Elucidation of the mechanism and role of Ang2 in lymphatic system development in this genetically engineered mouse model and parallel observations in families and individuals with congential LE-AD syndromes should contribute to understanding how Ang2 participates in the cascade of vascular growth factors involved in the formation and maintenance of the lymphatic vasculature and its interrelationships with blood vessel and heart development, along with how these events go awry in a wide variety of LE-AD syndromes. This understanding is likely to lead to improved detection, treatment, and possible prevention of some forms of congential and acquired LE-AD.

Ping Xia, Ph.D , M.D.

University of Arizona
Award Amount FY 02: $48,714

## Mechanisms of Cigarette Smoking on Human Infertility

It is estimated that in the United States one in six couples of reproductive age suffers from infertility. Cigarette smoking increases the risk of infertility in women. Women who smoked during their treatment of *in vitro* fertilization have an approximately 50% reduction in implantation rate and pregnancy rate compared with women who had never smoked. Also, the incidence of spontaneous abortion was higher in smokers (42.1%) than in non-smokers (18.9%). Furthermore, women smokers have higher frequencies of chromosomally abnormal oocytes and zygotes than nonsmokers. It has been demonstrated that constituents of cigarette smoke such as cotinine, a metabolite of nicotine, and cadmium are significantly increased in ovarian follicular fluids in smokers. In Arizona, approximately one-third of the women of reproductive age smoke cigarettes, which represents a significant health problem. The mechanisms of the deleterious effects of cigarette smoking on infertility are still unknown.

Establishment of successful pregnancy depends on good oocyte quality, which is determined by the healthy status of ovarian follicles. Our preliminary data have shown that productions of steroid hormones secreted by ovarian follicular granulosa cells and expression of cell death-related genes such as *fas* are related to oocyte quality, fertilization rate and embryo quality. The hypothesis of the proposed study is that smoking induces follicular atresia by interrupting hormone secretion and by accelerating programmed cell death in granulosa cells. Three aims will be focused to compare smokers with non-smokers at the 1) functional level, to analyze concentrations of steroid hormones and insulin-like growth factors in ovarian follicular fluids; 2) gene expression level, to characterize the gene expression profile of programmed cell death (apoptosis) in the ovarian granulosa cells; and 3) protein level, to detect the corresponding proteins expressed, as determined in the second aim. Further, results generated from these three aims will be compared to the patient information including oocyte quality, fertilization rate and embryo quality for both smokers and non-smokers. The long-term goal of these studies is to characterize smoking-induced molecular markers on the granulosa cells, which can indirectly predict oocyte quality for women smokers. Availability of such information will be of considerable value when counseling women regarding either preservation of their reproductive health or enhancement of their changes to conceive.

# SECTION G

## ANTICANCER DRUG DISCOVERY

## PROPOSALS RECEIVED

## FY 2001

| | | | |
|---|---|---|---|
| Ebbinghaus | University of Arizona | Triplex DNA-based Gene Therapy for Lung Cancer | $50000<br>50000 |
| Garcia | University of Arizona | A Phase I Dose-toxicity Trial of a Novel Cyclooxygenase II Inhibitor for the Prevention of Cervical Cancer | $199867<br>146710 |
| Guerriero | University of Arizona | The Stress Protein Hsp70 as a Target for Anticancer Therapy | $49801<br>49785 |
| Gunatilaka | University of Arizona | Discovery, evaluation and Development of Anti-cancer Drugs from Rhizosphere Microflora of Desert Plants | $220000<br>220000 |
| Hersh | University of Arizona | Treatment of Brain Tumors with Glioblastoma-derived Antigens Pulsed into Dendritic Cells or Dexosomes | $137500<br>137500 |
| Hoffman | University of Arizona | Advancement of Natural Products as Antitumor Drugs. | $198899<br>199349 |
| Hurley | University of Arizona | Et 743-Duplex DNA Adducts As Therapeutic Agents and Molecular Lures | $149677<br>149677 |
| Joshi | Arizona State University | Discovery and Development of Novel Plant-derived Therapeutics | $200000<br>200000 |
| Lukas | Barrow Neurological Institute | Novel Drug Treatments for Cerebellar Medulloblastoma and Small Cell Cancer of the Lung | $27500<br>27500 |
| Mash | University of Arizona | Rational Design and Production of Anticancer Drugs that Bind Cytosolic Akt | $200000<br>200000 |
| Pettit, G | Arizona State University | Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs | $400000<br>400000 |

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| Pettit, G | Arizona State University | Fast-track Development to Clinical Trials of New Anticancer Drugs | $349965<br>349965 |
| Pettit, R | Arizona State University | Novel Anticancer Agents from Soil DNA Libraries | $400000<br>400000 |
| Skibo | Arizona State University | Synthesis and Evaluation of New Antitumor Agents | $194578<br>178182 |
| Torrence | Northern Arizona University | 2-5A-Antisense for the Targeted Destruction of Cancer RNAs | $49993<br>49999 |
| Whitesell | University of Arizona | Heat Shock Proteins as Targets for Drug Discovery | $181599<br>185637 |
| Yang | University of Arizona | Camptothecin Anticancer Drug Target Interactions and the Consequent Rational Drug Development | $49940<br>49940 |
| Zhang | University of Arizona | Preclinical Development of Sulforaphane for Skin Cancer Prevention | $199885<br>199555 |

# SECTION H

## MEDICAL RESEARCH

## PROPOSALS RECEIVED

## FY 2001

| | | | |
|---|---|---|---|
| Adams | Arizona State University | Assessing the Possible Link Between Autism and Heavy Metal Toxicity | $149398 |
| Ahmad | University of Arizona | Effect of Tobacco Smoking on HIV-1 Pathogenesis and Disease Progression in Mothers and Infants Following Perinatal Transmission | $50000<br>50000<br>50000 |
| Ahmad | University of Arizona | Molecular Mechanisms of Pediatric AIDS Pathogenesis | $150000<br>150000<br>144280 |
| Ahmad | University of Arizona | Effect of Nicotine on HIV-1 Infection | $50000<br>50000<br>50000 |
| Ahmad | University of Arizona | Effect of Smoking on the Molecular Mechanisms of AIDS Pathogenesis | $150000<br>150000<br>150000 |
| Akporiaye | University of Arizona | Improvement of Dendritic Cell Cancer Vaccines by Elimination of a Tumor Derived Cytokine | $50000<br>50000<br>50000 |
| Appleton | Mayo Clinic Scottsdale | Tobacco Use and Cardiac Endothelial Dysfunction in Women, Treatment of Syndrome X | $14982<br>15432 |
| Backhaus | Arizona State University | A New Antioxidant Enzyme Therapy for Trauma-Induced, Ischemia-Reperfusion Injury | $49542<br>46473 |
| Baldwin | University of Arizona | Disruption of Cell-Cell Communications to Block Bacterial Pathogenicity | $50000<br>50000<br>50000 |
| Barker | University of Arizona | Human Papilloma Virus as a Predictor of Recurrent/Persistent Cervical Dysplasia after LEEP | $149301<br>149425<br>110419 |

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| Bernstein | University of Arizona | Delay of Aging Disorders by NAD+ Precursors Through Regulation of Antioxidant Enzymes, DNA Repair Enzymes and Survival/Apoptosis Pathways | $150000<br>150000<br>150000 |
| Bernstein | University of Arizona | Interactive Biologic Effects of Smoking Components (Benzo (a) pyrene, Nicotine) and Dietary Factors (Bile Acids) as Early Indicators of Progression Towards Gastrointestinal Malignancy | $300000<br>300000<br>300000 |
| Brown | University of Arizona | Alteration of Placental Structure and Immune Function by Maternal Smoking, Molecular Mechanisms | $149892<br>149709<br>149894 |
| Brown | University of Arizona | Role of Polyunsaturated Lipids in Cognitive and Cardiovascular Function and Dysfunction | $49995<br>49995<br>49995 |
| Burgess | University of Arizona | Use of Bronchial Epithelial Cells to Measure Genotoxic Effects of Cigarette Smoke | $149990<br>149701<br>149951 |
| Conroy | University of Arizona | Motor Vehicle Related Injury and Death to Arizona Children | $49888 |
| Coons | St. Joseph's Hospital | The Microangiopathy of Smoking in Nerve: An Additional Cause of Peripheral Nerve-Disease | $49999<br>49999 |
| DeLuca | University of Arizona | Effect of Nicotine of T Cell Development | $149674<br>149674<br>149674 |
| DeLuca | University of Arizona | Effect of Antenatal Tobacco Smoke Exposure on Human T Cell Development | $144872<br>144872<br>144872 |
| DeLuca | University of Arizona | Nicotine and Human Cellular Immune System Development | $408357<br>416391<br>430546 |

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| Denton | University of Arizona | A Unique Mass Spectrometer for Biomedical Studies | $66338<br>64812<br>64776 |
| Dixit | University of Arizona | A Preventative Model for Type 2 Diabetes and Renal Disease in Children, Focus on Obesity | $219219<br>212815<br>213640 |
| Fajardo | St. Joseph's Hospital | Local Steroid Use for the Prevention of Chronic Lung Disease (CLD) | $48870<br>49586<br>49879 |
| Flink | University of Arizona | Mechanisms of Cell Cycle Control, Axolotl Heart Regeneration | $49673<br>49664<br>49654 |
| Freeman | University of Arizona | Depression in Smokers and Nonsmokers, Omega-3 Fatty Acid Status and Pilot Treatment Study | $49941<br>49941<br>49941 |
| Friedman | University of Arizona | Survival of Mycobacterium Tuberculosis within Macrophages | $150000<br>150000<br>150000 |
| Futscher | University of Arizona | Transcriptional Repression as a Mechanism of Maspin Gene Inactivation in Breast Cancer | $46200<br>46200 |
| Galgiani | University of Arizona | Antigen 2/PRA in Vaccine Prevention of Diseases Caused by Coccidioides Immitis | $143551<br>98481<br>116894 |
| Gandolfi | University of Arizona | Mechanisms of PSA Regulation in Human Prostate | $128469<br>105674<br>109077 |
| Garland | University of Arizona | Active Immunotherapy of Lung Adenocarcinoma by Immunization with Vaccinia Virus Encoding the MUC-1 Tumor Antigen and IL-2 plus Concurrent GM-CSF | $149481<br>149481<br>149481 |

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| Glass | University of Arizona | Molecular Basis for the Role of Selenium in Biology | $50000<br>50000 |
| Gmitro | University of Arizona | Development and Clinical Evaluation of a Confocal Microendoscope | $50000<br>50000<br>50000 |
| Graves Jr. | Arizona State University West | The Impact of Nicotine on the Genetics of Aging II, Pleiotropy, Genetic Costs of Resistance and Fitness Consequences | $50000<br>50000<br>50000 |
| Grimes J. | University of Arizona | Designing Peptides for Stimulating Anticancer Immune Responses | $50000<br>50000<br>50000 |
| Guerriero | University of Arizona | Induction of Cell Death in Tobacco Related Cancers by a Novel Protein | $49801<br>49785<br>49785 |
| Gunatilaka | University of Arizona | Discovery, Optimization of Production and Evaluation of Novel Anticancer Drugs from Rhizosphere Microflora of Desert Plants | $195664<br>180514<br>187419 |
| Hamann | SmartPractice | Effect of Different Recall Modalities and Messages on Oral Health Maintenance and Oropharyngeal Cancer Screening Visits in Dental Practice in Arizona | $57442<br>17325 |
| Hartshorne | University of Arizona | Interaction of Campylobacter Jejuni with Host Cells | $141515<br>144345<br>147232 |
| Joens | University of Arizona | Characterization of Campylobacter Jejuni Genes Preferentially Expressed *In Vivo* | $49000<br>49000<br>49000 |
| Johnson | Arizona State University | The Effect of Cigarette Taxes on Maternal Smoking and Birth Weight | $49023<br>31225 |

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| Johnson | St. Joseph's Hospital | Nicotine Receptor Polymorphisms | $43582<br>44759<br>45984 |
| Johnson | St. Joseph's Hospital | Genetics of Familial Craniofacial Pain | $50000<br>50000<br>50000 |
| Joyce | Sun Health Research Institute | Smoking and Parkinson's Disease, Neuroprotectant or Artifact | $150000<br>150000<br>150000 |
| Katsanis | University of Arizona | Heat Shock Proteins as Stimulators of Anti-tumor Responses | $50000<br>50000<br>50000 |
| Kern | University of Arizona | The Mechanism of Post Resuscitation Myocardial Dysfunction, Potential Role of Inducible Nitric Oxide | $50000<br>50000<br>50000 |
| Kling | University of Arizona | Defining the Spectrum of Iron Status in Neonatal Rats | $150000<br>150000<br>150000 |
| Lake | University of Arizona | Initiating Immunity to Coccidioides Immitis, A Pulmonary Pathogen | $50000<br>50000<br>50000 |
| Lantz | University of Arizona | The Role of Inflamation in Chronic Obstructive Pulmonary Disease | $150000<br>150000<br>150000 |
| Lee | University of Arizona | Inoculating Young Adults Against Cigarette Advertising | $100324<br>112338<br>108605 |
| Lisse | University of Arizona | Osteoporosis and Smoking Cessation, An Intervention Study | $244410<br>181001<br>183728 |

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| Macia | Arizona State University | A Study of the Effect of Controlled Oxygen Delivery on Retinopathy of Prematurity | $49986<br>49959<br>49995 |
| Manciet | University of Arizona | The Use of Guided Imagery in Improving Patient Outcomes After Heart Surgery | $50000<br>50000<br>50000 |
| Manseau | University of Arizona | Molecular Biology and Anti-cancer Therapeutics of the Thioredoxcin/ P13 Kinase Signaling Pathway | $150000<br>150000<br>150000 |
| Massia | Arizona State University | Local Gene Therapy Targeting Vascular Graft Hyperplasia | $49999<br>49999<br>49999 |
| McDonagh | University of Arizona | Cardiopulmonary Mechanisms Underlying the Severe Cardiac Ischemic Injury Observed Following Exposure to Environmental Tobacco Smoke | $116321<br>110945<br>114274 |
| Miller | University of Arizona | Pharmacokinetics of Bupropion and Nicotine in Breast Milk and the Neonate | $49500<br>49997 |
| Miller | University of Arizona | Smoking in Pregnancy, The Role of Apoptosis | $48475<br>49786<br>49662 |
| Monroy | Northern Arizona University | Toxoplasma Gondii Oral Infection, Role of the Autonomic Nervous System | $34762<br>26820<br>27490 |
| Nagle | University of Arizona | Establishment of a Cancer Tissue and Serum Bank in Arizona for the Purpose of Improving Life for Men with Prostate Cancer | $131520<br>131520<br>131520 |
| Nakazato | University of Arizona | Development of an Arizona Organ Donor Protocol for Use of Dimethyl Sulfoxide to Improve Organ Function in Liver Transplantation | $149613<br>147686<br>135067 |

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| Olson-Garewal | University of Arizona | Traditional Navajo Medicine and Diabetes | $128627<br>91752 |
| O'Rourke | University of Arizona | Integrated Epidemiological Study of Valley Fever | $219797<br>242414<br>228128 |
| Panitch | Arizona State University | Bioresponsive Self-assembling Dextran-based Blood Substitutes for Trauma Care | $50000<br>50000<br>50000 |
| Pettit | Arizona State University | Development of New Anticancer Drugs for Improving Treatment of Tobacco Related Human Cancer | $100000<br>100000<br>100000 |
| Pettit | Arizona State University | Discovery and Development of Novel Anti-infective Agents | $149985<br>149974<br>149985 |
| Pierotti | Northern Arizona University | Role of Mechanical Load in Activating Satellite Cells | $45943<br>47931<br>47931 |
| Powis | University of Arizona | Thioredoxin Peroxidase, A Novel Mechanism for Protection Against Lung Toxicity | $50000<br>50000<br>50000 |
| Reid | University of Arizona | A Pilot Study of Lung Cancer Prevention with Selenium Supplementation | $128620<br>136289<br>103724 |
| Roe | University of Arizona | A Multicenter Study of the Effect of Smoking on Adenomatous Polyp Recurrence | $143254<br>145794<br>148376 |
| Roeske | University of Arizona | Agonist Specific Regulation of the Human Delta Opioid Receptor | $49500<br>49500<br>49500 |

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| Romanovsky | St. Joseph's Hospital | Nicotinic Anti-inflammatory System | $121298<br>125407<br>129690 |
| Romero | University of Arizona | Culturally Appropriate Tobacco Prevention for Youth | $49678<br>45223<br>28354 |
| Rowe | University of Arizona | Disequilibrium Mapping of Nicotine Dependence | $50000<br>50000<br>50000 |
| Sadrzadeh | University of Arizona | Tobacco Smoke Activates Neutrophils and Increases Oxidative Damage in Maternal and Fetal Blood | $133681<br>120469<br>122244 |
| Scheck | St. Joseph's Hospital | The Role of RIG ,Regulated in Gliomas, in the Therapy Resistance of Human Malignant Gliomas | $50000<br>50000<br>50000 |
| Scheck | St. Joseph's Hospital | Characterization of Cells Selected for Resistance to Temozolomide and BCNU in Human Malignant Gliomas | $50000<br>50000<br>50000 |
| Schram | University of Arizona | Biomarkers of Systemic Fungal Infections | $93067<br>92198<br>93150 |
| Schumacher | University of Arizona | Apoptosis of Hematopoietic Stem Cells in Umbilical Cord Blood | $49940<br>49940<br>49940 |
| Shapiro | University of Arizona | Smoking Cessation in Pregnancy, Motivational Counseling | $149786<br>148092<br>149288 |
| Sivakumar | St. Joseph's Hospital | Genotype and Phenotype Relationship in Malignant Hyperthermia | $50000<br>50000<br>50000 |

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| Sivakumar | St. Joseph's Hospital | A Double-Blind, Placebo-Controlled, Parallel Group, Pilot Study to Evaluate the Safety and Efficacy of TNFR:Fc (Enbrel) in Patients with Dermatomyositis | $135972<br>143140<br>101685 |
| Torrence | Northern Arizona University | New Antivirals for Virus Respiratory Diseases | $49922<br>49922<br>49939 |
| Trevor | University of Arizona | Generation of Effective Dendritic Cell-Lung Tumor Hybrid Vaccines for the Treatment of Lung Cancer | $115888<br>115151<br>118077 |
| Tubbs | Intrinsic Bioprobes, Incorporated | Proteomic Analysis of Nicotine Receptor Structure and Composition | $127884<br>127977<br>127927 |
| Vaillancourt | University of Arizona | Effects of Environmental biphenyls on the Brain, Cardiovascular, and Immune Systems | $147211<br>148453<br>148321 |
| Vaillancourt | University of Arizona | Analysis of Phospho-proteins Due to Nicotine Withdrawal | $49455<br>49470<br>49471 |
| Van Andel | Northern Arizona University | Evaluating Tobacco Smoke and Chlamydia Pneumoniae as Co-Factors in Atherosclerosis | $39490<br>24530<br>23870 |
| Wang | University of Arizona | Role of Neurokinin Substance P in Pathogenesis of Sidestream Cigarette Smoke | $49990 |
| Wesselius | Carl T. Hayden VA Med. Cntr. | Effect of Cigarette Smoking on Metal Sequestration by Human Alveolar Macrophages | $50000<br>50000 |
| Wilson | University of Arizona | An Integrative Analysis of Altered Membrane Traffic in Human Disease | $150000<br>150000<br>150000 |

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|---|---|---|---|
| Witte | University of Arizona | Rodent Model of ,Post-Mastectomy, Lymphedema, Pathophysiology, Treatment, and Prevention | $47992<br>48951<br>49941 |
| Witte | University of Arizona | Angiopoietin-2 and Lymphatic Development, Links to Lymphedema-Angiodysplasia Syndromes | $49514<br>49985<br>49484 |
| Wu | St. Joseph's Hospital | Role of GABA Receptor in MPP-Induced Dopamine Neuron Degeneration | $49861<br>49899<br>49538 |
| Wu | St. Joseph's Hospital | Function of a4 Nicotinic Acetylcholine Receptors | $148928<br>146876<br>148379 |
| Xia | University of Arizona | Mechanisms of Cigarette Smoking on Human Infertility | $48714<br>48877<br>48862 |
| Yamamura | University of Arizona | The Role of Calcium Calmodulin Dependent Kinase in Adenylyl Cyclase Superactivation upon Chronic Delta Opioid Treatment | $49500<br>49500<br>49500 |

# SECTION I

## INDEX

*Principal Investigators, New Awards and Continuing Contracts*