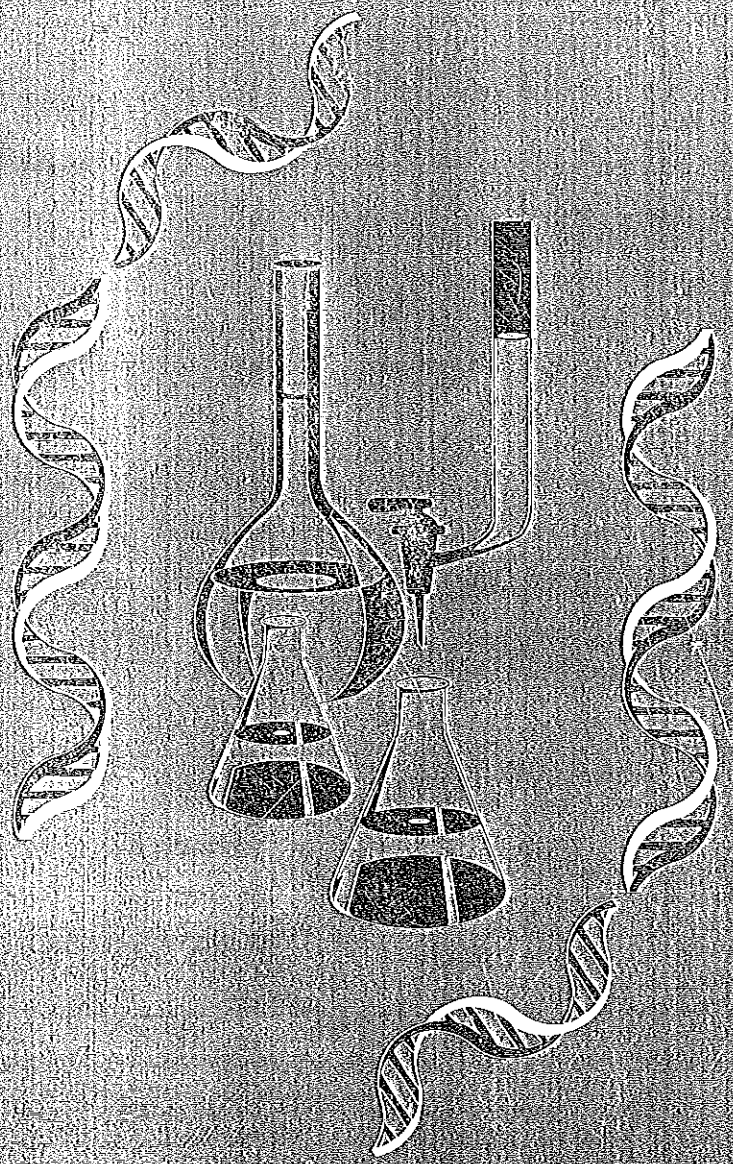
Arizona Disease Control Research Commission



2001 – 2002
Annual Report

January 2003

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
2001 – 2002

Jane Dee Hull, Governor

Henry Reeves, Ph.D., Chairman

COMMISSION MEMBERS

General Public
C. Eileen Bond, J.D.
Lyra McCoy, M.P.H.
Joseph A. Mislove, J.D.

Medical Community
William Crisp, M.D.
Eladio Pereira, M.D.
James L. Schamadan, M.D.

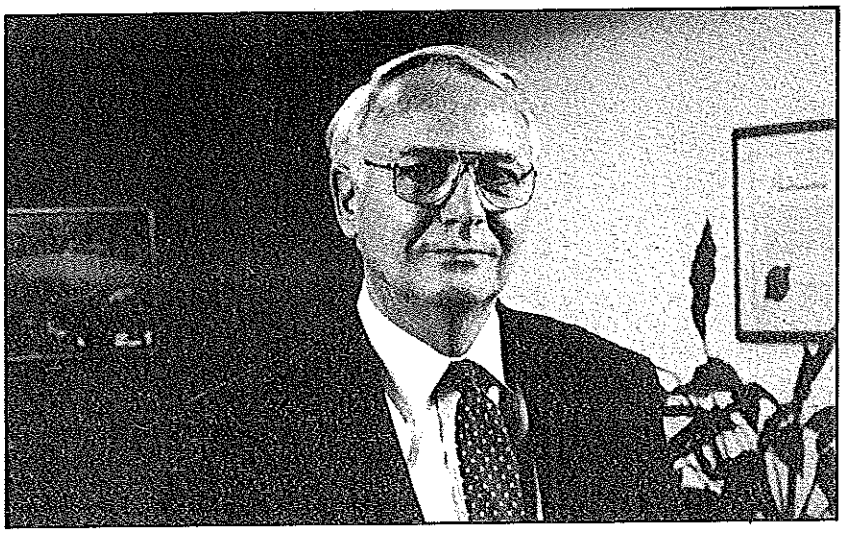
Scientific Research Community
T. Lon Owen, Ph.D.
Henry C. Reeves, Ph.D.
Walter H. Williams, Ph.D., M.D.

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 2003

TABLE OF CONTENTS



Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 2002 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 for 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 Commission awarded three contracts under the Parkinson's Disease Research initiative in March of 2002. The research began in April of 2002 and will continue through March 31, 2004. The Brain research projects were awarded in April and will begin on July 1, 2002. These projects will continue through June 30, 2004 using tobacco settlement funds. One contract received a three-year award and funds to complete the final year will come from the tobacco tax.

The four-year Anticancer Drug Discovery Program ended on June 30, 2002. This program has been very successful in bringing together scientists working on the cancer chemotherapeutic agents and chemotherapeutic regimens for treatment.

The original program set aside $10,000,000 to speed the introduction of promising agents into clinical trials. In all, approximately $11,600,000 was devoted to the program. $800,000 was used to update old equipment including a 20 year-old x-ray defraction unit and to purchase new state-of-the-art equipment including a recombinant DNA workstation and a very sensitive mass spectrometer. This equipment increased the number of samples that could be tested and decreased the time necessary to work out the structures of newly discovered compounds.

Twenty projects were funded during the four year period. Several new classes of compounds

were tested as were two new delivery systems designed to concentrate the drugs known to be effective at the site of the tumor. Three new compounds entered clinical trials either as single agents or to test new combinations of drugs for effectiveness. Two new compounds received patent protection.

These funds enabled the University of Arizona to outfit an entire laboratory complex devoted to natural compounds with anticancer activity found in desert micro-ecosystems. In all, nine new promising leads have come from this single laboratory.

One of the most important developments to emerge from this program has been the cooperation between institutions resulting in quarterly meetings held by the cancer researchers who are working on anticancer drug development. Presentations about on-going research have fostered collaborations between the universities and other institutions within Arizona. The result has been a strengthened basic science platform and an expanded clinical trials network to make experimental therapies available to patients throughout the state.

This program has been very successful in assisting researchers to compete successfully for federal funds. In the first three years our researchers have reported receiving 36.2 million dollars from federal and other outside funding agencies as a result of receiving state funds. Cancer is not a single disease, but many, and Arizona's effort has made a significant contribution to the cancer therapeutics knowledge base.

In October 2001 the ADCRC and the Flinn Foundation sponsored their second joint symposium entitled *Bridging Disciplines: The Key to Biomedical Research and Economic Development in the 21st Century*. Michael Finney, Vice President, The Michigan Economic Development Corporation was the keynote speaker. The symposium provided an opportunity for university, corporate and legislative leaders to learn about how the state of Michigan has become a biotechnology leader. This information was invaluable as we worked with a joint task force made up of members from the public, private and educational sectors to bring the Translational Genomics Research Institute (TGen) to Arizona. The Senate, under the leadership of the Honorable Susan Gerard, Chairman of the Health Committee, committed $500,000 per year for ten years to help support this project. One Commission member will serve on the TGen board of directors. The ADCRC was also named in a ballot proposition prepared by the legislature which, if passed, would raise the tobacco tax and commit additional funds for research programs and TGen.

The Commission continues to be involved in technology transfer and the patenting and licensing of discoveries funded with ADCRC monies. The Commission filed for two new patents this year in the areas of cancer drug and monoclonal antibody development. We are awaiting the issue of a third patent on compounds that may prove useful in the fight against AIDS.

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 look forward to another productive research year.

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 2001 – 2002 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 and a third term in 2002. His term will expire in May 2005.

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.

Lyra McCoy, M.P.H. — Mesa
Program Administrator,
Governor's Division of Drug Policy

Commissioner McCoy received her Masters Degree in Public Health with a specialization in Health Education and Promotion from the University of Arizona. Commissioner McCoy has been the Program Administrator for the Governor's portion of the Safe and Drug Free Schools and Communities program from the U.S. Department of Education for the past four years. The Governor's portion funds comprehensive science-based programs to provide preventive education in the areas of substance abuse and violence among youth throughout the state. Commissioner McCoy works closely with the statewide Governor's Alliance Against Drugs certification project and the Governor's Youth Commission. She is also involved in various projects within the Division of Drug Policy including the Arizona Program Design and Evaluation Logic Model efforts. Prior to joining the Governor's Office, Commissioner McCoy worked for the American Cancer Society and the Arizona Program for Nicotine and Tobacco Research in tobacco prevention with youth and tobacco policy change initiatives. She was appointed to the Commission in 2001 by Governor Hull, and her term expires in May 2004.

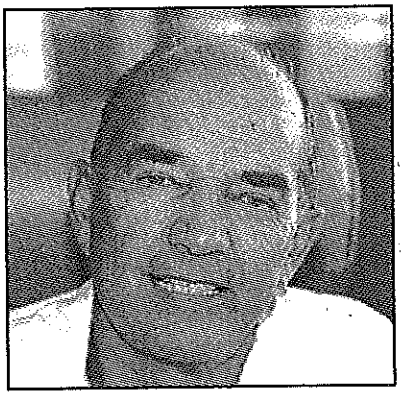
General Public

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 expired in May 2002.

Medical Community

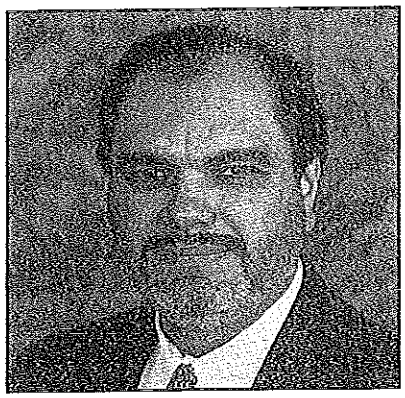
William Crisp, M.D. — Paradise Valley
Resident Education Gynecology/Oncology, Samaritan Regional Medical Center



Commissioner Crisp received his M.D. degree from George Washington University College of Medicine. He is Board Certified in Obstetrics and Gynecology and holds an Advanced Certificate in Gynecology Oncology. He also serves as an Adjunct Professor in the Cancer Research Institute and the Bioengineering Department at Arizona State University. He is the author of more than one hundred scientific publications and has served as President of both the Maricopa County and Arizona Medical Associations. Commissioner Crisp was first appointed to the Commission by Governor Mofford in 1988 and was reappointed by Governor Symington in 1991. He left the Commission in 1994 and was again appointed to the Commission in 2001 by Governor Hull. His term expires in May 2004.

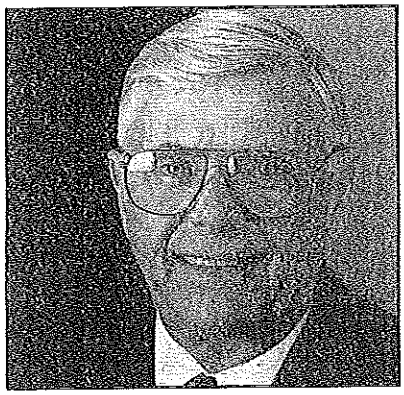
Medical Community

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



Commissioner Pereira received a 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, he joined the staff of Mariposa Community Health Center as a Scholar of the National Health Service. He returned to Emory University in 1990 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 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 the Commission in 1999 and reappointed him in 2002. His term will expire in May 2005.

James L. Schamadan, M.D. — Scottsdale
Special Assistant to the Governor for Homeland Security



Commissioner Schamadan received his M.D., *cum laude* from Ohio State University. He is Board Certified in Occupational and Environmental Medicine, and is a graduate of the Management Institute, University of California at Berkeley. He was a medical officer in Vietnam. He returned to join the Engineering faculty at Arizona State University where he developed the nation's prototype air ambulance system. In 1974 he was appointed as the first Director of the Arizona Department of Health Services. Commissioner Schamadan spent over a decade as President and Chief Executive Officer of the Scottsdale Memorial Hospitals where he shaped the growth of a new community health and hospital system. He has published numerous scientific articles and co-authored two textbooks. He is a recognized authority on heat stress disease. He was a member of the American Voluntary Medical Team on its humanitarian mission to Kuwait during Desert Storm. He was appointed to the Commission by Governor Hull in 2002, and his term expires in May 2005.

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, reappointed in 2001, and his term expires in May 2004.

Walter Williams; Ph.D., M.D. Tucson
Professor, Department of Nuclear Medicine
and 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 May 2003.

Summary of 2001-2002 Commission Activities

The Commission had 64 contracts in four programs—tobacco-related research, anticancer drug discovery, unrestricted medical research and Parkinson's disease research—with medical researchers in Arizona as of July 2001. In addition to the regular Commission programs, ADCRC will be supporting the Translational Genomics Research Institute with a $500,000 annual award for a period of ten years. 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 medical research and brain research projects awarded in 2002 can be found in Section E. Lay summaries for new unrestricted medical research projects and Parkinson's disease research are found in Section F. The medical research projects begin in July, FY 2003 while the Parkinson's disease research projects began on April 1, FY 2002. The summaries provide an overview of the new research.

Approximately 696 Requests for Proposals (RFPs) for 2002-2003 awards were mailed to potential applicants in September 2001. The amount available for new unrestricted medical research was approximately $1,800,000 and an additional $1,000,000 was available for Parkinson's disease and brain research. In response to the RFPs, the Commission received 5 Parkinson's disease research proposals and 120 unrestricted medical research proposals. Section G lists the research proposals received in response to each RFP.

In November and December the medical research proposals were sent to a panel of national and international scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more than 120 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 2002 the Commission selected 17 proposals for funding. Parkinson's disease research proposals were evaluated by a four-person panel of experts who made their recommendations to the Commission after interviewing each of the 5 principal investigators. The Commissioners selected 3 proposals to receive contract awards. The number of cooperative projects funded increased again this year resulting a smaller number of contracts selected with increased funding going to individual projects. During 2002-2003 the ADCRC will be managing 64 contracts.

SECTION A

CONTINUING CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR TWO

David Bearss, Ph.D.

University of Arizona
FY 2002: $43,439

The 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 a better understanding of the disease through the use of animal models. In this study we have focused on the development of a transgenic mouse model of pancreatic cancer by driving the expression of cancer causing genes in the pancreas. These models have been characterized and are being further evaluated for their potential as predictors of human pancreatic cancer. We hope, through continuing with this study, we will be able to better understand this terrible disease and make new progress against it.

Eugene W. Gerner, Ph.D.

University of Arizona
Award Amount FY 2002: $150,000

Interdisciplinary Basic Science Program in Colon Carcinogenesis

During the past year, we have treated Min (multiple intestinal neoplasia) mice with tobacco-specific carcinogens to evaluate the effects of these agents on intestinal carcinogenesis. To test the hypothesis that these carcinogens are affecting intestinal vascular function, we have created a novel model of intestinal carcinogenesis by crossing Min mice with nitric oxide synthase 2 (NOS2) knockout mice. We have found that NOS2 influences intestinal tumor formation in a manner dependent on dietary arginine levels. Gene expression profiling studies have been conducted in both Min and Min X NOS2 (-/-) mice. We have found that the expression of a number of novel genes associated with cell turnover (especially apoptosis) and angiogenesis are altered in Min mice. Some of these are NOS2-dependent and others are NOS2-independent. Our results underscore the importance of arachidonic and amino acid metabolism in processes leading to intestinal tumor function in mice. These data support previous work from our group identifying genes in these metabolic pathways as excellent targets for intestinal, including colon, cancer prevention and treatment.

Publications:

Fultz KE, Gerner EW. APC-dependent regulation of ornithine decarboxylase in human colon cancer tumor cells. **Molecular Carcinogenesis** 34:10-18, 2002.

Gerner EW, Ignatenko NA, Fultz KE. Inducible gene expression strategies for applications in gene therapy I. In: *Pharmaceutical Perspective of Nucleic Acid-Based Therapeutics* (SW Kim and R Mahato, eds.) **Harwood Academic Pulishers**, 2002: 15-32.

Gerner EW, Ignatenko NA, Besselsen DE. Preclinical Models for Chemoprevention of Colon Cancer. In: *Recent Results in Cancer Research* (Boyle P, *et al.* eds.) **Springer-Verlag, Heidelberg,** (In Press).

Kramer DL, Gerner EW. Therapeutic strategies targeting polyamines. In: *Cancer Chemoprevention. Volume 1. Promising Cancer Chemopreventive Agents.* (GJ Kelloff, ET Hawk, CC Sigman, eds.) **The Humana Press, Inc.**, (In Press).

Lawson KR, *et al.* Toxicity of the polyamine analog CHENSpm is modulated by the FAD-dependent polyamine oxidase. **Clin Cancer Res** 8:1241-1247, 2002.

Martinez JD, *et al.* The molecular biology of cancer. In: *Burger's Medicinal Chemistry and Drug Discovery* (6^{th} edition) (D. Abraham, ed.) **Wiley**, (In Press).

Quinones H, List A, Gerner EW. Selective exclusion by the polyamine transporter as a mechanism for the differential radioprotection of amifostine derivatives. **Clin Cancer Res** 8: 1295-1300, 2002.

Taylor PM, Gerner EW. Posttranscriptional regulation of COX-2 by genetic and intestinal luminal risk factors in colon tumor cells. **Molecular Carcinogenesis** (Accepted with Revisions).

Taylor Parker M, Gerner EW. Polyamine-dependent posttranscriptional regulation of COX-2. **Biochimie** (In Press).

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY 2002: $144,871

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

C-myc is an important oncogene in human cancer. In previous studies we have shown that we can target the transcriptional regulatory, or switch, regions of this gene to shut off synthesis. We have now demonstrated, in mice bearing human tumors that overexpress c-myc, that down-regulation on this gene leads to slowing of tumor growth. During the second year of the award we have explored the structure-activity relationship of a series of c-myc switch molecules. In program 1 we have designed and synthesized a series of compounds and examined their fluorescence and DNA binding activity. In program 2 we have evaluated their G-quadruplex c-myc-interactive properties and selectivity. 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 which could have a direct effect on treatment of cancer in Arizona citizens.

Publications:

Grand CL, *et al.* The cationic porphyrin TMPyP4 downregulates c-MYC and hTERT expression and inhibits tumor growth *in vivo*. **Molecular Cancer Therapeutics** 1:565-576, 2002.

Jean M. Schmidt, Ph.D

Arizona State University
Award Amount FY 2002: $130,587

In vivo Efficacy Evaluation of New Anticancer Drugs

Tests of six anti-cancer drugs, selected because of promising cell culture cytotoxicity results, have progressed in a SCID *in vivo* xenograft system. A very aggressive human large-cell lung tumor, H460, has been used in xenograft to test several drugs, two of which definitely retard tumor development dramatically. Activity of these anticancer drugs has also been examined in human colon cancer xenografts with promising results. The drugs Auristatin PYE and Auristatin 15 PE, both synthetic, are the most effective so far. Pancratistatin prodrug, sodium narcistatin, (both plant products) an aaptamine, derived from a marine sponge; and phenstatin prodrug, a synthetic tubulin inhibitor, are also being examined in xenografts and for cell-cycle inhibition with flow cytometry. Several other SCID xenograft systems have been established for future work including FADU, a human tobacco-related pharyngeal tumor.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY 2002: $138,299

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 two years of work on this project, drugs targeting histological cancer types afflicting many Arizonans, namely leukemia, melanoma and non-small-cell lung cancer, have been developed. Patents are pending on new classes of compounds including cyclopent[b]indoles, indoles, and new DNA recognition agents. Our approach to developing these drugs has been to exploit levels of the reducing enzyme DT-diaphorase in our drug designs. A drug activated by this enzyme can target a cancer possessing high levels of DT-diaphorase such as melanoma and lung cancer. Exciting new developments in the past year include peptide-conjugated drugs that are actively taken up by specific cancers and novel heat shock protein (HSP 90) inhibitors that exploit high levels of this protein in certain cancers such as leukemia. The combination of peptide conjugation and DT-diaphorase activation has resulted in enhanced activity against melanoma and lung cancers.

Henry I. Yamamura, Ph.D.
(Previously Paul F. Consroe, Ph.D.)

University of Arizona
Award Amount FY 2002: $49,522

Molecular Mechanism of Cannabinoid Action at the Human CB1 Receptor

Smoking has been implicated in several forms of cancer including lung cancer. This has major health consequences for the residents of Arizona since about 25% of the state 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 surface known as the cannabinoid CB1 receptor. These receptors interact with G-protein to produce their effects. We have previously demonstrated that a specific G-protein ($G\gamma 2$) appears to be involved in cannabinoid antinociception using antisense technology. We now show that the cannabinoid receptor antagonist SR141716A activates cAMP formation. The molecular mechanism of SR141716A augmentation of cAMP formation is being investigated.

Robert Berg, M.D.

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

Optimal Treatment of Prolonged Ventricular Fibrillation: CPR 1st *vs.* Defibrillation 1st

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. Our dual objectives in the second year of this study were to determine 1) whether a brief period of cardiopulmonary resuscitation prior to defibrillation would improve readiness of the myocardium for defibrillation and ultimate outcome, and 2) whether defibrillation with an automated external defibrillation (AED), which is easy to use by minimally trained individuals, is as effective as a traditional manual defibrillator (those typically used in hospitals). Provision of precountershock CPR (CPR 1st) tended to improve the readiness for defibrillation with either type of defibrillator, but did not improve the outcome. Outcome was far superior with manual defibrillation than with an AED. New approaches to improve outcomes with the increasingly used AEDs deserve further investigation.

Abstracts:

Berg RA, *et al.* Precountershock cardiopulmonary resuscitation improves readiness for defibrillation in a swine model of prehospital ventricular fibrillation. Critical Care Medicine 29 (12):A73, 2001.
31st Critical Care Congress, Society of Critical Care Medicine, San Diego, CA, January 26-30, 2002.

Berg RA, *et al.* Worse outcome with automated external defibrillation in a swine model of prehospital VF cardiac arrest. Critical Care Medicine 29 (12): A73, 2001. 31st Critical Care Congress, Society of Critical Care Medicine, San Diego, CA, January 26-30, 2002.

Berg RA, *et al.* Delay in the provision of chest compressions with the use of an Automated External Defibrillator by EMS personnel after out-of-hospital cardiac arrest. American College of Emergency Physicians, Seattle, WA, October 7-8, 2002.

Xavier LC, *et al.* Superior outcome with immediate manual defibrillation *versus* automated external defibrillation in a swine model of prehospital ventricular fibrillation cardiac arrest. JACC 39:282A, 2002.

Qin Mary Chen, Ph.D.

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

Molecular Mechanisms of Oxidant and Nicotine Induced Cardiac Toxicity

We study the synergistic effect of oxidants and nicotine in inducing cardiomyocyte hypertrophy. Measurements of gene expression failed to find an increase in βMHC with oxidants plus or minus nicotine. The data on ANF gene are somewhat inconsistent and are perhaps largely affected by the dose of oxidants. Three branches of MAP kinases are activated by oxidants ERKs, p38 and SAPKs. Nicotine did not activate these kinases or enhance the activation of these kinases by oxidants. Using a commercially available assay, we could not detect a significant increase of calcineurin activity by oxidants. The reporter gene assay showed 2-3 fold induction of NF-AT3 promoter activity by oxidants, but the data needs to be verified by gel shift assay. It is possible that the endogenous level of calcineurin and NF-AT3 in cultured cardiomyocytes are too low for the conventional assay. Alternative approaches are necessary for obtaining definitive data.

Publications:

Chen QM, *et al.* Molecular mechanisms of cardiac hypertrophy induced by toxicants. Cardiovasc Toxicol 1:267-283, 2001.

Tu VC, Bahl JJ, Chen QM. Signals of oxidant induced cardiac myocyte hypertrophy: Key activation of phosphatidylinositol 3 kinase and p7086 kinase. J Pharm Exp Therap 300:1101-1110, 2002.

Chen QM and Tu VC. Apoptosis and heart failure: mechanisms and therapeutic implications. Am J Cardiovasc Drugs 2:43-57, 2002.

Abstracts:

Tu VC, Chen QM. Distinct roles of MAPKs and P13K in oxidant induced hypertrophy. Toxicologist 61:A#469.

Janet L. Funk, M.D.

University of Arizona
Award Amount FY 2002: $105,135

Investigation of a Novel Neuroprotective Agent in Stroke

Although stroke is a major cause of death and disability in Arizona, little progress has been made in preventing the neurological defects caused by strokes. As research begins to unravel the processes leading to nerve death in stroke, it has become apparent that these destructive forces might be interrupted by harnessing the body's own defense systems. Our laboratories have now identified a new substance that is produced by the brain in response to stroke. This protein, which is produced by blood vessels in injured brain, is able to increase the size of blood vessels thus allowing more blood to flow. Initial treatment studies suggest that this protein protects injured brain. Future experiments are planned to find out whether treatment with this protein soon after the onset of stroke (ie. when people come to the emergency room with strokes) can limit brain injury and, thus, be used as a new treatment.

Abstract:

Ritter L, *et al.* Parathyroid Hormone-related protein (PTHrP) induction after permanent middle cerebral artery occlusion: A vasodilator with neuroprotective effects. Society for Neuroscience, 2002, (Accepted).

Ronald J. Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY 2002: $360,943

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

This program of three separate research projects seeks to establish 1) 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; 2) whether nicotinic acetylcholine receptors (nAChR), which are targets of tobacco nicotine action and critically involved in natural chemical signaling throughout the body, are on micro-vessels or other components of the BBB; and 3) some features of nAChR that are involved in BBB function and are likely to contribute to effects of stroke. A clinical component of the project which was to establish effects of tobacco use and nicotine exposure on incidence, severity, progression, and treatment of stroke, and on recovery from stroke was terminated due to low subject enrollment. The broad thesis of the research program is that tobacco use and nicotine exposure affect the structure and transport characteristics of the BBB, and that these effects have deleterious consequences during stroke.

Studies done in the second year continue to build evidence for expression of nAChR on blood vessels that constitute the BBB and affect BBB function. This evidence comes from studies of human micro-vascular endothelial cells grown in cell culture and histochemical staining of human or rodent brain tissues for nAChR subunit gene expression as messenger RNA, nAChR, subunits as immunoreactive proteins, and nAChR complexes as radioligand binding sites. Studies using bovine or rodent samples also identify effects of nicotine exposure on molecular elements involved in vessel formation and show evidence of nAChR expression. Collectively, data obtained to date is consistent with the project's central hypothesis that nicotine exposure alters BBB and brain micro-vascular function. This indicates important roles for nAChR in these effects. There is promise that these effects can lead to better understanding and improved strategies for prevention and treatment of stroke. Insight might also be gained into how to regulate function of the BBB, perhaps through manipulation of nAChR signaling, to facilitate therapeutic drug access to the brain.

Abstracts:

George AA, *et al.* Radioligand binding autoradiography and *in situ* hybridization to detect nicotinic acetylcholine receptors and their subunit transcripts in cell lines and mouse brain. Soc Neurosci A27:980, 2001.

Hawkins BT, *et al.* Rat cerebral microvessels express nicotinic acetylcholine receptors. Soc Neurosci A28, (In Press).

John W. Regan, Ph.D., M.D.

University of Arizona
Award Amount FY 2002: $49,621

FP Prostanoid Receptor Isoforms in Human Heart Disease

The goal of this project is to discover if isoforms of the FP prostaglandin receptor exist in humans and to determine if they are expressed in the heart. Prostaglandin receptors that are activated by $PGF_{2\alpha}$ are called FP receptors and two variants, or isoforms, are known to exist in sheep. Prostaglandins, such as $PGF_{2\alpha}$, are linked to cardiac hypertrophy; therefore, to understand the role of $PGF_{2\alpha}$ in this process, it is important to know if FP receptor isoforms also exist in humans. In our previous progress report we described the successful cloning of a novel human FP receptor isoform that we named "FP_s." During the period of the present report we have been successful in the preparation of antibodies that specifically recognize this receptor. We have used these antibodies to show that FP_s is selectively expressed in endothelial cells of the human placenta. The location of FP_s in endothelial cells suggests a role for this receptor in cardiovascular function.

Publications:

Fujino H, Srinivasan D, Regan JW. Cellular conditioning and activation of β-catenin signaling by the FPB prostanoid receptor. **J Biol Chem**, (In Press).

Srinivasan D, Fujino H, Regan JW. Differential internalization of the prostaglandin $F_{2\alpha}$ receptor isoforms: Role of protein kinase C and clathrin. **J Pharmcol Exp Ther** 302:219-224, 2002.

Weber TJ, Markillie LM, Chrisler WE, Vielhauer GA, Regan JW. Modulation of clonal selection processes by the prostaglandin $F_{2\alpha}$ receptor. **Mol Carcinogenesis**, (In Press).

D. Larry Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY 2002: $126,000

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

Increased circulating cholesterol is known to promote risk of coronary artery disease. It is now emerging that cholesterol promotes production and accumulation of amyloid β (Aβ) deposited in the hallmark pathologic lesion of Alzheimer's disease (AD), the senile plaque, perhaps by shifting away from normal metabolism of amyloid β protein precursor (AβPP) to A3. Previous studies employing the cholesterol-fed rabbit model of AD demonstrated that induction of AD-like Aβ accumulation in brain could be reversed by co-administration of cholesterol lowering drugs or removing cholesterol This finding prompted the initiation of an AD Cholesterol-Lowering (Statin) Treatment Trial. We now present data that identify a previously unrecognized role for dietary water quality on the severity of neuropathology induced by elevated cholesterol.

Neuronal accumulation of Aβ induced by increased circulating concentrations of cholesterol in the New Zealand white rabbit is attenuated when distilled drinking water is administered compared to use of tap water. The numbers of neurons in cholesterol-fed rabbits that exhibited Aβ immunoreactivity, relative to normal chow-fed controls, increased ~2.5 fold among animals on tap water but only ~1.9 fold among animals on distilled water. This yielded a statistically significant ~28% reduction due to the use of distilled water. In addition, the subjectively assessed intensity of neuronal Aβ immunoreactivity was consistently reduced among cholesterol-fed rabbits allowed distilled drinking water compared to cholesterol-fed rabbits on tap water. As intensity of antibody immunoreactivity is likely related to concentration of antigen, the identified difference among cholesterol-fed rabbits allowed distilled drinking water may hold greater importance than a significant reduction in numbers of affected neurons. The effect on neuronal Aβ immunoreactivity intensity was observable among cholesterol-fed rabbits reared and allowed tap water when performing studies in three distinct locales. Pilot data suggest the possibility of increased clearance of Aβ from the brain, identified as increased blood levels among cholesterol-fed rabbits administered distilled water compared to animals on tap water. The agent(s) occurring in tap water excluded by distillation, promoting accumulation of neuronal Aβ immunoreactivity is(are) yet undisclosed, but arsenic, manganese, aluminum, zinc, mercury, iron and nitrate have tentatively been excluded because they were not identifiable (below detection limits) in the tap water of the three locales where the cholesterol-induced neuropathologic difference was observable. These findings suggest that water quality may impact on human health in the setting of increased circulating cholesterol levels and could illustrate a truly simple life-style change that could be of benefit in AD.

Edward D. French, Ph.D.

University of Arizona
Award Amount FY 2002: $124,046

Nicotine Dependence and Dopamine Neurons: Electrophysiological and Molecular Studies

During the past year we continued our systematic examination of the effects of nicotine on nerve cells within the brain's reward center. Nicotine acts on nicotinic acetylcholine receptors to alter the excitability of neurons within that portion of the brain known to play a key role in the abuse liability of drugs. The existence of several subtypes of nicotinic acetylcholine receptors provides potential targets for the development of novel therapeutic agents to treat addiction to tobacco containing products. This year we shifted our experimental focus from assessments in the rat to those in the mouse. The mouse shares considerable genomic homology to that of the human providing an ideal model to use to examine the effects of various genetic alterations on nicotine receptor pharmacology. Our approach continues to combine electro-physiological, behavioral and molecular biological techniques toward delineating the effects of acute and chronic nicotine exposure on dopamine and non-dopamine neurons within the brain's pleasure/reward center. We have established that acute nicotine administration in the mouse produces an inhibition of dopamine neuronal activity, an effect also reflected by a reduction of locomotor activity. After seven days of nicotine treatment both the electrophysiological and behavioral effects increase. In the mouse long tern nicotine exposure actually increases neuronal activity in the brain reward center. The effects of nicotine can be prevented by selective pharmacological antagonists. We are presently attempting to identify through molecular biological methods whether the electro-physiological and behavioral changes resulting from chronic nicotine administration result from alterations in the population of the subtypes of nicotinic acetylcholine receptors. We are seeking to identify the cellular sites and mechanisms by which nicotine becomes an addictive substance and the neuronal effects of chronic use. It is our hope that these results will lead to the development of new pharmacological therapies to treat nicotine dependence which affects approximately a quarter of the population of Arizona.

Publication:

Sandoval KE, French ED, *et al.* Exploring the role of neuronal nicotinic acetylcholine receptors on ventral tegmental area dopamine neurons in the ICR mouse: An *in vivo* electrophysiological study. Society for Neuroscience 2002, (Online).

Abstract:

George AA, *et al.* Radioligand binding autoradiography and *in situ* hybridization to detect nicotinic acetylcholine receptors and their subunit transcripts in cell lines and mouse brain. Soc Neuroscience A27:980, 2001.

Hugh Miller, M.D.

University of Arizona
Award Amount FY 2002: $138,137

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

Postpartum relapse continues to be a major source of lost opportunities that encumbers many women with long-term health consequences. The impact on Arizona's women, children and families is undoubtedly dramatic but remains largely unnoticed amidst many other pressing health concerns. We have spent the last year actively enrolling patients from local area hospitals. The trial called for the randomization of postpartum women to standard of care, relapse prevention counseling with a placebo, or bupropion SR. We overcame some initial difficulty in acquiring clinical trial material from GlaxoWellcome and now have well-established procedures that we will use through the end of this trial. Enrollment is nearly complete and long-term follow-up is underway. Completion of this trial will prepare the way for evidence-based interventions directed at postpartum relapse prevention.

James F. Collins, Ph.D.

University of Arizona
Award Amount FY 2002: $49,500

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

Surfactant lines the lungs and allows them to inflate normally. Smoking adversely affects surfactant production and lung function. Surfactant producing cells in the lung intake Pi via a phosphate transporting protein called NaPi-IIb. Our novel data have demonstrated that nicotine and cigarette smoke exposure decrease expression of the NaPi-IIb gene in isolated lung cells and in laboratory rodents, respectively. We surmise that this reduction in gene expression may be related to the smoking-induced perturbations in surfactant production. Other experiments are underway to decipher precisely how the NaPi-IIb gene is regulated by cigarette smoke exposure in transgenic mice created specifically for the purpose. Further proposed experiments will seek to determine the precise role that this gene plays in cigarette smoke exposure related decreases in surfactant production. These studies are highly relevant to the State of Arizona as pulmonary diseases directly related to smoking cost the taxpayers millions of dollars.

Dominick DeLuca, Ph.D.

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

Nicotine Effects on Human Stem Cell Differentiation *In Vitro*

Little work has been done to determine the potential effects 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.

The purpose of the proposed research is to evaluate the effects of nicotine on the developing immune cells–T cells, B cells, and monocytes. We have developed an *in vitro* fetal thymus organ culture (FTOC) system that mimics the growth and differentiation of both human and murine immune cells. In the past year we have shown 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. This study 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 2002: $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 understand the effects of constituents of cigarette smoke on ovarian function. We determined that nicotine decreases production of an important ovarian hormone, andro-stenedione. Since androstenedione is an essential precursor for estrogen synthesis, inhibition of androstenedione production by nicotine may contribute to lower estrogen production and infertility in women who smoke. Our original hypothesis was that nicotine exerts negative effects on ovarian function by a mechanism called oxidative stress. However, we were unable to detect several markers of oxidative stress in ovarian tissues, leading us to question whether this mechanism is active in the ovary. In the next year we plan to test whether nicotine induces atresia (cellular death) in follicles via oxidative stress or by an alternate mechanism. Continued progress on these studies will enhance our understanding of mechanisms underlying the negative effects of smoking on fertility in women.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY 2002: $150,000

Analysis of Autoantibodies to T-cell Receptor in Rheumatoid Arthritis

The study of rheumatoid arthritis (RA) is of particular importance to Arizonan citizens since there is an abnormally high prevalence (5%) of RA in the State of Arizona. We have found that individuals with RA have elevated levels of autoantibodies (lgM class) directed against variable domains of T cell receptors (TCR) and consequently have generated monoclonal autoantibodies (mAAbs) to these Tcr epitopes. We hypothesized that the antibodies might exert a regulatory or protective function and carried out detailed analyses of the antigenic specificity of the molecules and functional studies to test for a direct immunoregulatory role. The mAAbs bound to peptide-defined epitopes of variable domains of TCRα, β, and γ chains as well as to immunoglobulin light chains. Functional *in vitro* studies showed that these human mAAbs decreased the production of the pro-inflammatory cytokine interleukin-2 (iL-2) and were apparently nondestructive to the cells. These results raised the encouraging possibility that mAAbs of this type prove useful as therapeutic agents in human inflammatory autoimmune diseases.

Publications:

Adelman MK, Marchalonis JJ. Endogenous retroviruses in systemic lupus erythematosus: Candidate lupus viruses. Clin Immunol 102:107-116, 2002.

Marchalonis JJ, *et al.* T-cell receptor derived peptides in immunoregulation and therapy of retrovirally-induced immunosuppression. Cri. Rev. Immunol 21:57-74, 2001.

Marchalonis JJ, *et al.* Natural recognition repertoire and the evolutionary emergence of the combinatorial immune system. The FASEB J 16:842-848, 2002.

Robey IF, *et al.* Human monoclonal natural autoantibodies against the T-cell receptor inhibit IL-2 production in murine T cells. Immunology 105:419-429, 2002.

Robey IF, *et al.* Specificity mapping of human anti-T cell receptor monoclonal natural antibodies: Defining the properties of epitope recognition promiscuity. The FASEB J 16:642-652, 2002.

SECTION B

CONTINUING CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR THREE

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 2002: $149,964

Effects of Antioxidant Nutrients and Smoking on Type Specific HPV Persistence

In the US in 1995, approximately 15,800 new cases of invasive cervical cancer were diagnosed and 4,900 women died. Although cervical cancer can be prevented through routine participation in Pap smear screening programs, there are tremendous costs associated with the need for diagnostic follow-up and treatment when abnormalities are found. In Arizona the number of women diagnosed with abnormal Pap smears in the last decade has significantly increased. To decrease health care costs and patient burden, strategies that could prevent cervical dysplasia are needed. These approaches include identifying relevant risk factors, such as nutritional factors and smoking, and modifying these factors to decrease overall cervical dysplasia risk.

Research has definitively shown that infection with the human papillomavirus (HOV) is a cause of most cases of cervical cancer. Although a woman's risk for cervical cancer is 10-20 fold higher if she has HPV infection, HPV infection alone is insufficient to cause cervical cancer. The women at highest risk are those who consistently test positive for HPV infections over time (persistent infection) and have a higher concentration of the virus (viral load).

However, there is little information currently available on what factors allow HPV infections to persist and progress to cervical cancer. Nutritional status, in particular antioxidant nutrients, and smoking may be such factors. Both factors have consistently been associated with cervical dysplasia and cervical cancer. In addition, smoking has been shown in numerous studies to adversely affect antioxidant nutrient status. Therefore, to adequately investigate HPV persistence, nutritional status and smoking status must be simultaneously assessed and considered.

Data resulting from this study have clearly indicated that antioxidant nutrients, such as carotenoids and vitamin E, decrease risk of cervical cancer by decreasing the duration and risk of persistent HPV infections and by increasing the clearance of these infections.

Publications:

Giuliano AR, *et al.* Clearance of oncogenic human papillomavirus (HPV) infections: Effect of smoking. Cancer Causes & Control 13(8), 2002.

Giuliano AR, *et al.* Incidence, prevalence, and clearance of type specific HPV infections: The young women health study. Journal Infectious Diseases 186(4):462-469, 2002.

Inserra P, *et al.* Ethnic variation of the p53 codon 72 polymorphism, HPV persistence, and cervical cancer risk. International Journal of STD & AIDS, (In Press).

Sedjo RL, *et al.* Dietary and plasma vitamin E and their association to HPV persistence. Cancer Causes and Control, (In Press).

Sedjo R, *et al.* Folate status: Is there an association with persistent HPV infection and cervical dysplasia? Nutrition: International J Applied and Basic Nutritional Sciences, (In Press).

Sedjo RL, *et al.* Plasma and dietary carotenoids as risk factors for human papillomavirus persistence. Cancer Epidemiology Biomakers and Prevention 11(9), 2002.

Sedjo RL, *et al.* Folate and vitamin B12 as risk factors for human papillomavirus persistence. Cancer Epidemiology Biomarkers and Prevention 11:353-359, 2002.

Iman Hakim, M.D.

University of Arizona
Award Amount FY 2002: $102,962

The Role of High Tea Consumption in the Modulation of DNA Oxidative Damage in Smoker

Tea drinking has been associated with decreased occurrence of cancer and heart disease. The goal of this study was to test the hypothesis that increased tea consumption, whether green or black, could reduce oxidative DNA damage as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG). A Phase II randomized, controlled, dietary intervention trial was designed to study the effect of high consumption (4 cups per day) of decaffeinated green or black tea on urinary 8-OHdG over a 4-month period. A total of 133 smokers completed the 4-month intervention with no differences in adherence by intervention group. Among smokers with detectable damage at baseline, those consuming tea had decreased urinary 8-OHdG levels of 4 months (21.5 % and 6.5 % decrease in green and black tea group, respectively), while those randomized to water had a slight increase (2.7%). More complete analyses will control for baseline antioxidants and 8-OHdG levels.

Bertram L. Jacobs, Ph.D.

Arizona State University
Award Amount FY 2002: $150,000

Specific Induction of dsRNA Mediated Suicide in Lung Cancer Cells

The overall goal of the research is to develop an otherwise harmless virus that can specifically kill lung cancer cells. This virus could then be potentially used as a novel, specific, anti-cancer agent. The research focus has shifted to identifying novel strains of vaccinia virus that are tumor specific in their replication due to being sensitive to the anti-viral effects of interferon. Many cancer cells fail to respond to treatment with interferon, whereas non-tumor cells routinely respond to treatment with interferon. Since interferon-treatment of cells that respond to interferon (i.e., normal cells) can block replication of interferon-sensitive viruses, an interferon-sensitive strain of vaccinia virus should be able to preferentially replicate in ono-responsive tumor cells but fail to replicate in responsive normal cells. We have prepared and characterized six variants of vaccinia virus that have potential as tumor specific viruses. Work is continuing to optimize use of these viruses in experimental animals.

Jesse Martinez, Ph.D.

University of Arizona
Award Amount FY 2002: $49,990

p-53-Dependent Apoptosis in Lung Cancer

During the previous funding period we determined that the p53 tumor suppressor could interact with several members of the 14-3-3 family of proteins. We determined that one of the 14-3-3 proteins, 14-3-3 gamma, that p53 interacts with can cause abnormal DNA replication resulting in polyploidy and chromosomal instability when over-expressed in lung cancer cells. This is consistent with recent evidence suggesting that several of the 14-3-3 proteins, including 14-3-3 gamma, may be directly involved in DNA replication. Over-expression of 14-3-3 gamma normally lead to apoptosis; however, mutations in p53 suppress apoptosis. Hence, a lack of p53 and increased 14-3-3 gamma may promote chromosomal instability in lung cancer. Importantly, we have found 14-3-3 gamma is over-expressed in some lung cancer cell lines suggesting that it may play a role in lung tumorigenesis and that it may be a useful therapeutic target.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY 2002: $150,000

Aberrant Expression of Redox Associated Proteins NF-kB (p65), Thioredoxin and Inducible Nitric Oxide Synthase as Biomarkers of Colon Cancer Risk

We have evaluated the protein expression of key anti-apoptotic redox proteins (e.g. NF-kB, NOS2, thioredoxin) in the flat mucosa of patients with and without colon cancer and determined that they are aberrantly expressed. We have also prepared and characterized colonic epithelial cell lines resistant to deoxycholate (NaDOC)-induced apoptosis at the molecular and cellular levels and found NF-kB and NOS2 to be up-regulated. NF-kB was inhibited with antisense oligonucleotides which sensitized cells to NaDOC-induced apoptosis. We also determined that the NOS/NO/GC/cGMP /PKG signaling module contributes to apoptosis resistance. Guanylate cyclase (GC) was cleaved by caspase-6 during NaDOC-induced apoptosis of sensitive cells, underscoring the importance of GC as a survival protein. Nicotine was found to increase oxidative stress, activate NF-kB and GRP78, induce apoptosis and sensitize cells to genotoxic/xenobiotic stresses by NaDOC. We conclude that key redox-related proteins may be of value as intermediate biomarkers of colon cancer risk.

Publications:

Bernstein C, *et al.* DNA repair/proapoptotic dual-role proteins in five major DNA repair pathways: Fail-safe protection against carcinogenesis. **Mutation Research** 511:145-178, 2002.

Bernstein C, Payne CM, *et al.* Activation of the metallothionein IIA promoter and other key response elements by ursodeoxycholate in HepG2 Cells. Relevance to the cytoprotective function of ursodeoxycholate. **Pharmacology** 65:2-9, 2002.

Bernstein H, *et al.*. Patchy field defects of apoptosis resistance and dedifferentiation characterize the flat mucosa of colon cancer patients. **Annals of Surgical Oncology** 9(5):505-517, 2002.

Crowley-Weber CL, Payne CM, *et al.* Development and molecular characterization of HCT-116 cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. **Carcinogenesis,** (In Press).

Ramsey L, *et al.* Perils of immuno-histochemistry: Variation in staining specificity of commercially available COX-2 antibodies on human colon tissue. **Digestive Diseases & Sciences,** (In Press).

Washo-Stultz D, *et al.* Role of mitochondrial complexes I and II, reactive oxygen species and arachidonic acid metabolism in deoxycholate-induced apoptosis. **Cancer Letters** 177:129-144, 2002.

Donato F. Romagnolo, Ph.D.

University of Arizona
Award Amount FY 2002: $49,500

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-protein may favor the accumulation of DNA damage and the onset of sporadic breast cancer. 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 present in the environment and may assign to the BRCA-1 gene a role of sentinel marker against low-dose/chronic exposure to this class of environmental xenobiotics.

Publications:

Jeffy BD, *et al.* Activation of the aromatic hydrocarbon receptor pathway is not sufficient for transcriptional repression of BRCA-1: Requirements for metabolism of benzo[*a*]pyrene to 7*r*, 8*t*-Dihydroxy-9*t*, 10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. **Cancer Res** 62:113-121, 2002.

Jeffy BD, Chirnomas RB, Romagnolog DF. Epigenetics of breast cancer: Polycyclic aromatic hydrocarbons as risk factors. **Environ Mol Mutagenesis**, 39:2-3, 2002.

Abstract:

Arizona Cancer and Southwest Environmental Health Sciences Centers Meetings, The University of Arizona, Tucson, AZ, 2002.

Seth D. Rose, Ph.D.

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

Enzyme Active Site Tailored Anti-Cancer Drugs

Cancer cells undergo unrestrained cell division as a result of biochemical processes that have lost their normal control mechanisms. To combat this, inhibition of a particular enzyme in cancer cells has been a widely pursued therapeutic strategy. Our approach is to prepare chemical compounds that precisely fit into the enzyme to specifically target it within cells. In addition, the compounds were designed to have the novel feature that they could destroy the enzyme's activity by undergoing a chemical reaction with the enzyme. This year nearly two dozen compounds of this type in four different chemical categories were prepared, and many of them were tested for activity, along with compounds we made previously. Several were found to effectively inhibit the enzyme and/or the growth of human cancer cells in culture. This work may lead to new anti-cancer agents for the benefit of Arizona residents.

Danny L. Brower, Ph.D.

University of Arizona
Award Amount FY 2002: $45,286

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. This study is aimed at generating and characterizing a set of mutations in integrin genes to 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 identified approximately 60 new integrin structural mutations, and have determined the molecular defect in all of these. These mutants already have been useful in studies to determine how integrins work at the molecular level, and will continue to be so.

Publications:

Jannuzi AL, *et al.* Disruption of the C-terminal cytoplasmic domain of the βPS integrin subunit has dominant negative properties in developing *Drosophila.* Mol Biol Cell 13:1352-1365, 2002.

Mary C. Davis, Ph.D.

Arizona State University
Award Amount FY 2002: $18,924

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

The aim of this study was to examine whether two risk factors for cardiovascular disease, smoking and menopause, affect physiological stress responses among middle-aged women. Sixty-seven female smokers and nonsmokers matched in age participated in a laboratory session during which they first relaxed and then completed three challenging behavioral tasks. Throughout the session cardiovascular, lipid, and cortisol measures were assessed. The findings for cardiovascular data indicate that blood pressure increased to a similar degree during stress among smokers and non-smokers. Stress-related blood pressure increases appeared to be caused by increased constriction of blood vessels in smokers, but by increased blood flow from the heart in non-smokers. These differences were especially apparent among the premenopausal group of women. The pattern of responding that characterized smokers (*i.e.*, constriction of the vasculature) is potentially important as it may be more detrimental to cardiovascular health over the long term than a pattern of responding that involves increased blood flow. These findings suggest that smokers may experience an elevated heart disease risk compared to nonsmokers in part because they tend to respond to stress with vascular constriction.

Eugene Morkin, M.D.

University of Arizona
Award Amount FY 2002: $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 in 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 cardiomyocyte 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. Results indicate that three-weeks after grafting, the collagen scaffold was completely integrated with the injured myocardium. The scaffold prevented cardiac dilation and improved function by shifting the ventricular pressure-volume curve toward normal. Angioneogenesis was present within the graft with mature small arteries 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 2002: $50,000

Role of Gap Junction Mediated Communication in Preventing Endothelial Dysfunction

The mechanism by which smoking leads to diminished blood vessel responses is not well understood, but a deficiency of gap-junction mediated communication between vascular cells is one possibility. Gap junctions are aggregations of intercellular channels composed of a proteins called connexins. We examined the effects of disrupting communication in the blood vessel wall, using mice which lack specific connexins. Our results show that connexin37 and connexin40 are collectively critical for endothelial communication and that they are co-dependent on each other for optimal expression in endothelium. Elimination of connexin40 in particular was found to cause a striking decrease in the levels of connexin37 protein and to reduce endothelial coupling in a surprisingly age-dependent manner. Ablation of both connexin37 and connexin40 eliminated endothelial communication entirely and resulted in mice with severe vascular abnormalities that died perinatally. Thus, endothelial gap-junction mediated communication is critical for the development and/or maintenance of the vasculature.

Publications:

Simon AM, McWhorter AR. Vascular abnormalities in mice lacking the endothelial gap junction proteins connexin37 and connexin40. Developmental Biology, (In Press).

Abstracts:

McWhorter AR, Simon AM. Dye-coupling levels in aortic endothelium of vascular connexin knockouts. International Gap Junction Meeting. Honolulu, HI, 2001.

Marvin J. Slepian, M.D.

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

Microtubule Dependent Integrin Function: Role in Atherosclerosis and Restenosis

The migration of smooth muscle cells (SMCs), cells of the arterial wall, into the lumen of the artery leading to narrowing, is a vital mechanism in the development of atherosclerosis. The interactions of SMC's 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) reduced focal adhesion kinase (FAK) phosphorylation and increased the expression of p53 and c-myc. These findings suggest that alterations of integrin-microtubule interactions impacts outside-in integrin signaling. Adding to our previous results, it appears that CA-4 effects are mediated via alteration of both inside-out and outside-in integrin signaling. *In vivo* local delivery of CA-4 was shown to limit arterial renarrowing following balloon injury. These findings support our working hypothesis for novel activity of CA-4 involving selective disruption of microtubule-dependent $\beta 3$ integrin functions. Defining mechanisms regulating integrin function will open the door for Arizona citizens to new anti-atherosclerotic therapies.

Ronald J. Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY 2002: $149,802

Molecular Bases for Nicotine Dependence

Tobacco use, driven by nicotine dependence, remains a significant health care problem in the state, nationally, and internationally. This project continues to work to establish effects of extended nicotine exposure on numbers and function of its principal biological targets in the brain and body, the diverse family of nicotinic acetylcholine receptors (nAChR).

Studies done over the course of this project support the initial 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, and our work shows that different nicotine-like drugs can either mimic or block effects of nicotine at specific forms of nAChR. We think that the most important and relevant findings concern effects on nAChR function. We found that prolonged nicotine exposure produces losses in nAChR function that become progressively deeper as nicotine treatment times are lengthened. Our studies cover a range of times of nicotine exposure and show loss of nAChR function whether measured using electrophysiological or chemical ion flux assays. The types of nAChR affected include those found in muscle, in the autonomic nervous systems, and in the brain.

The information obtained is of potential use in the design of strategies and therapies to block nicotine dependence or to relieve unpleasant effects of nicotine withdrawal, thereby promoting cessation to tobacco use and control of tobacco-related disease. They also are relevant to the anticipated use of chronic nicotinic ligand therapy to treat neuropsychiatric disorders. Moreover, from our studies, we postulate that tobacco use in many cases involves nicotine self-administration to treat clinical or subclinical depression, anxiety, attentional disorders, etc. Moreover, we postulate that successful smoking cessation therapy needs to address not only nicotine dependence by delivering nicotine in amounts and at rates provided by tobacco products, but also underlying neuropsychiatric pathology in at least a subset of tobacco users.

Publications:

Gentry CL, Lukas RJ. Local anesthetics noncompetitively inhibit function of four distinct nicotinic acetylcholine receptor subtypes. J Pharm Exper Thera 299:1038-1048, 2001.

Gentry CL, Lukas RJ. Regulation of nicotinic acetylcholine receptor numbers and function by chronic nicotine exposure. Current Drug Targets. CNS & Neurolog Disorders 1:359-385, 2002.

Pacheco MA, *et al.* Characterization of human $\alpha 4\beta 2$ neuronal nicotinic receptors stably expressed in SH-EP1 cells. Neurochem Res 26:683-693, 2001.

Reitstetter R, Lukas RJ, Gruener R. Dependence of nicotinic acetylcholine receptor recovery from desensitization on the duration of agonist exposure. J Pharmacol Exper Ther 289:656-660, 1999.

Sabbagh MN, *et al.* The nicotinic acetylcholine receptor, smoking, and Alzheimer's disease. J Alzheimer's Disease, (In Press).

Shytle RD, *et al.* Nicotinic acetylcholine receptors as targets for antidepressants. Molec Psychiat 7:525-535, 2002.

Ronald M. Lynch, Ph.D.

University of Arizona
Award Amount FY 2002: $49,998

Effect of Nicotine on Hypothalamic Glucose Responsive Neurons

A primary reason smokers do not stop smoking is weight gain following cessation of nicotine drugs. Neurons in the hypothalamus of the brain detect changes in nutrients and hormones to set caloric intake and maintain body weight. These neurons are few in number and, therefore, difficult to isolate. Our goal was to quickly isolate these neurons to evaluate gene expression. In initial studies we identified markers for the sensory neurons and analyzed their distribution in the hypothalamus. Knowing cell specific markers allowed us to target a light emitting protein, Green Fluorescent Protein (GFP) to these neurons in mice. The GFP was expressed in the nutrient sensing cells; however, its levels were too low to allow easy isolation of the cells. The targeting method has been improved, and new mouse models are being developed. With these targeted models we ultimately hope to evaluate neuronal changes associated with this important aspect of nicotine addiction.

Publications:

Tompkins LS, *et al.* Regulation of secretory granule pH in insulin secreting cell lines. Am J Physiol Cell Physiol 283(2): C429-37, 2002.

Paul A. St. John, Ph.D.

University of Arizona
Award Amount FY 2002: $46,427

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 continued to examine the internalization of cell-surface nicotinic receptors that we previously showed is caused by sustained exposure to nicotine or other nicotinic agonists. We also have begun to examine the expression of nicotinic receptors on cultured central nervous system neurons. Additional work performed under subcontract to Dr. Mary I. Johnson examined the toxic effects of nicotine and alcohol, alone and in combination, on cultured sympathetic ganglion cells.

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY 2002: $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 nervous system (SNS) outflow to immune cells in lymphoid organs and, thus, alter immune functions that may contribute to disease pathology. We found that nicotine increases arthritis severity by altering SNS pathways that modulate immune functions. Disease severity was increased following chronic nicotine treatment started at adjuvant challenge to induce arthritis in Lewis rats. Nicotine treatment shifted production of T helper cell cytokines towards a Th1 cytokine profile that would be expected to promote inflammation in arthritic joints. Nicotine treatment also promotes production of macrophage pro-inflammatory cytokines and reduced production of macrophage anti-inflammatory cytokines in arthritic rats. These data suggest chronic nicotine exposure as a result of smoking or treatments to quit smoking exacerbate arthritis. Preliminary findings indicate that nicotine alters these immune functions, at least in part, by changing sympathetic signaling with immune cells in lymphoid organs.

Publication:

Lorton D, Lubahn C, Bellinger DL. Potential use of drugs that target neural-immune pathways in the treatment of rheumatoid arthritis and other autoimmune diseases. Current Drug Targets. **Inflammation & Allergy** 1:403-432, 2002.

Abstracts:

Bellinger DL, Madden K, Lorton D. The influence of the sympathetic nervous system on primary antibody response in young and old mice. Soc. Neurosci. A26: 634.17, 2001.

Felten DL, *et al.* Effects of ganglionic blockade, or β-adrenergic receptor antagonists and agonists on severity of adjuvant arthritis during different disease phases. Soc. Neurosci A26:844.15, 2001.

Lubahn C, *et al.* Chronic treatment with adrenergic agents attenuates the inflammation and bone destruction associated with adjuvant arthritis. Soc. Neurosci A26:844.16, 2001.

Lubahn C, *et al.* Chronic treatment with adrenergic agents attenuates the inflammation and bone destruction associated with adjuvant arthritis. **Arizona Alzheimer's Research Center Annual Meeting**, A59, 2002.

SECTION C

CONTRACTS

ANTICANCER DRUG DISCOVERY

YEAR ONE

FY 2002

Scot W. Ebbinghaus, M.D.

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

Triplex DNA Based Gene Therapy of Lung Cancer

The HER-2/neu oncogene is commonly over-expressed in numerous types of cancer including non-small cell lung cancers. Inhibition of HER-2/neu expression has beneficial therapeutic effects. The antigene strategy using inter-strand DNA triplex formation to inhibit oncogenic expression is promising. The specific aims of this proposal include 1)construction of a vector containing an engineered reverse transcriptase (RT) gene that produces a single stranded DNA (ssDNA) from mRNA and 2) verification of the production of ssDNA and its ability to form triplex with the HER-2/neu promoter. We have cloned a plasmid containing the RT gene and the sequence to express ssDNA into a green fluorescent protein (GFP) expression vector. *In vitro* gel shift assays have shown that the proposed ssDNA expressed by the GFP construct successfully binds the duplex target with high affinity. Primer extension assays are currently being optimized in an attempt to identify ssDNA production in cells transfected with the GFP expression vector.

Vince Guerriero, Ph.D.

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

The Stress Protein Hsp70 as a Target for Anticancer Therapy

Cancer is 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. This laboratory has recently identified a novel human gene that codes for a protein called HspHP1 that could be used to stimulate cell death in cancer cells. The purpose of the research described here is to further investigate the utility of this novel protein in the treatment of cancer. The first year of the work has resulted in defining a region at one end of HspBP1 that is essential for full activity. Further experiments are in progress to more clearly define this region. These results will be used to design proteins with potent activity that can be used to treat cancers.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 2002: $220,000

Discovery, Evaluation and Development of Anti-Cancer Drugs from Rhizosphere Microflora of Desert Plants

The overall goals of this project are to discover, evaluate and develop novel anti-cancer drugs from microorganisms associated with rhizospheres of Sonoran desert plants growing under semi-extreme conditions. During the course of the first year of this project, terrecyclic acid A and a novel dihydroxanthone, the two antitumor compounds previously discovered by us, have been isolated in sufficient quantities for mechanistic and animal studies. Animal studies indicated that the novel dihydroxanthone is effective against the Lewis non-small cell lung cancer mouse model and is non-toxic; whereas, terrecyclic acid A was found to be less effective. Mechanistic studies employing cell cycle analysis and confocal microscopy of NCI-H460 (non-small cell lung cancer) cells treated with the novel dihydroxanthone and terrecyclic acid A suggested that these compounds may exert their anticancer activity by a novel mechanism of action. We have also evaluated 500 fungal extracts in two new target-oriented *in vitro* bioassays involving angiogenesis and heat shock response activation. Of those tested, 7 extracts were found to be active in anti-angiogenesis assay, and 35 were active in the primary heat shock response activation assay. The latter extracts are currently being evaluated in a secondary Hsp90 assay, and those showing activity in this secondary assay and strong antiangiogenic activity will be prepared on large-scale and subjected to bioactivity-guided fractionation to obtain sufficient quantities of active compounds for structure elucidation and further biological evaluation. Discovery of a novel anti-cancer agent active in Lewis non-small cell lung cancer mouse model with low toxicity is significant as we are interested in compounds active in lung cancer which will have an impact on the tobacco-dependent portion of Arizona's population.

Evan M. Hersh, M.D.

University of Arizona
Award Amount FY 2002: $137,500

Treatment of Brain Tumors with Glioblastoma Cell Derived Antigens Pulsed into Dendritic Cells or Dexosomes

During the first year of the project fresh glioblastomas were accumulated and stored. It was found that their growth in the test tube was too slow to allow sufficient cells to be accumulated for vaccines. Glioblastoma cell lines were obtained and characterized for the expression of tumor-associated antigens that can stimulate an immune response.

Techniques to develop lysates and eluates (extracts) were established. Multiple lysate preparations produce approximately 0.2 milligrams of protein per million cells. Eluates produced approximately 70 micrograms of protein per 100 million cells. These were considered sufficient amounts for vaccine.

Dendritic (immune) cells (DCs) were prepared and electrofusion technology was developed to fuse them with gliobalstoma cells. DCs were also successfully pulsed with fluorescent-labeled lysates. Dexosomes were produced from the U251 cell line. However, the numbers were insufficient for vaccine production. Tumor antigen peptides were identified from the EGFRV-III antigen, and one was selected for further development into dexosomes and dendritic cells.

We are currently conducting functional assays of lymphocytes stimulated by dendritic cells pulsed with lysates and eluates. These results should result in the development of a candidate for a glioblastoma vaccine within the next year.

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY 2002: $149,677

Et 743-Duplex DNA Adducts as Therapeutic Agents and Molecular Lures

Ecteinascidin 743 (Et 743) is a marine natural product that is approved for treatment of soft tissue sarcomas. In previous studies we have demonstrated that the molecular target of Et 743 is a protein complex involved in DNA repair. This repair complex is sequestered by Et 743, and this may provide an opportunity to enhance the activity of other DNA-reactive drugs by increasing the lifetime of drug–DNA complexes in cancer cells. During the first year of the award we have designed and constructed a DNA duplex molecule containing three Et 743 molecules. These Et 743–DNA adducts have been used as a molecular lure to trap DNA binding proteins that bind specifically to the adduct structures. An initial study using LC-MS-MS has identified several of these proteins belonging to the DNA repair group.

Ronald Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY 2002: $27,500

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

Cerebellar medulloblastoma (CMED) is the most common brain tumor in children. Small cell carcionoma of the lung (SCCL) is a deadly lung tumor. Both of these tumors are thought to originate from neuron-like cell precursors, and both seem to grow due to lost or interrupted signals to induce maturation of precursor cells, causing the cells to continue dividing.

Nicotinic acetylcholine receptors (nAChR) are chemical signal 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.

Initial studies to achieve these goals have shown that human CMED cells do not express nAChR as functional or ligand binding entities. However, at least one gene encoding a component of nAChR is expressed in some form by these cells. Continuing work is determining whether cell survival and rates of division of CMED or SCCL cells are affected after exposure to drugs that activate or block nAChR signaling when tested alone or in combination with other treatments. These studies will demonstrate whether drugs targeting nAChR can provide novel therapies to treat CMED and SCCL.

Eugene A. Mash, Jr., Ph.D.

University of Arizona
Award Amount FY 2002: $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 conditions where normal cells will die through a process of programmed cell death. We are studying a protein called Akt that is the key to turning off the cell survival signaling pathway in cancer cells. It has been shown that inhibition of the function of Akt in cancer cells restores that normal process by which abnormal cells die. Modulation of Akt is, therefore, regarded as a promising strategy for anticancer drug therapy. Compounds related to a lead compound previously identified by us were synthesized and tested for Akt binding activity. Two promising drug candidates have emerged and are currently in refinement.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2002: $400,000

Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs

In May, 1998, Governor Hull signed legislation directing the ADCRC to accelerate the discovery and development in Arizona of new anticancer drugs to clinical trials. That is the overall objective of this research. Five new anticancer drugs discovered in the ASU Cancer Research Institute and currently at various levels of development have been selected for accelerated preclinical research leading to clinical trials. As these promising new drug candidates move toward the clinic, available resources will be devoted to the scale-up, procurement, and processing of other promising plant, marine organism, and microorganism anticancer constituents. The availability of these leads in larger quantities will accelerate the isolation of the new anticancer drugs, ensuring a steady stream of new drug prospects moving toward clinical trials. The five drug candidates selected were isolated primarily from terrestrial plants and marine animals. The candidates are combretastatin A-1 prodrug, pancratistatin prodrug, hydoxyphenstatin prodrug, tyrostatin phosphate prodrug, and fluorcomstatin prodrug. The rapid advance of these new drugs to human cancer clinical trials should lead to a series of important advances in improving human cancer treatment. The next priorities will be from the list of ASU-CRI discovered anticancer drugs proposed for the new contract period.

Publications:

Holwell SE, *et al.* Combretastatin A-1 phosphate a novel tubulin-binding agent with *in vivo* antivascular effect in experimental tumors. **Anticancer Research** 22:707-712, 2002.

Pettit GR, *et al.* Antineoplastic Agents 465. Structural Modification of Resveratrol: Sodium Resverastatin Phosphate, **J Med Chem,** 45:2534-2542, 2002.

Pettit GR, *et al.* Isolation and structure of pedilstatin from a Republic of Maldives *Pedilanthus sp.* **J Nat Prod,** (In Press).

Pettit GR, *et al.* Antineoplastic Agents 460. Synthesis of combretastatin A-2 prodrugs. **Anticancer Drug Design** 16:165-194, 2001.

Pettit RK, *et al.* Antifungal and cancer cell growth inhibitory activities of 1-(3', 4', 5-trimethoxyphenyl) -2-nitro-ethylene. Mycoses 45:65-74, 2002.

Thamm DH, *et al.* Preclinical study of dolastatin-10 in dogs with spontaneous neoplasia. Cancer Chemother. Pharmacol 49:251-255, 2002.

Luke Whitesell, M.D.

University of Arizona
Award Amount FY 2002: $181,599

Heat Shock Proteins as Targets for Drug Discovery

The genetic mutations that cause cancer often result in the production of abnormal versions of cellular proteins that are involved in the control of tumor growth and survival. A particularly effective 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 (Hsp 90). Over the past year, we have developed new molecular techniques to identify Hsp-binding drugs, and we have used these techniques to evaluate the activity of a panel of novel compounds that were synthesized in our labs and that of Dr. Ed Skibo at Arizona State University. At this point we have identified three compounds that are at least 10-fold better at binding Hsp90 than the known Hsp90-active drug novobiocin. In the coming year we will continue to synthesize and screen more derivatives. The activity of the three best compounds that we identify will be evaluated using cancer cells growing in culture dishes and in mice.

Abstracts:

Whitesell L, *et al.* Heat shock protein function as a target for anticancer drugs discovery. Proceedings of the 2001 AACR-EORTC International Conference on Molecular Targets and Cancer Therapeutics, Abstract #203, Miami Beach, FL, 2001.

Marron M, *et al.* Targeting Hsp90 function: Identification of a novel small molecule inhibitor of tyrosine kinase activity in tumor cells. 1st International Conference of the Hsp90 Chaperone Machine, Arolla, Switzerland, 2002.

SECTION D

CONTRACTS

MEDICAL RESEARCH

YEAR ONE

FY 2002

Alyssa Panitch, Ph.D.

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

Bioresponsive Self-Assembling Dextran-Based Blood Substitutes for Trauma Care

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) control of bleeding is not treated, these substitutes do not include 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. We have shown that peptide-dextran conjugates consisting of peptide for the DC11b/CD18 binding pocket (A-domain) inhibit inflammatory cell adhesion to activated endothelial cells in static cell culture. We have also shown that dextran conjugated to assembly peptides of fibrin will associate with fibrin upon clotting *in vitro*. Future work involves optimization of peptide domains and *ex vivo* perfusion studies.

Publication:

Brandon LS, Panitch A. Biologically-based self-assembling hydrogels. Mat Res Soc Proc Vol. 724, 2002.

Harris Bernstein, Ph.D.

University of Arizona
Award Amount FY 2002: $300,000

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

On the basis of 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. In order to advance prevention and treatment, we completed several studies that identified and clarified the molecular events that occur early in the progression to these cancers. In particular, we elucidated the significance of defects in DNA repair and programmed cell death, as well as the role of bile acids and the smoking component, nicotine, in this progression. Abnormal areas in normal-appearing flat colonic mucosa (field defects) appear to predispose individuals to colon cancer. In a study of these field defects we reported two specific promising early indicators of cancer risk. We also reported on the importance of obtaining multiple biopsy samples to assess colon cancer risk because of the patchy nature of the field defects.

Publications:

Bernstein C, Bernstein H, Payne CM, Garewal H. DNA repair/proapoptotic dual-role proteins in five major DNA repair pathways: Fail-safe protection against carcinogenesis. **Mutation Research** 511:145-178, 2002.

Bernstein C, Payne CM, Bernstein H, Garewal H. Activation of the metallothionein IIA promoter and other key response elements by ursodeoxycholate in HepG2 cells. Relevance to the cytoprotective function of ursodeoxycholate. **Pharmacology** 65:2-9, 2002.

Bernstein H, Holubec H, Payne CM, Roe DJ, Cui H, Warneke JA, Jacobson EL, Garewal H, Earnest DL, Bernstein C. Patchy field defects of apoptosis resistance and dedifferentiation characterize the flat mucosa of colon cancer patients. **Annals of Surgical Oncology** 9(5):505-517, 2002.

Crowley-Weber CL, Payne CM, Gleason-Guzman M, Watts GS, Futscher B, Kunke K, Waltmire C, Dvorakova K, Bernstein C, Craven M, Garewal H, Bernstein H. Development and molecular characterization of HCT-116 cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. **Carcinogenesis**, (In Press).

Ramsey L, Fass R, Garewal H, Hart NK, Payne CM, Bernstein H, Bernstein C. Perils of immunohistochemistry: Variation in staining specificity of commercially available COX-2 antibodies on human colon tissue. Digestive Diseases and Sciences, (In Press).

Washo-Stultz D, Crowley-Weber CL, Dvorakova K, Bernstein C, Bernstein H, Kunke K, Waltmire CN, Garewal H, Payne CM. Role of mitochondrial complexes I and II, reactive oxygen species and arachidonic acid metabolism in deoxycholate-induced apoptosis. Cancer Letters 177:129-144, 2002.

M. Bonner Denton, Ph.D.

University of Arizona
Award Amount FY 2002: $66,338

A Unique Mass Spectrometer for Biomedical Studies

The goal of this project is to confirm the hypothesis that hydrogen (H_2) laser ionization mass spectrometry (MS) has significant advantages to solving biomedical problems (*i.e.* analysis of drugs, anti-tumor agents, chemical warfare agents, etc.) by time-of-flight (TOF) MS. The instrument development phase of the project has been completed which included bringing the H_2 laser back on line and interfacing the laser to the Jordan TOF MS. Initial tests are in progress to determine the system's optimum operating characteristics. Three custom sample probes, compatible with the Jordan MS, have been designed and constructed to enable the analysis of biologically important solid and liquid samples by photoionization. In addition, a Mariner™ TOF with conventional electrospray interface has been installed to facilitate studies comparing H_2 laser photoionization with commercial ionization techniques.

Bernard W. Futscher, Ph.D.

University of Arizona
Award Amount FY 2002: $46,200

Transcriptional Repression as a 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 and, therefore, is a significant health issue in the United States and Arizona. Basic research studies performed in our laboratory have determined that specific molecular changes to the maspin gene are responsible for it inappropriately being turned off. Maspin is important in breast cancer because it blocks metastatic tumor growth. Our goal is to determine if our basic laboratory findings translate to breast cancer in the real world. To date we have analyzed 10 breast cancer specimens in detail, and the results indicate that our basic hypothesis appears correct. Analysis of more samples will be performed in year two to increase the power of our results. The delineation of the molecular mechanisms 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.

Arthur F. Gmitro, Ph.D.

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

Development and Clinical Evaluation of a Confocal Microendoscope

This research project is aimed at development and evaluation of a new type of instrument called a fluorescence confocal microendoscope for imaging the lung. The ultimate goal is to demonstrate that the instrument can improve the accuracy of diagnosis of lung disease. Specific objectives of the work are to complete development of the instrumentation, evaluate the instrument of imaging lung tissue, and show the feasibility of using the instrument for *in vivo* lung imaging in an animal model. The focus of the research in year one has been on the instrument development and the evaluation of fluorescent dyes for imaging lung tissue. The design of a microendoscope catheter with a miniature objective and focusing mechanism has been completed and fabrication is proceeding. Studies of the imaging properties and cytotoxicity of acridine orange fluorescent dye have also been completed.

Publication:

Rouse AR, Gmitro AF. Development of a Fiber Optic Confocal Microendoscope for Clinical Endoscopy. BIOS 2002, SPIE Proc. 4613:244-253, 2002.

Abstract:

Gmitro AF, Rouse A, Kano A. *In vivo* Fluorescence Confocal Microendoscopy. 2002 IEEE International Symposium of Biomedical Imaging, Washington, D.C. July 7-10, 2002.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY 2002: $195,664

Discover: Optimization of Production and Evaluation of Novel Anticancer Drugs from Rhizosphere Microflora of Desert Plants

The overall goal of this project is to discover novel anticancer drugs from microorganisms associated with rhizospheres of desert plants growing under semi-extreme conditions. During the course of the first nine months of the project, roots of 28 species of desert plants representing 12 families and 24 genera have been sampled, and 1536 bacteria and 149 fungi have been isolated adding to our library of the Sonoran desert rhizosphere microorganisms. Of the new collections, 200 bacteria and 149 fungi have been cultured and their extracts were prepared. These extracts were screened for their potential anticancer activity using 4 cell-based [NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS cancer), and WI-38 (primary fibroblast)] and two novel target-oriented *in vitro* bioassays for inhibition of angiogenesis and activation of heat shock response. Extracts derived from two fungi which were found to inhibit the growth of a least one cancer cell line by >90% were selected for dereplication and detailed investigations. Bioassay-guided fractionation of the ethyl acetate extracts of these fungi yielded 3 compounds with significant anticancer activity. Structure elucidation and further anticancer evaluation of these compounds are currently in progress. 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 most elderly and/or tobacco-dependent portion of Arizona's population because these cancers are more prevalent in the elderly and in tobacco users.

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

University of Arizona
Award Amount FY 2002: $131,520

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

The Arizona Cancer Prostate Cancer Tissue and Serum Bank has been established. In the last year we were able to procure snap frozen section biopsies from 104 prostate cancer patients. In addition, we obtained 4-6 frozen blocks from 125 radical prostatectomy specimens. In addition, the prostatectomy specimens were totally imbedded, and from this collection we have constructed a prostate tissue micro array consisting of 94 samples representing various stages and precancerous conditions. In addition, we have created an electronic computer database which is on the web and available to all investigators in Arizona. Currently, we are in the process of migrating in all of the data, which represents 686 patient biopsies and 428 radical prostatectomies which were collected previously.

Publications:

Hao J, *et al.* Cell line-specific translation of two laminin 5 β3 chain isoforms. Gene 283:237-244, 2002.

Nagle RB. New approaches to tissue analysis. J Histochemistry and Cytochemistry 49:1-2, 2001.

Parrish AR, *et al.* Culturing precision-cut human prostate slices as an *in vitro* model of prostate pathobiology. Cell Biology and Toxicology 18:205-219, 2002.

Schmeltz M, *et al.* PEAZ-1: A new human prostate neoplastic epithelial cell line. Prostate 48:79-92, 2001.

Scott KM, *et al.* Diagnostic frozen prostate sextant biopsies: An approach for preserving protein and RNA for additional studies. Prostate 44:296-302, 2000.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2002: $ 100,000

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

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 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 was continued. Further development toward clinical trials of our most promising and advanced anticancer drugs was proposed for ADCRC support. Drugs such as auristatin 15-PE and auristatin M; dolastatin 15, 16 and 17; combretastatin A-1, A-2, A-3, D-1, and D-2 prodrugs; auristatin C; cephalostatins 1 and 7; and the marcistatin prodrugs are moving forward. For dolastatin 16, synthesis of the phosphate prodrugs was successful and will be scaled up. For combretastatin A-1, the scale up total synthesis is now in progress and will be developed to clinical trials. For combretastatin A-2 and A-3, the synthesis of the phosphate prodrug has been achieved and it will be scaled up. Concerning the narcistatin prodrugs, we now have enough for the next stages of animal studies and the animal anticancer work is now in progress. We continued to isolate and/or synthesize sufficient quantities of these exciting anticancer drugs to ensure adequate supplies for National Cancer Institute (NCI) preclinical research, a necessary step for clinical decision making and further development beyond our Institute's resources.

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY 2002: $49,673

Mechanisms of Cell Cycle Control: Axolotl Heart Regeneration

The effects of myocardial infarction pose a serious health threat because damaged heart tissue is incapable of repair due to a block in cardiomyocyte cell division. To more clearly understand the biochemical mechanisms governing the block of human cardiac muscle DNA replication and mitosis, cellular factors that control proliferation are being studied in an amphibian model of heart regeneration. Preliminary results indicate that specific cell division gene products such as cyclin B and CDC10 are present in the non-injured adult heart. Presence of cyclin B in the non-proliferative state is unusual since in other species adult heart cells are irreversibly blocked in G1 phase of the cell cycle and do not contain mitotic cyclins. Additionally, curing experimental wounding of the heart, up-regulation of a novel form of cyclin B which contains a serine/threonine kinase domain, and mRNA related to skeletal muscle myogenic 5 has been observed. A clearer understanding of the role these two factors play in cardiomyocyte repair will be useful in developing a gene therapeutic approach to remodel human damaged myocardium resulting from infarction or human cardiovascular disease.

Publications:

Flink I. Cell cycle reentry of ventricular and atrial cardiomyocytes following apical ventricular amputation in the axolotl, *Amblystoma mexicanum:* Confocal microscopic immunofluorescent image analysis of bromodeoxyuridine-labeled nuclei. **Anatomy and Embryology** 205:235-244, 2002.

Karl B. Kern, M.D.

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

The Mechanism of Post Resuscitation Myocardial Dysfunction: Potential Role of Inducible Nitric Oxide

After cardiac arrest and successful Cardiopulmonary Resuscitation (CPR), the heart struggles to pump effectively. The mechanism of this failure of the heart pumping function is not known. We are investigating the role of one type of enzyme system within the heart, the nitric oxide synthase (NOS) enzymes, that if overly stimulated, could lead to such heart failure. We have found that after resuscitation, chemical stimulants called inflammatory cytokines, known to stimulate the nitric oxide synthase enzymes, are increased in the venous blood of the heart. Measurement of two forms of NOS enzymes following cardiac arrest shows that they are very active during the exact time heart pump function is most compromised. Preliminary data suggest that blocking the NOS enzymes can improve heart pumping function recovery much sooner than in untreated subjects. If proven effective, blockade of the NOS system could improve outcome for the thousands of Arizonans suffering cardiac arrest each year.

Marlys H. Witte, MD

University of Arizona
Award Amount FY 2002: $49,514

Angiopoietin-2 and Lymphatic Development: Links of Lymphedema-Angiodysplasia Syndromes

Lymphatics parallel blood vessels and return leaked plasma in tissue back to the blood stream. Lymphatic failure from obstruction or growth defects (angiodysplasia, AD) promotes lymphedema (LE), a brawny disabling tissue swelling (many thousands afflicted in Arizona, millions worldwide). Various protein growth-modulating factors–VEGF, ANG2 and FOXC2–together control both lymphatic and blood vessel growth. Related gene alterations produce specific LE-AD syndromes in man and/or genetically-engineered mice. We have documented by enhanced lymphatic imaging the specific pattern of severe lymphatic and lymph node under development along with a double row of eyelashes (distichiasis) in Foxc2-deficient mice closely mimicking familial LE-distichiasis patients with FOXC2 gene mutations. Greater understanding of how these gene defects produce contrasting LE-AD syndromes in mouse models should result in improved understanding and management of human LE-AD syndromes.

Publications:

Erickson R, *et al.* Clinical heterogeneity in lymphedema-distichiasis syndrome with FOXC2 gene mutations. **J Med Genetics** 38:761-766, 2001.

Gale NW, *et al.* Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by angiopoietin-1. **Developmental Cell** 3:411-423, 2002.

Holberg CJ, *et al.* Segregation analyses and a genome-wide linkage search confirm genetic heterogeneity and suggest oligogenic inheritance in some Milroy congenital primary lymphedema families. **Am. J. Med. Genetics** 86: 303-312, 2001.

Kriederman B, *et al.* Angiopoietin-2 and lymphatic development. In: *Progress in Lymphology - XVIII.* Proc. 18th Int'l. Congress of Lymphology, September 3-7, Genoa, Italy, 2001. Campisi C, (ed), **Lymphology** 35, 2002, (In Press).

Myloyde T, *et al.* Magnetic resonance imaging (MRI) of the lymphatic system: Experimental studies and clinical background. In: *Conquering Lymphatic Disease: Setting the Research Agenda* Witte M. (ed.). Chaite W, *et al.* (co-eds.) The University of Arizona, 181-187, 2002.

Witte CL, Witte MH. Physiology and imaging of the lymphatic vascular system. In: *Vascular Surgery; A Comprehensive Review*, 6th Edition, Chapter 43, Moore, WS (ed.) W.B. Saunders Company, Philadelphia, Pennsylvania, 2002: 837-859.

Witte M, Angiodysplasias and molecular lymphology. In: "Conquering Lymphatic Disease: Setting the Research Agenda" Witte M. (ed.) Chaite W, *et al.* (co-eds.) The University of Arizona, 35-42, 2001.

Witte MH, *et al.* Lymphangiogenesis and lymphangiodysplasias: From molecular to clinical lymphology. In: *The Biology of Lymphangiogenesis.* Wilting J (guest ed.) Microscopic Research and Technique 55:122-145, 2001.

Witte MH, *et al.* Molecular lymphology and genetics of lymphedema-angiodysplasia syndromes. In: *Lymphology* [Lehrbuch der Lymphologie]. Foldi M, Kubik S (eds.) Urban & Fischer Verlag, Munchen, Germany, 2002: 695-727.

Witte M, Witte C. Clinical Vignettes. In: *Conquering Lymphatic Disease: Setting the Research Agenda* Witte M. (editor). Chaite W, *et al.* (co-eds.) The University of Arizona, 43-51, 2001.

Abstract:

Northup KA, Witte MH. Syndromic classification of hereditary lymphedema. J Inv Med 50(1):171 & 50(2):157A, 2002.

Ping Xia, Ph.D., M.D.

University of Arizona
Award Amount FY 2002: $48,714

Mechanism of Cigarette Smoking on Human Infertility

Cigarette smoking increases the risk of infertility in women. In Arizona, approximately one-third of the women of reproductive age smokes cigarettes, which represents a significant health problem. The mechanisms to the deleterious effects of cigarette smoking on infertility are still unknown. It has been demonstrated that cotinine, a metabolite of nicotine, is significantly increased in ovarian follicular fluids in smokers. Cotinine was reported to inhibit progesterone secretion by follicular granulosa cells. We demonstrated the cotinine acts through growth arrest and DNA damage, which indicated by inducing a gene expression of Gadd45 (growth arrest and DNA damage inducible protein) in granulosa cells. Additionally, IGF system is involved in regulation of ovarian follicular development. Our results showed that insulin-like growth factor (IGF)-II was significantly increased in the follicular fluids in smokers. In the next two years we will further demonstrate the signal transduction and gene expression in the follicular granulosa cells treated with components of cigarette smoke.

Linda L. Garland, M.D.

University of Arizona
Award Amount FY 2002: $128,620

A Pilot Study of Lung Cancer Prevention with Selenium Supplementation

Lung cancer remains the leading cause of cancer-related death for men and women in the U.S. in the year 2002. Approximately 85% of all lung cancers are linked to tobacco use. While one's risk of lung cancer decreases significantly with quitting smoking, the more than 40 million former smokers in the U.S. today remain at elevated risk of lung cancer even 20 years or more after quitting and may benefit from strategies to help prevent lung cancer.

Selenium, a miro-nutrient, has shown promise as an anti-cancer agent. A study conducted at the University of Arizona by Dr. Clark and colleagues showed that persons with relatively low levels of blood selenium who took a daily selenium supplement developed fewer lung cancers than those taking a placebo. How selenium acts as an anti-cancer agent is now well understood, but we hypothesize that it may act to alter abnormal changes in DNA, specifically, abnormal DNA methylation that has been related to lung cancer development. Thus, we are currently conducting a study of daily selenium supplementation in the form of selenized yeast tablet versus placebo yeast tablet in 200 former smokers. We are collecting sputum cells and cheek cells before and after 12 months of supplementation for DNA extraction to test whether selenium will modulate abnormal DNA methylation patterns associated with lung cancer development.

Thomas O. Baldwin, Ph.D.

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

Disruption of Cell-Cell Communication to Block Bacterial Pathogenicity

Bacteria are not merely single cellular organisms acting as individuals; they signal each other and can act coordinately. This is accomplished via a quorum sensing system, which allows pathogenic bacteria to achieve a population density sufficient to overwhelm their host when the pathogenicity genes are expressed. It is now clear that antibiotics alone will not eliminate the threat of bacterial disease, as the occurrence and spread of antibiotic resistance within pathogens is outstripping the development of new antibiotics.

We have identified 40 proteins that are up-regulated and 30 proteins that are down-regulated when the quorum sensing system of our model bacterium is activated. Using the same technique, we will now be able to identify the components of the quorum sensing of selected bacterial pathogens including *Helicobacter pylori* (peptic ulcers), *Bordetella pertussis* (whopping cough), *E. coli* (food poisoning), and *Xenorhabdus luminescens* (which may produce an antagonist to pathogenic bacteria).

Karl H. Schram, Ph.D.

University of Arizona
Award Amount FY 2002: $93,067

Biomakers of Systemic Fungal Infections

The goal of this project is to identify and quantify biomarkers of systemic fungal infections in pediatric bone marrow patients. Initial studies have focused on the development of methods for the isolation of cyclic peptides and other intermediary metabolites of fungi/yeasts of clinical importance. Fungal pellets of *Aspergillus fumigatus, A. terrus* and *Candida albicans* have been supplied by Dr. Tomas Walsh of the NIH. The extracts are being examined by GC/MS and electrospray LC/MS for the presence of cyclic peptides or other compounds useful as biomarkers of systemic fungal/yeast infections. Blank patient blood samples are being extracted in the same manner to obtain a background of the components present and quantitative procedures are being developed using blank patient blood samples spiked with model cyclic peptides.

The goal sets for the first year of this project have been met or exceeded. The laboratory has been set-up and supplied and personnel hired, including two outstanding technicians in addition to Dr. Petra Miketova who oversees the research. Methods for the extraction of cyclic peptides and other potential biomarkers of fungal infections have been developed and applied to blank medium, fungal medium and fungal samples. Analysis of the medium and fungal extracts using electrospray (ESI) time of flight (TOF) mass spectrometry (MS) indicate the presence of hither to unidentified compounds in the extracts of *Aspergillus fumigatus*. Methods used for the extraction of medium and fungi have also been applied to spiked human blood samples and blank blood samples. Analysis of the spiked blood samples shows the methods are working, but additional clean-up steps need to be performed because of the presence of interfering background components.

J. Kristin Olson-Garewal, M.D.

University of Arizona
Award Amount FY 2002: $124,615

Traditional Navajo Medicine and Diabetes

This proposed study is a retrospective case review comparing 294 Navajo diabetics who have used traditional Navajo medicine with an equal number of Navajo diabetics who have used only allopathic (Western) medicine. The study involves a home interview of subjects by a bilingual (Navajo/English) interviewer regarding the participants' attitudes, beliefs, and experience with Traditional Navajo Medicine (TNM). In addition, a medical chart review will be done to abstract information relevant to the management of diabetes. In the event the patient has not been treated for diabetes within the allopathic medical system, a finger stick hemoglobin A1C will be done.

The first year of the study accomplished final approval of the project by the Navajo Human Research Review Board and training and/or hiring of seven interviewers located throughout the eight regions of the Navajo Nation. A major change in recruiting strategy was required of the Review Board after initial approval. The original plan was for the Indian Health Service Centers to send out recruiting letters to all known diabetics. The possibility was raised that this strategy would possibly bias the study population to exclude those diabetics who used TNM exclusively. Thus, the recruiting methodology was changed to flyers and posters which the study interviewers, interested medicine men, and Indian Health Service diabetic clinics have all agreed to distribute.

A delay in startup has necessitated a three month extension of year one. Immediately at the beginning of year two, the study will be started in the Southern areas of the Reservation (Winslow, Arizona and Gallup, New Mexico). The responses to the first twenty five interviewers will be reviewed before the study is deployed across the entire Navajo Nation.

Kemmons A. Tubbs, Ph.D.

Intrinsic Bioprobes, Inc.
Award Amount FY 2002: $127,884

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, complex proteins called nicotinic acetylcholine receptors (nAChR), found throughout the nervous system, can exist in several different forms reflecting their composition from different building blocks called subunits. Our understanding of the precise subunit composition of several important forms of nAChR, their associations with other kinds of cell proteins, as well as how nAChR subunits are changed during or after their synthesis is not known. Collectively, these deficiencies in our knowledge compromise our understanding about how nAChR function is altered by prolonged and/or repeated exposure to nicotine. These effects contribute to the habitual use of tobacco products (and related adverse health and economic consequences) by a significant number of adult Arizonans. To address these deficiencies in our understanding about structure and composition of diverse forms of human nAChR, we have developed and refined sophisticated techniques to generate and characterize complex proteins like nAChR that reside in cell membranes. Additionally, we are using the refinement of molecular biology techniques to engineer other nAChR subtypes in full-length, truncated and epitope-tagged forms. Assimilation of expressed protein characterization using mass spectrometry (MS) techniques is proceeding. MS analysis is a central component of a new technology for studies of proteins called proteomics. Work accomplished to date has used these proteomic techniques to initiate characterization of diverse forms of nAChR containing different subunit building blocks. MS and related techniques have been applied 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 tissues. MS techniques are being applied to identify and characterize other cellular components that interact with specific forms of important nAChR. To this end, initial studies are under way to use nAChR subunits as bait to capture those interacting and assembly partners.

Mary Kay O'Rourke, Ph.D.

University of Arizona
Award Amount FY 2002: $219,797

Integrated Epidemiological Study of Valley Fever

Recently, Valley Fever (coccidioidomycosis) rates in Arizona have doubled. Our goal is to evaluate recent cases of the disease, identify contributing factors, and improve public health interventions. During the seven months of funding, we altered the survey to accommodate the reduced budget, switched from a door-to-door to a telephone methodology, altered and translated (English to Spanish) all study materials and questionnaires, sought and gained approval from the internal review board responsible for the protection of human subjects, trained interviewers and collected data to evaluate the sampling method, and identified sampling areas using census and soil composition information. We initiated evaluation of the laboratory test by purchasing equipment and supplies, training laboratory technicians, and identifying critical issues in recent work (ours and others). Our progress is consistent with the time line outlined in the proposal. When completed, the project will identify new strategies Arizona residents can use to reduce cocci exposure.

Garth Powis, D. Phil.

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

Thioredoxin Peroxidase, A Novel Mechanism for Protection Against Lung Toxicity

Reactive oxygen species (ROS) damage lung tissue. ROS are present in tobacco smoke, or they can be produced in lung cells themselves by uncoupling of normal respiration in the mitochondria by chemicals in smoke. The mitochondria have mechanisms to remove ROS, and one of the most important is the thioredoxin-2 (Trx-2)/peroxiredoxin system. We have shown that mice with both Trx-2 genes activated die early during embryo development at a time when mitochondrial respiration is just starting. Mice with one copy of the Trx-2 gene inactivated develop normally but have decreased levels for the Trx-2 protein, and we predict they will be sensitive to oxidant damage. The mouse model we have developed points to the importance of Trx-2 in protecting against ROS damage and will be an invaluable tool in studying the role of Trx-2 in a number of diseases including lung disease and cancer.

SECTION E

NEW CONTRACT AWARDS

BRAIN AND MEDICAL RESEARCH

BEGINNING FY 2003

AND

PARKINSON'S DISEASE RESEARCH

BEGINNING 4TH QUARTER FY 2002

Rodney D. Adam, Ph.D.

University of Arizona
Award Amount FY 2003: $49,999

Gene Expression in Giardia Lamblia

Giardia lamblia is a common parasitic cause of diarrhea throughout the world. It is the most common cause of water-borne outbreaks of diarrhea in the United States and is a common cause of diarrhea in backpackers and in day-care centers. Unlike most of the bacteria and viral causes of diarrhea, giardiases is frequently chronic, resulting in symptoms that persist for weeks or months. In addition to diarrhea and abdominal pain, impaired food absorption and weight loss are common. Chronic giardiasis has also been associated with impaired growth in children. The reason for the inability of the body to eliminate the infection despite what should be an effective immune response is probably the occurrence of antigenic variation. Giardia has on its surface a protein coat that probably represents the most important target of the immune system. However, the organism is able to switch its protein coat from one form of the protein to another, requiring the infected person to then produce another set of antibodies. Although these proteins are similar biochemically, antibodies for one do not recognize the other. Giardia has a family of 150-300 genes that produce these proteins, called variant-specific proteins (VSPs). Antigenic variation occurs when Giardia switches from one VSP gene to another. We know that the DNA of the gene does not change when a gene is turned on or off, so it is likely an epigenetic mechanism–a heritable trait that does not involve a change in the DNA sequence. Epigenetic forms of inheritance are known to be involved in certain types of cancer and in developmental biology, as well as in many other situations.

The major goal of this project is to understand how Giardia controls the expression of its surface proteins of VSPs. The hypotheses addressed in this project propose that the specific chromosomal location of the VSP genes is important in the control of their expression and that the mechanism of control involves histone acetylation and deacetylation. We have evidence from our laboratory that a VSP gene artificially placed into a location outside the chromosome (as a plasmid) can be expressed in organisms not otherwise expressing that gene. Therefore, we believe that the location at a certain place in the chromosome is important in controlling expression. We plan to further evaluate this hypothesis by introducing VSP and other genes into Giardia as plasmids and into the chromosome. By doing so, we will be able to determine the importance of the chromosomal location in controlling expression. Modifications of histones (the proteins that surround the chromosomes) are the most commonly documented means of epigenetic inheritance that fit our observations with Giardia. Therefore, we also plan to determine whether the most common histone modifications are involved in controlling VSP gene expression. A better understanding of how Giardia controls these surface proteins will help us understand how the infections persist and may result in effective immunization or novel drug treatments.

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY03: $175,000

Molecular Mechanisms of HIV-1 Infection in Immature and Mature Mononuclear Cells

AIDS in children is a serious problem in the United States and a catastrophe worldwide. New cases of HIV-1 infection in women of childbearing age in the United States have increased by 63% in the past three years. Some of these infected women may transmit HIV-1 to their infants even with the current AZT treatment. This number may further increase due to the lower success rates of highly active antiretroviral treatment (HAART) observed in hospitals and clinical practices. In the state of Arizona, the number of AIDS cases is increasing at a significant pace and Tucson and Phoenix, Arizona have been included in the top 50 metropolitan areas in the country with highest annual rates of AIDS. The majority of HIV-1 infected infants (immature hosts) develop AIDS faster than infected adults (mature hosts). Furthermore, with the lower success rates of HAART and development of multidrug resistant HIV-1, more HIV-1 infected infants may rapidly progress to AIDS. The multidrug resistant HIV-1 is likely to be transmitted to several infants, which may not be treatable with the current lines of available antiretroviral drugs. Therefore, there is a need to develop new lines of better and effective drugs, which will only be possible if the molecular mechanisms of HIV-1 infection in immature and mature hosts' cells are elucidated. The population of Arizona is on the rise and so is AIDS; therefore, infected infants receiving HAART may develop resistance and rapidly progress to AIDS, and new neonates may become infected with drug resistant HIV-1. This will have a greater impact on the Arizona Health Care System. With no cure or vaccine at hand, research that investigates the molecular mechanisms of HIV-1 infection in immature and mature hosts would be helpful in obtaining information to develop new strategies in combating the growth and development of AIDS.

The majority of HIV-1 infected infants develop AIDS faster compared to infected adults, including differences seen in clinical manifestations. The reasons are not clearly understood but may be partially explained due to the relative immaturity of the immune systems in neonates and infants. However, the differences in the molecular mechanisms on HIV-1 infection in immature (neonates and infants) and mature (adults) hosts' mononuclear cells are not clearly understood, making it difficult to develop strategies for prevention and treatment of HIV-1 infection in children. Our hypothesis is that HIV-1 replicates more efficiently in and destroys immature mononuclear cells more rapidly compared with mature mononuclear cells, which results in a more rapid disease progression in neonates and infants than in adults. In the proposed studies, comparison of the molecular mechanisms of HIV-1 infection in immature (cord) and mature (adult) blood mononuclear cells will be determined, including the destruction of T-cells precursors in a thymus organ culture system. The specific aims are to: 1) compare the kinetics of HIV-1 replication in immature and mature mononuclear cells, 2) study the entry and post entry events of HIV-1 infection in immature and mature mononuclear cells, 3) determine the cell activation and proliferative capacities of and HIV-1 gene expression in immature and mature

mononuclear cells, and 4) study the effect of HIV-1 infection on T-cell development in immature and mature mononuclear cells. The insights generated by these studies may contribute to a better understanding of the differences and similarities of the molecular mechanisms of HIV-1 infection in immature and mature hosts' mononuclear cells as well as destruction of target cell precursors. These results should be helpful in developing strategies for the treatment and prevention of HIV-1 infection in infants and adults by means of better antivirals and vaccine, respectively.

Molly A. Brewer, D.V.M., M.D., M.S.

University of Arizona
Award Amount FY 2003: $167,748

Fluorescence Spectroscopy as a Biomarker for Prevention/Early Diagnosis of Ovarian Cancer

Ovarian cancer is the most common cause of death from gynecologic cancer in the United States. According to the American Cancer Society, in 2001 there will be at least 26,800 new cases of ovarian cancer and 14,000 women will die from this disease. Despite recent advances in treatment, the 5-year survival rate remains poor due to the inability to diagnose ovarian cancer before it has spread to other areas in the body besides the ovary. The majority of women with ovarian cancer are diagnosed after the cancer has spread to the upper abdomen; the survival rate is dismal in these women with 70% or more dying of their cancer. Thus, prevention and early diagnosis merit at least as much attention as treatment of the disease once it has occurred.

Using family history and genetic testing, we can identify those women at higher than average risk for developing ovarian cancer. However, the current screening in women at increased risk of ovarian cancer is limited in detecting early cancers. The three current screening tests–pelvic exam, ultrasound, and the blood test CA125–fail to adequately and safely screen those women at moderate to high risk who often have a 10-40% or greater chance of developing ovarian cancer. This group of women needs effective methods to prevent ovarian cancer or to detect ovarian cancer either as a precancer or in an early, and thus more curable, stage. Not only do these moderate to high risk women routinely go unrecognized in Arizona, but no alternative exists to offer better screening than the current methods.

We propose developing optical methods (using fluorescence light coming from tissue) to examine the ovary and provide a more accurate diagnosis for early ovarian cancer to women who are at a moderate to high risk for developing ovarian cancer. Building on our experience with prevention of ovarian cancer using medications, we will use a cell culture model to better understand how cells become inactive. We propose that the opposite mechanisms occur during early cancer development (unrestrained growth), so understanding the timing of the molecular events that occur and correlating them with the changes of fluorescence light will allow us to build a model for what is occurring inside the cells as they progress from normal to pre-cancers.

We will then use this information to study pieces and slices of ovary that we remove surgically from patients. This tissue will be examined with the same light that we have used in the cell experiments. We will keep this tissue alive long enough to explore what happens when it is exposed to drugs that prevent the cells from growing and to drugs that stimulate growth. We will study the different signatures that originate from cells in the tissue over time. Achieving these objectives over the 3 years will give us tremendous information on how to later create a device that will scan the surface of the ovary to find abnormal areas and biopsy these areas to determine if a small cancer is developing.

Marlene P. Freeman, M.D.

University of Arizona
Award Amount FY 2003: $49,988

Omega-3 Fatty Acids for Postpartum Depression

In general, women are more likely than men to experience major depressive episodes throughout the lifespan, and the postpartum period is a particularly vulnerable time. Ten to twenty percent of new mothers suffer from postpartum depression. Postpartum depression affects not only the mother, but also the infant and family. Infants of depressed mothers are more likely to develop insecure attachments, cognitive and behavioral problems, and are more likely to be abused or neglected. Postpartum depression is a disorder with broad public health implications, with consequences that impact on almost every aspect of child development. Some women, as well as pediatricians, may be unwilling to accept standard antidepressant medications while breast-feeding, because long-term effects to standard antidepressants on the nursing infant are unknown. Omega-3 fatty acids are long-chain fatty acids found in some plants and seafood. Omega-3 fatty acids have a number of health benefits that are well-documented in the medical literature. In order to ensure adequate supplies of omega-2 fatty acids for the developing baby, maternal omega -3 fatty acids are selectively transferred to the developing baby during pregnancy. The baby, who needs omega-3 fatty acids for brain and visual development, relies on the mother's supply during pregnancy and breast-feeding. Omega-3 fatty acids are essential fatty acids; they cannot be manufactured by the body and must come from dietary sources. Despite their importance during pregnancy and lactation, intake of omega-3 fatty acids by pregnant and lactating women in the U.S. is inadequate, only reaching 20-60% of intake recommended by members of a National Institutes of Health workshop. Epidemiological data and treatment data of mood disorders provide compelling support for this study of omega-3 fatty acids for postpartum depression.

The overall objective of the research is to determine the efficacy of omega-3 fatty acids in treating postpartum depression. The hypotheses are 1) omega-3 fatty acids will be an efficacious treatment for postpartum depression, as demonstrated by significant reduction of depressive symptoms compared to placebo, and 2) oral administration of omega-3 fatty acids will increase

maternal red blood cell omega-3 fatty acid content in women with postpartum depression and correlate with response to treatment. The *rationale* includes data that suggest a role of omega-3 fatty acid deficiency in major depression and postpartum depression and depletion of omega-3 fatty acids during pregnancy. Studies have demonstrated the efficacy of omega-3 fatty acids in mood disorders. This study involves an area of study–postpartum depression–in which minimal treatment data are available. It involves a promising but understudied compound with many potential health advantages. This project is a double-blind, placebo-controlled, randomized trial of omega-3 fatty acids in 100 women with postpartum depression. Patients will be randomly assigned to omega-3 fatty acids or placebo, a non-active comparison. This study will provide meaningful data regarding the efficacy of omega-3 fatty acids in the treatment of postpartum depression. Postpartum depression affects the mother and her infant. Since breast-feeding offers advantages to mother and baby, many mothers would like to breast-feed. However, breast-feeding while receiving treatment with antidepressants may pose risks. Optimal care of postpartum depression would be efficacious and safe for both the mother and baby. Treatments that would provide additional health benefits to the mother and baby would be ideal. Omega-3 fatty acids appear to meet all of these criteria and deserve assessment in a randomized controlled trial.

Jorge A. Giron, Ph.D.

University of Arizona
Award Amount FY 2003: $50,000

Molecular Characterization of Type IV Pili Produced by Enterohemorrhagic Escherichia Coli 0157:H7: The Etiologic Agent of the Hemolytic Uremic Syndrome

Enterohemorrhagic *Escherichia coli* (EHEC) serotype 0157:H7 is recognized as a significant enteric pathogen that has been implicated in numerous outbreaks in the United States, Canada, Northern Europe, Japan, and South America. Due to the alarming emergence of this pathogen, the state of Arizona maintains a constant surveillance of the appearance of 0157:H7 strains. The bacteria are acquired through consumption of contaminated food (beef and vegetables) or fruit juices. When they reach their target sites in the human intestine, they can cause bloody diarrhea that can progress to hemorrhagic colitis. The most severe manifestation of this infection is the development of the Hemolytic Uremic Syndrome (HUS) that is characterized by renal failure, commonly resulting in death. This infectious disease affects humans of all ages, but the young and old are the groups most susceptible to HUS. Although it is known that the bacteria produce a potent toxin called Shiga toxin, no information is available about the attachment factors (called pili) that allow the bacteria to stick to and colonize the intestine. This project aims to characterize the pili structures produced by these bacteria and to elucidate their role in adherence and colonization of epithelial cells *in vitro*. The hypothesis is that the adhesive properties of EHEC 0157:H7 rely on the production of type IV pili that enable these bacteria to recognize host cell receptors and to promote colonization of the intestine.

The main goals of this investigation are to 1) further characterize the type IV pili produced by EHEC 0157:h7 and to demonstrate their contribution of the pathogenic scheme of the bacteria; 2) provide clues about the structure-function properties of these pili by studying their biochemical and genetic basis and the biological consequences of pili production; and 3) elucidate the nature and biology of the pili structures responsible for the colonization of the gut by EHEC. This will contribute to the diagnostics for the infection and to the development of effective preventive strategies directed towards controlling the emergence of 0157:H7 infections in Arizona.

The objectives are a) to purify and characterize biochemically and antigenically the type IV pili produced by EHEC 0157:H7; b) to study the function of the genes involved in production of these pili; and c) to provide clues of the biological relevance of pili production within the context of pathogenesis.

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY 2003: $175,000

HPV Infection in Men (HIM) Study: A Prospective Cohort Study

Human papillomavirus (HPV) is a common sexually transmitted infection. It is the virus that causes invasive cervical and vulvar cancers in women, and penile and anal cancers in men. In addition to cancer, HPV infections are the cause of genital warts affecting both men and women. In 2000, 12,800 new cases of cervix cancer were diagnosed and 4600 women died of invasive cervical cancer in the US with higher incidence and mortality rates documented among different ethnic groups. In Arizona, Hispanics have more than twice the rate of cervical cancer than white, non-Hispanic women. In addition, other cancers caused by HPV are increasing such as anal cancer which has risen 35% in the past 15 years in women. HPV related cancers are totally preventable. We have the technology to screen, diagnose, and treat pre-cancerous lesions. The detection of these lesions is the basis for Pap smear screening in women. However, not all those at risk participate fully in screening programs. In addition, there are tremendous costs associated with the testing of women with normal cells, repeat Pap testing of false positive tests, and with diagnostic treatment and follow-up when abnormalities are found. The annual cost of managing all HPV related disease is estimated to be $750 million in the US and in excess of $6 billion worldwide. In order to prevent disease and decrease patient burden and health care costs, strategies to prevent HPV related diseases are needed. One promising approach is the development of an HPV vaccine for men and women. As we have sufficient data from HPV natural history studies conducted among women to design vaccine trials, the testing of these vaccines in women is progressing rapidly. Unfortunately, little is known about the natural history of HPV infection in men. Therefore, vaccine efficacy in men is lagging by several years. A prospective study which assesses HPV infection and immune response is required to move vaccine programs forward in men, a prevention strategy that will benefit both men and women.

The goal of this proposed research is to further our understanding of HPV infection in men. We propose to conduct a prospective cohort study of young men followed over an 18-month period to determine the incidence of new HPV infections, the persistence of HPV infections over time, the prevalence of 27 different genotypes of HPV, and the development of HPV antibodies. We will also identify sociobehavioral factors associated with HPV infections in men.

Results from this study will provide much needed information about the natural history of HPV infections in men, which will be used in the development of vaccination programs. In addition, the study will provide an educational forum about the most commonly acquired sexually transmitted infection of which few men are aware.

Lokesh Joshi, Ph.D.

University of Arizona
Award Amount FY 2003: $175,000

Recombinant Protein Therapeutics

Cardiovascular disease and cancer are the leading causes of death in US and in the state of Arizona. Recently, two novel therapeutic proteins with great promise of limiting mortality caused by these diseases have been discovered. Until now, mammalian systems have been the only source of these two and most other therapeutic proteins. However, there are concerns of possible disease transmission from contaminating viruses, prions and other infectious agents when these therapeutic proteins are produced in mammalian systems. Plants are able to produce human proteins that have therapeutic applications and do not pose the risk of transmitting animal diseases. Plants can be grown in large-scale operations to produce kilogram quantities of medically important proteins for patient care. We will primarily use tobacco plants as 'green bio-factories' to develop recombinant protein therapeutic modalities. This represents a novel approach that has significant biotechnology potential.

We hypothesize that biologically active proteins of medical importance can be successfully produced in plants. Plant biotechnology enables easy, economic and large-scale production of medically important macromolecules. We plan to establish this project with two candidate molecules that are active targets for cardiovascular disease and cancer.

There are three main objectives of this research proposal: 1) to produce two therapeutic proteins in plants, TAT-HSP20, for improved and safer vascular bypass surgeries, and Gc-MAF, a molecule that can help reduce and possibly eliminate cancerous tumors; 2) to perform biological activity characterization of these two proteins made in plants to confirm that they will function appropriately; and 3) to develop novel drug delivery methods for these therapeutic proteins.

The ultimate goal of this proposal is to advance protein based therapeutics to treat specific human diseases.

Lynn J. Manseau, Ph.D.

University of Arizona
Award Amount FY 2003: $49,971

Intramolecular Regulation of Cappuccino and Identification of Effectors

Cancer, hereditary deafness, renal aplasia and limb defects are all health issues important to the state of Arizona. The study of *cappuccino (capu)*, a gene implicated in these health concerns, will lead to greater understanding of the development of these diseases. Although genes related to *capu* are found in humans, we study it in fruit flies because of the ease of genetic and molecular analyses. Through our analyses in fruit flies, we know that *capu* is a member of the formin family of proteins thought to link signaling to regulation of the internal framework of the cell. Other members of the formin family are self-regulating. One end of the protein binds to the other end of the protein, keeping it turned off. Interaction with a signaling protein releases the ends of the protein from each other, turning on the protein. By removing the ends of the protein, we can artificially turn on the protein. This will turn on proteins downstream. By suppressing this mutant phenotype, we can identify downstream proteins in the pathway. If we can identify suppressor proteins of known function, it will help us understand how *cappuccino* related genes in human function in cancer, hereditary deafness, renal aplasia and limb defects. The project goals are to 1) identify regions of the cappuccino protein necessary for the self-regulation described above, 2) make flies that express an unregulated, turned-on form of *capu* by deleting the ends of the protein and analyzing the appearance of these flies; and 3) identify suppressor mutants of the unregulated, turned-on form of *capu* and determine what proteins are encoded by these suppressors.

Stephen Massia, Ph.D.

Arizona State University
Award Amount FY 2003: $169,929

Local Gene Therapy Targeting Vascular Graft Hyperplasia

Cardiovascular disease remains a major health problem in the nation and in Arizona. The use of tobacco products, especially cigarette smoking, is a major remediable risk factor for the development of coronary artery and peripheral vascular disease. Bypass graft placement is widely used as an interventional therapy for treating obstructions in arteries due to atherosclerotic disease. One major problem with this therapy is that it doesn't treat the disease and the bypass can rapidly become obstructed. Another problem is that synthetic graft replacements do not work as well (have a higher risk of becoming obstructed) as natural vessels, e.g. saphenous veins, harvested from the patient. Unfortunately, many patients have multiple bypass surgeries, deplete their supply of available natural vessels and depend on the use of synthetic vascular grafts. The major driving force behind the failure of synthetic vascular grafts is the body's immune reaction to implanted synthetic materials that promotes improper healing. This healing response promotes the development of intimal hyperplasia or abnormal thickening of the artery wall where it is connected (sutured) to the synthetic graft resulting in severe constrictive renarrowing or failure of the graft to restore blood flow. At the cellular level, intimal hyperplasia is a result of accelerated growth of smooth muscle cells (SMCs) within the artery wall followed by SMC migration to the luminal or internal blood-contacting surface of the artery with concurrent increased cell and tissue growth at the luminal interface. Currently, little progress has been made toward developing strategies to reduce occlusive hyperplasia in synthetic vascular grafts. The proposed study will develop a new gene therapy strategy where genes that target the inhibition of graft hyperplasia are delivered locally from the synthetic grafts. This approach has potential for decreasing the failure rate of synthetic vascular grafts.

Occlusive hyperplasia (blockage by scar tissue ingrowth) in synthetic vascular grafts requires the migration of vascular tissue cells, primarily vascular smooth muscle cells (SMC's). Therefore, a potential approach for treating graft hyperplasia would be to genetically alter SMC function via local gene therapy in vascular tissues conjoined with the synthetic graft so that migration and subsequent hyperplasia development is impeded. The overall goal of the proposed study is to develop a local gene therapy approach for limiting cell migration and luminal narrowing in synthetic vascular grafts. We hypothesize in the proposed work that the porous space of synthetic vascular grafts can be filled with materials that dissolve and gradually release genes that impede cell migration and graft blockage. It is further hypothesized that this strategy will be an effective means for limiting blockage in synthetic vascular grafts. Gel materials will be developed to fill porous synthetic grafts and locally release genes. The final studies will evaluate how effectively the prototype gel materials delivers genes from synthetic grafts placed in a well established animal model for testing synthetic vascular performance. Therapeutic benefit of the gene therapy will also be assessed in this animal model.

Megan M. McEvoy, Ph.D.

University of Arizona
Award Amount FY 2003: $50,000

Structural Studies of the Apical Protein Complex Formed During Asymmetric Cell Division

The development of higher organisms into the complex and diverse creatures we see around us requires that there are ways in which cells can become different from each other during the growth of the organisms. When a cell divides, instead of making two identical copies of itself, there must occasionally be ways of generating two different kinds of cells. One way a cell can accomplish this is by localizing factors to one side of the cell or the other, and then dividing such that those factors are located in only one of the two new cells. These factors, which are usually proteins, will then act to control the development of the cell into one of two kinds. This process is called intrinsic asymmetric cell division. In order to work effectively, the correct protein complexes must form at the appropriate times in the proper places during the cell's cycle of division. At present, the ways in which the factors are localized within the cell is not very well understood. An excellent model system for studying this process is found in the neural stem cells of the fruit fly, *Drosophila melanogaster*. Asymmetric cell division has been observed in many organisms including humans. Understanding the mechanisms by which stem cells divide can have important implications in the treatment of cancer because of the potential for stem cells to proliferate and form tumors.

The goal of this research is to try to understand intrinsic asymmetric cell division by determining the structures of the proteins that are involved in this process. An atomic level description of proteins can yield insight into the ways proteins work and, in this case, may yield some clues as to how the protein complexes form and are localized. In particular, the structures will shed some light on 1) how these proteins can select their cognate partners from amongst the many proteins within the cell, 2) what structural features are present that allow these proteins to bind to multiple targets, and 3) how these interactions are regulated. The primary method for determining the structures will be solution nuclear magnetic resonance spectroscopy. Two proteins have been selected for initial structural characterization because of their central role in this process. They both form complexes with several other proteins in the system and are required for the cell to divide in the correct direction. Detailed structures of these proteins will allow an understanding of the protein-protein interactions which are vital to this fundamental biological process. Since related proteins have been identified in other organisms and/or cell types, structural and biochemical results in this system will be of utility in understanding this widespread and complex process.

Bradley S. Moore, Ph.D.

University of Arizona
Award Amount FY 2003: $50,000

Engineered Biosynthesis of "Unnatural" Natural Products for Drug Discovery

Natural chemical constituents of living organisms called natural products have historically been employed to treat human infections and diseases. As the discovery rate of new biologically active natural products slows in comparisons to the increased rate of infectious diseases that are developing resistance toward traditional antibiotics, it is imperative that the discovery rate of novel drug candidates increases. In order to assure the constant flow of new chemical entities for the drug discovery, we have initiated a program to generate novel drug candidates from genetically engineered microorganisms. My group is focused on engineering "unnatural" natural products, compounds that are difficult or impossible to obtain by any other currently available means, by genetically modifying the genes that encode the synthesis of structurally novel and biologically active bacterial metabolites. In this proposal, we aim to expand the chemical engineer's genetic toolbox to include terpene biosynthesis genes in order to engineer a new generation of novel "unnatural" natural products for biological evaluation as new antimicrobial and anticancer agents.

This proposal addresses two important issues in generating new biosynthetic products for drug discovery efforts. First, the combinatorial biosynthetic repertoire will be expanded to include terpene-based structural diversity. This major natural product pathway to important therapeutic agents such as the anticancer agent Taxol has to date been neglected and promises to yield structurally novel small molecules. Second, engineered small molecules possessing terpene residues are likely to have biological properties not addressed by polyketides and peptides alone, thus expanding combinatorial biology into new therapies. Specifically, we propose to study how the antimicrobial anticancer natural product marinone and its derivatives are biosynthesized in bacteria at the chemical, biochemical, and genetic levels. With this information in hand, we aim to genetically engineer novel hybrid polyketide-terpenoid compounds in surrogate bacterial host stains for biological evaluation using the new methodology of combinatorial biology with the marinone biosynthetic machinery.

Michael H. Ossipov, Ph.D.

University of Arizona
Award Amount FY 2003: $174,071

Fentanyl-Induced Paradoxic Pain, Antinociceptive Tolerance and Receptor Down-Regulation

Chronic or persistent pain remains a significant problem in our society and is an important concern to the people in Arizona. Pain adversely impacts quality of life and the ability of an individual to function to his or her fullest level of productivity, thus imposing an enormous socioeconomic burden. Severe chronic pain may come from a variety of causes, including cancer, nerve injuries and diseases such as post-herpetic neuralgia or tic douloureux. Currently, the most effective means of treating pain requires the use of opiate analgesics (narcotics) such as morphine. Recently, a transdermal patch containing fentanyl, which is a very potent and efficacious opioid analgesic, has been introduced to provide constant pain relief over 3 days at a time. A patch containing sufentanil, even more potent than fentanyl, is expected to be marketed soon. Unfortunately, the use of opioids is associated with the development of tolerance to their analgesic effects such that ever-increasing doses are needed to maintain a constant level of pain relief. However, increasing the doses of the analgesic may only be done until side-effects such as mental clouding and constipation become intolerable. Recently, it was discovered that the long-term use of an opioid analgesic may actually cause a paradoxic *increased* sensitivity to pain or even cause abnormal pain itself. We believe that this development of paradoxical abnormal pain caused by powerful analgesics such as fentanyl and morphine may be the basis for the tolerance that develops. As pain increases, then the need for the analgesic increases, which is interpreted as tolerance. This increased need for opiate may trigger further increases in abnormal pain which would be manifested as increased tolerance to the action of the analgesic. Gaining an understanding of the processes that are involved in driving the development of opiate-induced abnormal pain and tolerance will help us to develop treatments to take full advantage of opiate-mediated analgesia while minimizing the development of abnormal pain and tolerance.

The primary objective of the proposed studies is to increase our understanding of the means through which tolerance to the actions of opiate analgesics develops. We plan to achieve this objective through the use of standard laboratory techniques to allow us to correlate the changes in responses to the opiate analgesics with changes in the numbers and/or function of specific receptors (specialized proteins on cell surfaces that react with drugs) for opiates. The experiments described in this application will test the hypothesis that prolonged administration of opiates causes a reduction in functional receptors that in turn leads to increased input of pain signals to the central nervous system. This paradoxic pain leads to increased need for analgesics and appears as tolerance. These objectives will be met by analyzing changes in receptor population, blocking the activity of pain-transmitting nerves and by blocking the effect of the chemical messengers of these neurons. Behavioral responses will be used to measure analgesia, and the release of neurotransmitters from spinal cord preparations will be analyzed as measures of opiate receptor

function. We believe that the prolonged administration of opiates leads to decreased function of the receptors through which opiate analgesics exert their action, and this allows increased, rather than decreased, activity of pain sensory fibers. Several techniques will be used including behavioral observations of analgesia, visualization of receptor population by using dyes bound to antibodies that bind selectively to these proteins, and by the use of cell cultures that express the opiate receptors. This is a collaborative effort among members of the Department of Pharmacology and the Department of Anesthesiology. The expertise of the individuals in these departments include an understanding of behavioral pharmacology and functional neuroscience, cellular and molecular biology and, importantly, a critical understanding of clinical management of pain.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY 2003: $175,000

Role of cGMP-Dependent Protein Kinase (PKG) in Apoptosis Resistance and Colon Cancer Biomarker Development

Each year in Arizona about 900 people die from colon cancer (the second leading cause of cancer deaths in the state). On the basis of these current mortality rates, about 64,000 of Arizona's current residents (about 1.6%) will die of cancer of the colon, unless there are significant improvements in prevention and treatment. Colon cancer is caused, in part, by lifestyle factors such as a diet rich in fats and red meat and low in fruits and vegetables. However, the risk for colon cancer on an individual basis has not been adequately assessed. Our group was the first to determine that the normal appearing mucosal lining of the colon cancer from 50% of patients with colon cancer or at high risk for colon cancer show defects in the ability of colon cells to die after treatment with a DNA-damaging agent. The consequences of this biologic defect is that cells with damaged DNA can replicate, increasing the risk of developing mutations, some of which lead to cancer. This inability to die (referred to as apoptosis resistance) occurs in the early phase of malignant progression to adenocarcinoma of the colon and esophagus. We would now like to expand our ability to detect high risk patients (including the remaining 50%) using a combination of molecular and cellular techniques. Toward this goal, we have developed death-resistant colon cell lines in our laboratory that mimic the condition that we observed in the actual lining of the colon of high risk patients and have already characterized these cell lines using a variety of molecular and cellular techniques. The production of nitric oxide (NO) appears to play a key role in the death-resistance of colon cells; however, the key downstream effect of NO are not known for the colon cells at the present time. The expression of fifteen key genes that affect the cell death pathway was determined to be altered in these resistant cell lines. One of these proteins whose expression was significantly up-regulated is protein kinase G (PKG), an enzyme that alters protein function by adding a phosphate group at key positions (process of phosphorylation). PKG also happens to be a key enzyme that is activated by NO. Since the

concentration of PKG in colon cells is 10-100X that present in most other cell types, it may represent a master controller of gene expression and protein function in the colon and is responsible, in part, for the observed death resistance. We plan to determine if a targeted micro array of these fifteen genes (including PKG) can be used to effectively identify Arizona residents, on an individual basis, who may be at risk for colon cancer. Our research team has worked together for more than 10 years and consists of basic scientists working closely with physicians. We have co-authored 25 publications and have been very successful in obtaining Federal funding from the National Cancer Institute as a result of funding received from the ADCRC. We anticipate that the present project will be equally as successful if funded.

Resistance to cell death is a defect in the mucosal lining that predisposes individuals to colon cancer. Our main hypothesis is that PKG plays a key role in resistance to cell death of colonic epithelial cells through the modulation of gene expression and phosphorylation of proteins. Our short–term goal is to understand if a targeted PKG/Apoptosis Resistance Micro array can be used to identify patients with or at high risk for colon cancer. This micro array assay will be compared to apoptosis resistance, which is a live cell bioassay that currently identifies only 50% of patients with colon cancer. Our *long-term goal* is to develop reliable biomarkers that assess colon cancer risk on an individual basis and can be used on archived tissue biopsies obtained during simple colonoscopy. The availability of such biomarkers would be invaluable for guiding patients toward appropriate dietary and smoking behavioral changes and for monitoring progress during cancer chemoprevention treatment.

Patricia E. Penn, Ph.D.

La Frontera Center, Inc.
Award Amount FY 2003: $109,946

Comparing Smoking Cessation Treatments for Persons with Schizophrenia and Other Psychotic Disorders

A 1998 survey showed that 21.9% of Arizona's population smoke cigarettes. Nearly one-half of the cigarettes smoked in Arizona are smoked by someone with a mental illness. Data from studies conducted across the country indicate that cigarette smoking among individuals with schizophrenia or other serious and persistent mental disorders is three times higher than in the general population. Moreover, most persons with a serious mental illness die of smoking-related illnesses. Most persons with a serious and persistent mental illness are supported by public funds. If fewer of these persons smoked, a significant savings of health care funds could occur. Treatments designed to help people stop smoking have not been extensively studied in persons with serious mental illnesses. In fact, the majority of the research studies on treatment to help people stop smoking have specifically excluded persons with psychiatric disorders. Changing smoking behavior in this population has been a challenging problem for the following reasons:

- Most persons with a serious mental illness smoke.

- Patients with a past or present history of schizophrenia are unlikely to stop smoking on their own.
- Increased dopamine (a neurotransmitter) from smoking may affect psychiatric symptoms.
- Smoking decreases blood levels of many medications used to treat mental illnesses
- There are many social and behavioral factors associated with smoking in persons with serious mental illnesses.
- Existing community-based smoking cessation programs have not been readily accessible to this population.

In a study conducted at La Frontera Center – the comprehensive community behavioral health organization that will serve as the site for this study – 89% of 112 clients with a psychiatric illness and substance abuse disorder reported smoking cigarettes. A subsequent survey of 50 clients with serious mental illness who smoke revealed that 57% were interested in quitting smoking. These people reported smoking an average of 24 cigarettes per day and spending $18 per week on cigarettes. This translates to approximately $1,000 per person per year. This is a very significant amount of money when one considers that approximately half of these individuals live on income of less than $4,500.00 per year.

This study compares two treatment approaches designed to help a group of adults with schizophrenia and other similar mental illnesses stop smoking. Nicotine Replacement Therapy (NRT), also known as "the patch," has been extensively tested and shown effective and very safe in helping adults without mental illnesses quit smoking. It has not yet been tested extensively in persons with serious mental illnesses. Another intervention, Contingent Reinforcement, is a non-drug behavioral intervention that has been shown to be effective in treating other addictions. In some previous studies it has shown promise as a way to help people quit smoking. Contingent reinforcement means giving people rewards for not smoking. Study participants will be given a small monetary incentive at each clinic visit if they have not been smoking. In addition to helping people to stop smoking, with the obvious benefits that provides, we will be asking about changes participants notice in their physical and psychological symptoms, money saved, and quality of life.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY 2003: $250,000

Development of New Anticancer Drugs for Improving Human Cancer Treatment

Human cancer constitutes some 200 related diseases that continue to cause a catastrophic number of deaths (nearly 600,000 annually) accompanied by tremendous personal and economic disasters. 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 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 will be continued. Further development toward clinical trials of one of our promising and advanced anticancer drugs such as auristatins 15-F and 15-DMO; auristatin M; dolastatins 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 National Cancer Institute (NCI) preclinical research necessary for clinical decisions and further development beyond our Institute's resources. The ADCRC research will also include continuing the synthesis of vitally important clinical supplies of our anticancer drug discoveries for the NCI such as dolastatin 10.

Barry M. Pryor, Ph.D.

University of Arizona
Award Amount FY 2003: $49,986

Characterization of *Alternaria* Isolates Associated with Allergenic Asthma

The objectives and hypotheses of this study are based, in part, upon specific findings from an on-going long-term epidemiological study on the natural history of childhood asthma, the Tucson Children's Respiratory Study (CRS). An important finding of the Tucson CRS has been that an allergen produced by the fungus *Alternaria alternata* was the single allergen most strongly correlated with the development of asthma in children living in semiarid regions (southern Arizona). This finding is consistent with other studies performed in the Middle East and in Australia, as well. However, these findings are not without a certain level of uncertainty with regard to the correct identification of the fungus that produces these allergens, A. *alternata*

Nearly all critical taxonomic work on *Alternaria* species has come from studies involving plant pathogenic species. Considerable morphological diversity has been documented in small-spored species like A. *alternata*. Historically, most small-spore *Alternaria* species similar to A. *alternata* have been described as A. *alternata* due to the inability of mycologists to reconcile this morphologically diverse and complex group of fungi into a comprehensive taxonomic classification system. Within the last 10 years Dr. E. G. Simmons, recognized world-wide as the foremost authority on *Alternaria* fungi, has developed culture conditions and defined morphological criteria that permits the consistent and reliable identification of *Alternaria* species. One important finding of his work is that typical A. *alternata* is not common and most plant pathogenic fungi identified as A. *alternata* are, in fact, misidentified. This finding is particularly significant for medical mycology where the advances from plant pathology research have not yet been incorporated. Most importantly, this has a significant impact on specific findings of the Tucson CRS and on continued research in the relationship between childhood sensitization to *Alternaria* allergens and asthma in semi-arid environments such as southern Arizona.

The proposed project will investigate the morphological, molecular, and immunological diversity of small-spored *Alternaria* species associated with the onset of childhood asthma in and around the vicinity of Tucson, AZ, and to compare the characteristics of these isolates with those of typical A. *alternata* isolates (type cultures) and isolates from which commercial allergen preparations are made for clinical sensitization testing. Specific objectives are 1) to obtain a collection of small-spored *Alternaria* species representative of the species diversity found in the Tucson area, and examine the diversity of recovered isolates using morphological and DNA characteristics, 2) to determine allergen content of recovered isolates using immunological and molecular techniques, and 3) to compare the sensitivity of asthmatic children to allergens extracted from *Alternaria* isolates collected in the survey, type A. *alternata* isolates, and isolates used in commercial allergen preparation. The following hypotheses will be tested: 1) most *Alternaria* isolates collected in the Tucson survey are morphologically and/or molecularly distinct

from type A. *alternata*; 2) most *Alternaria* isolates collected in the Tucson survey produce allergens that are not produced by type A. *alternata* isolates or by isolates used in commercial allergen preparations; and 3) the sensitivity of asthmatic children to allergens extracted from *Alternaria* isolates collected in the survey differs from the sensitivity to allergens extracted from type A. *alternata* isolates or from isolates used in the production of commercial allergen preparations. If data support these hypotheses, then the potential for commercial development of improved *Alternaria* allergen preparations for use in standardized allergen sensitization test is suggested and would be evaluated in future studies.

Theodore P. Trouard, Ph.D.

University of Arizona
Award Amount FY 2003: $50,000

3D Diffusion-Weighted Radial MRI of the Brain

Diffussion-weighted magnetic resonance imaging (DW-MRI) is a clinically useful neuroimaging tool that enables non-invasive measurement of the motion of water within the human brain. Because the motion of water within the brain is extremely sensitive to the microscopic architecture and integrity of brain tissue, DW-MRI is being actively used to evaluate a number of neurological disorders, as well as evaluate therapies aimed at correcting them. The ability to use non-invasive tools for diagnosis and evaluation of neurological diseases is important to all persons, particularly to an aging population.

Because of technical problems associated with motion in living systems, DW-MRI in the clinical setting is typically carried out using methods that are insensitive to motion. However, these methods yield low-resolution (i.e blurry) images. To realize the full potential of DW-MRI for neuroimaging applications, the spatial resolution of the techniques needs to be improved.

The primary objective of this research project is to develop a method for high-quality, high-resolution 3 dimensional (3D)DW-MRI of the human brain. In previous work, we have developed a DW-MRI method that combines novel data acquisition and image reconstruction methods and allows 2D DW-MRI to be carried out with high spatial resolution without artifacts due to motion. We called this method DIFRAD-FSE (Diffusion-weighted radial acquisition of data with fast spin echo) and have applied it to many clinical studies of neurological disorders. Our results demonstrate that DIFRAD-FSE allows DW-MRI to be carried out with much higher in-plane resolution than currently used methods. In this project we will exploit all of the unique aspects of DIFRAD-FSE to develop a new 3D MRI method, 3D-DIFRAD-FSE, that will allow diffusion-weighted imaging of the brain to be carried out with high spatial resolution in 3 dimensions with good signal-to-noise ratio (SNR) without artifacts from motion. This new MRI method will enable the human brain to be studied in more detail than is currently possible and will have a significant impact on the diagnosis, evaluation and treatment of many neurological disorders.

Danzhou Yang, Ph.D.

University of Arizona
Award Amount FY 2003: $49,940

DNA and Topoisomerase I Interactions of Novel Homocamptothecin Anticancer Drugs

The camptothecin (CPT) derivatives are among the most promising anticancer drugs recently introduced in the clinic. Topotecan (TPT) has been approved for the treatment of advanced ovarian cancer and for second-line therapy in small cell lung cancer. Irinotecan (CPT-11: Camptosar) has been approved for the treatment of colorectal carcinoma. Current clinical trials indicate that camptothecin derivatives will be useful in a variety of other human malignancies. Campthothecin derivatives are renowned for their unique mechanism of action, inhibition of DNA topoisomerase I (topol). Homocamptothecins (hCPT) are a group of novel CPT analogues with a modified seven-member lactone ring by the insertion of a methylene group in the E-ring. hCPTs fully conserve the topoisomerase I inhibiting activity and stimulate high levels of DNA cleavage. This E-ring modification enhances the stability of the lactone ring, which, in the case of classical CPTs, opens to an inactive carboxylate form at physiological pH. This enhanced stability may account for the superior antitumor activity of hCPT *in vitro* and *in vivo*. The irreversibility of E-ring hydrolysis of hCPTs should also reduce the toxicities seen in CPTs with the classical six-member E-ring. In addition, this E-ring modification decreases the drug binding to human serum albumin and, therefore, increases the active drug level in human plasma. Little is known about the molecular level details of the interactions of hCPT with target DNA and topol and the different mechanisms of action by hCPT and classical CPT. We propose in this application to explore the structural and molecular level details of the DNA and topol interactions of the homocamptothecins. From this structural and molecular information we expect to obtain a better understanding of the mechanism of action that accounts for the superior biological activity of these compounds. This information can be used to develop improved hCPTs and possibly other new camptothecin analogues that will help better treat cancer patients in Arizona.

The overall goal of the proposed research it to explore the structural and molecular-level details of the DNA and topol interactions of the homocamptothecins, and from those details, to obtain a better understanding of their mechanism of action. We will use high-field NMR spectroscopy, molecular computational modeling in combination with the protein and DNA biochemical assays and HPLC methods for the project. The objectives of the proposed research are 1) to study the DNA interactions of hCPTs and test how DNA interactions affect the stabilities of hCPTs, 2) to elucidate the three-dimensional structures of hCPT-DNA complexes by using NMR spectroscopy and computer molecular modeling, 3) to study the interactions of hCPTs with DNA and topol in the ternary complexes, and 4) to obtain some structural information of the DNA and topol interactions of hCPTs.

Jeffrey Joyce, Ph.D.

Sun Health Research Institute
Award Amount FY 2002: $705,000

Arizona Parkinson's Disease Center

Parkinson's disease (PD) is a progressive neurologic disorder characterized by tremor when the person is sitting or standing at rest, rigidity or stiffness, and slowness of movement. As the second most common age-related neurologic disorder, the social and economic impact of PD is substantial and will increase even more as our population ages. The symptoms of PD are caused by the loss of cells in the brain that manufacture dopamine (DA), a chemical messenger molecule used for signaling between certain nerve cells, and are well managed, at least initially, by currently available drugs. For many patients, however, present therapies become progressively less effective with time, do not impact depression and dementia (which can co-occur with PD), and, perhaps most importantly, only address the symptoms of PD, not its underlying causes. As a result, there is little or nothing anyone can presently do to prevent or slow down PD. Several avenues of research, however, have begun to suggest pathogenic (disease-causing) mechanisms of PD. By understanding these mechanisms better, it may be possible to come up with neuroprotective agents that offer lasting benefits for PD patients or perhaps even prevent it entirely. This is the common theme that links all the elements of our proposed Arizona PD Center. In addition, in accord with the ADCRC RFP (Request for Proposal), we have structured our Center along the lines of an NIH-funded PD Center of Excellence including clinical cores, and we have been mindful in designing the cores and projects that they should provide the basis for a successful NIH application in two years.

Four institutions have joined together to create the proposed Arizona PD Center: Sun Health Research Institute, Mayo Clinic Scottsdale, Barrow Neurological Institute, and Arizona State University. Each institution provides documented expertise in the projects and cores within the Center, and their investigators have been working together for four years on various PD-related studies. Three research projects are proposed that are thematically linked by their attempts to understand fundamental pathogenic mechanisms in PD and, in so doing, make possible new neuroprotective strategies for PD. Project 1, D_3 *preferring agonists provide neuroprotection in PD through elevation of BCNF*, tests the hypothesis that DA losses in PD reflect a failure of certain growth factors to support DA neurons. A corollary to be tested is that a class of compounds called D_3 agonists may up regulate these growth factors, restoring their ability to protect DA neurons. Project 2, *Is age-related proteasome inhibition the cause of Parkinson's disease?*, tests the hypothesis that increased oxidative stress leads to abnormal protein aggregation that, in turn, causes pathologic inclusions such as Lewy bodies to accumulate within DA neurons and kill them. Project 3, *Selective glial attack on DA neurons*, tests the hypothesis that cells called glia,

which normally support neurons, become activated in an inflammatory sense in PD and become responsive to a novel factor secreted by DA neurons that invites glial attack. All three mechanisms to be tested in the three projects are thematically linked as mainstream research areas, the understanding of which could lead to new neuroprotective strategies for PD. Moreover, they have several common bridges that will promote interactions and information-sharing within the projects. Three core components are also included to support the research projects. The Clinical Core will provide detailed clinical evaluation on all subjects that come to autopsy so that these subjects can be categorized as being controls or having PD and no other clinical disorder. The Neuropathology Core will provide Arizona PD Center clinicians and researchers with accurate neuropathologic diagnoses on all study subjects. The Administrative Core will provide basic financial oversight, statistical and database support, as well as integration of the Center components through joint seminars and meetings of the investigators.

Yong Shen, M.D., PH.D.

Sun Health Research Institute
Award Amount FY 2002: $147,500

Gene Targeting Approaches Reveal Dopamine Cell Death and Protection by Estrogen Therapy: Implication for Parkinson Disease

Parkinson's Disease (PD) is a chronic and progressive degenerative brain disorder. It is mainly characterized by a lack of dopamine, a chemical found in the brain, and by loss of cells that produce dopamine in selective brain regions. Although the reason for losing dopamine cells in Parkinson's disease is unknown, a combination of environment and inherited factors, as well as gender and aging, is thought to contribute to the development of the disease. For example, tumor necrosis factor alpha (TNFα) is a protein within the body that can bind to TNFα specifically. Studies show that the level of TNFα and TNF receptor type I are elevated in PD patients. Studies on animal models for Parkinson's disease demonstrate that TNFα is particularly toxic to dopamine cells in the brain. We believe that TNFα-induced dopamine cell death is through TNF receptor-1 which activates a death signal in dopamine cells. However, the dopamine cells are protected by estrogen, a female hormone. Interestingly, the incidence and prevalence of Parkinson's disease are higher in men than in women. Estrogen has been discovered to have a protective effect on neuronal degeneration, and the neuroprotective property of estrogen in Parkinson's disease has been suggested as a potential treatment for Parkinson's disease. In order to understand the mechanisms of TNFα-induced dopamine cell death and the neuroprotective action of estrogen in Parkinson's disease, this proposal–in a TNF receptor knockout mouse model–using estrogen treatment on the cells of the mice will not only provide a viable alternative model to 6-hydroxydopamine and MPTP, severe and dangerous toxins which induce dopamine cell death, but also prove useful for screening possible neuroprotective and therapeutic drugs for treatment of Parkinson's disease.

Tumor necrosis factor alpha (TNFα), a protein within the body that can chronically enhance inflammation, has been reported to be toxic to dopamine cells in the Parkinson's brain. To understand this mechanism and to develop effective drugs to stop this dopamine cell death, we will examine whether TNFα induced dopamine cell death is through its receptors, TNF receptor-1 and II (TNFR-I and TNFR-II), by using our TNFR-I and TNFR-II knockout mouse models and gene-delivery of viral vectors over-expressing the TNF recetpors. We expect that dopamine cells are more sensitive to TNFα in both TNFR-I over-expression and TNFR-II knockout models based on findings from our and other reports that TNFR-I contains an intracellular death domain, whereas TNFR-II may have a survival domain. Furthermore, we will examine how estrogen protects dopamine cells. Specifically, we will examine whether estrogen can block TNFα binding to TNF receptors which either activate death genes, inhibit the binding complex producing free radicals, or both. Thus, the hypothesis we will test in this proposal is that TNFα-induced dopamine cell death is mediated by TNF receptor differential expression in brains with Parkinson's disease. Protective effects of estrogen on dopamine cells may be regulated through blocking biochemical cascades of death induced by TNFα-TNF receptor complexes in dopamine cells.

Michael Sierks, Ph.D.

Arizona State University
Award Amount FY 2002: $147,500

Controlling α-Synuclein Aggregation as a Tool for Studying Parkinson's

The protein α-synuclein has been strongly correlated with Parkinson's Disease (PD) because it is a major component of Lewy Body aggregates, a hallmark feature of PD. Two different mutations in the α-synuclein gene are associated with early onset cases of PD, and transgenic animal models expressing α-synuclein develop PD symptoms. α-synuclein is a natively unfolded protein having a random 3-dimensional structure; however, it can adopt a number of different folded conformations depending on the environment. One of these folded structures, the β-sheet form, facilitates formation of numerous aggregated morphologies including large fibril structures found in Lewy Bodies, spherical protofibril structures, and smaller aggregates or oligomers. While all of these structures occur, their respective roles in the progression of PD is not known. We do not know whether any of the different aggregate structures are toxic to nerve cells, or whether they represent a protective strategy designed to neutralize a potentially toxic species. Currently, there are no methods available to control formation of any of the aggregate structures and, thus, no method of testing their effects on progression of PD. Identifying the toxic form of α-synuclein is essential for developing a successful therapeutic. For example, inhibiting fibril formation may increase the concentration of the smaller oligomeric and protofibril structures, which would actually precipitate progression of PD if these forms were the toxic species. There is, therefore, a critical need to develop a tool whereby we can control formation of different morphologies of α-synuclein and study how presence of the individual morphologies affects the progression of PD.

The long-term objective of this proposal is to generate multifunctional antibody fragments that can be used to prevent accumulation of toxic forms of α-synuclein, a protein correlated with development of PD. These antibodies can be used in conjunction with other therapeutics to develop a non-invasive long-term successful therapeutic treatment for PD and other neurological disorders associated with α-synuclein aggregation. Our hypothesis is that misfolding of α-synuclein into specific toxic morphologies is a fundamental step in the progression of PD, and developing antibody based proteins to control formation of the individual α-synuclein morphologies will provide a means of identify the toxic α-synuclein forms and can serve as part of a potential therapeutic treatment for controlling PC. In order to test this hypothesis, we will develop a microscopic technique that will allow us to both visualize individual α-synuclein morphologies and to recover antibody fragments that bind specifically to a particular morphology. We will utilize a novel combination of two very different state of the art technologies to achieve the objectives of this proposal: first, we will use phage display technology, a powerful technique that enables us to generate large pools of antibodies to different regions of α-synuclein; second, we will use single molecule fluorescence microscopy to image all the different α-synuclein morphologies, then detect and recover antibodies bound to a specific morphology.

SECTION F

PROPOSALS RECEIVED

FY 2002

Adam	University of Arizona	Gene Expression in Giardia Lamblia	$49999 49999 49999
Adams	Arizona State University	Assessing the Possible Link Between Mercury Toxicity and Autism	$174992 115290
Ahmad	University of Arizona	Molecular Mechanisms of AIDS Pathogenesis	$175000 175000 175000
Ahmad	University of Arizona	Molecular Mechanisms of HIV-1 Infection in Immature and Mature Mononuclear Cells	$175000 175000 175000
Allen	Arizona State University	Structure of the Survival Motor Neuron Protein	$50000 50000 50000
Allen	Arizona State University	Structure of the Nicotinic Acetylcholine Receptor	$50000 50000 50000
Arnett	University of Arizona	Physical Activity and the Physical Environment	$175000 175000 175000
Baldwin	University of Arizona	Dietary, Oxidative, and Inflammatory Mediators of Cardiovascular Disease in Obstructive Sleep Apnea	$138199 138599 117291
Bayles	University of Arizona	Hearing and Alzheimer's Disease: Impact and Intervention	$172347 172345 174488
Braun	University of Arizona	A Novel Model for the Study of Type 2 Diabetes	$50000 50000 50000

Brewer	University of Arizona	Fluorescence Spectroscopy as a Biomarker for Prevention–Early Diagnosis of Ovarian Cancer	$167748 143548 149048
Brophy	Arizona State University	Improving Post-Surgical Functional Outcomes with Exercise Rehabilitation in Patients with Peripheral Vascular Disease	$174035 174035 174035
Brower	University of Arizona	Identification of Genes Involved in the Regulation of Integrin Activity	$49882 49882 49882
Brown	University of Arizona	Maternal Influences on Early Human T-Cell Differentiation: Enhanced Decidual IL-4 Production	$50000 50000 50000
Brown	University of Arizona	Alteration of Placental Structure and Immune Function by Maternal Smoking	$175000 175000 175000
Burd	University of Arizona	Isolation of Adult Bone Marrow-Derived Stem Cells Capable of Neuronal Replacement in Neurodegenerative Disease	$49390 49390 49390
Burgess	University of Arizona	Acute Lung Injury Following Smoke Exposure: Predictive Value of Sputum Biomarkers	$175000 175000 175000
Carey	University of Arizona	Nonverbal Learning Disability and Pediatric Brain Tumor Survivors	$49584 44575 44575
Chen	University of Arizona	The Association of Hormonal Factors with Mammographic and Changes in Mammographic Densities	$174798 174341 174600
Clark	Arizona State University	The Use of *Scutellaria Baicalensis* Secondary Metabolites for the Treatment of Human Malignant Brain Tumor	$175000 175000 175000
Cone-Wesson	University of Arizona	Infant Hearing for Modulated Tones	$49823 49796 49819

Davis	Arizona State University	Stress Vulnerability, Physiological Reactivity, and Body Fat Distribution in Young Adults	$119612 109221 98838
DeLuca	University of Arizona	Effect of Antenatal Tobacco Smoke Exposure on Human T Cell Development	$175000 175000 175000
DeLuca	University of Arizona	Nicotine Regulation of T Cell Development	$400000 400000 400000
DeLuca	University of Arizona	Effect of Nicotine on T Cell Development	$175000 175000 175000
Deroin	Southwest College of Naturo-pathic Medicine	Chronic Hepatitis C: Combination Botanical Intervention. A 6-month Randomized, Double-Blind, Controlled Trial	$171967 171747 171747
Dixit	University of Arizona	A Preventative Model for Type 2 Diabetes and Renal Disease in Children: Focus on Obesity	$48045 49145 49145
Erickson	University of Arizona	N-Acetyltransferase, Folate Metabolism, and Orofacial Clefting	$95611 98557 103793
Freeman	University of Arizona	Omega 3 Fatty Acids for Postpartum Depression	$49988 49988 49658
Fregosi	University of Arizona	Influence of Prenatal Nicotine Exposure on the Control of Breathing in Neonates	$50000 49997 49999
Galbraith	University of Arizona	Analysis of Global Gene Expression to Dissect Endcrine Pathophysiology	$175000 175000 175000
Gandolfi	University of Arizona	Role of Selenoamnio Acids in Prostate Cancer	$133278 136682 140235

Garcia	University of Arizona	Human Papilloma Virus as a Predictor of Recurrent/Persistent Cervical Dysplasia after LEEP	$173606 174349 126774
Giron	University of Arizona	Molecular Characterization of Type IV Pili Produced by Enterohemorrhagic Escherichia Coli 0157:H7: The Etiologic Agent of the Hemolytic Uremic Syndrome	$50000 50000 50000
Giron	University of Arizona	Role for Pili and Flagella Produced by Stenotrophomonas Maltophilia in Adherence and Biofilm Formation	$50000 50000 50000
Giuliano	University of Arizona	HPV Infection in Men (HIM) Study: A Prospective Cohort Study	$175000 175000 175000
Glass	University of Arizona	Molecular Basis for the Role of Selenium in Biology	$50000 50000
Groves	University of Arizona	Gender Differences in Transport and Nephrotoxicity of Cysteine Conjugates	$50000 50000 50000
Grow	Midwestern University	Nicotine Inhibition of Acetylcholine Receptor Clustering at the Neuromuscular Synapse	$48963 49719
Hakim	University of Arizona	A Multibiomarker Approach to Study the Effects of High Tea Consumption on Oxidative Stress	$167100 170413 171030
Hoffman	University of Arizona	Advancement of Natural Products as Novel Chemotherapeutic Agents	$174559 174559 174559
Hoffman	Arizona State University	Brain and Autoimmunity	$50000 50000 50000
Hogue	Arizona State University	RNA-Host Protein Interactions in Coronavirus RNA Replication	$50000 50000
Horton	University of Arizona	Structure of the Human DNA Damage Recognition Complex	$43404 49923 49745

Jacobs	Arizona State University	Development of a Single Vaccine for Protection Against Both Smallpox and Anthrax	$400000 400000 400000
Joens	University of Arizona	Characterization of *Campylobacter Jejuni* Genes Expressed Preferentially *In Vivo*	$49425 49368 49309
Johnson	Barrow Neurological Institute	Nicotine Receptor Polymorphisms	$50000 50000 50000
Joshi	Arizona State University	Recombinant Protein Therapeutics	$175000 175000 175000
Kaemingk	University of Arizona	Markers of Central Nervous System Injury Following Traumatic Brain Injury	$172904 174071 153941
Kail	Southwest College of Naturo-pathic Medicine	Asthma: Combination Botanical and Nutrient Therapy. A 3-month Randomized, Double-Blind, Controlled Trial	$170295 170075 170075
Katsanis	University of Arizona	Immunotherapy of Cancer Using Chaperone Protein-Based Approaches	$175000 175000 175000
Katsanis	University of Arizona	Identification of Schizophrenia Phenotypes using fMRI	$49947 49944 49402
Kim	Southwest College of Naturo-pathic Medicine	Type 2 Diabetes: Botanical Intervention. A 6-month Randomized, Double-Blind, Controlled Trial	$174088 173868 173868
Lake	University of Arizona	Dendritic Cells and Coccidioidomycosis (Valley Fever)	$50000 50000 50000

Li	Sun Health Research Institute	Estrogen and Alzheimer's Disease: Novel Transgenic and Knockout Animal and Cellular Models	$174969 174574 174425
Lohman	University of Arizona	Prevalence and Prevention of Osteoporosis in Postmenopausal Women at High Risk	$174687 174230 174559
Lopez	University of Arizona	Increasing Access to Breast and Ovarian Cancer Genetic Risk Assessment and Counseling via Telemedicine	$147722 172695 174508
Lopez	University of Arizona	Improving the Clinical Accuracy of Cervical Dysplasia Assessment Via Telemedicine and Adjunctive Laboratory Techniques	$162994 126998 114868
Lorson	Arizona State University	The Role of the SMN/p53 Complex in Spinal Muscular Atrophy	$49500 49500 49500
Lorton	Sun Health Research Institute	Nervous System Regulation of the Immune System in Rheumatoid Arthritis	$175000 175000 175000
Lue	Sun Health Research Institute	Immunotherapy and Alzheimer's Disease: The Interaction of Human Microglial Fcγ Receptors with Antibody-Opsonized Amyloid	$50000 50000 50000
Lukas	Barrow Neurological Institute	Receptors for Nicotine Involved in Reward and Mood	$145262 149730 149922
Lukas	Barrow Neurological Institute	Molecular Bases for Nicotine Effects	$149945 149988 149622
Malan, Jr.	University of Arizona	Endocannabinoid Regulation of Pain Sensitivity in Inhibition of Nerve Growth Factor Release	$116596 113528 118057
Manseau	University of Arizona	Intramolecular Regulation of CAPPUCCINO and Identification of Effectors	$49971 49971 49971

Marshall	University of Arizona	Quantitative Histometry, Growth Factors, Bone Mineral Density and Mammographic Densities	$174769 174942 174569
Martinez	University of Arizona	Role of 14-3-3 in Lung Cancer	$143735 142965 142965
Massia	Arizona State University	Local Gene Therapy Targeting Vascular Graft Hyperplasia	$169929 175000 173350
McEvoy	University of Arizona	Structural Studies of the Apical Protein Complex Formed During Asymmetric Cell Division	$50000 50000 50000
McQueen	University of Arizona	Neurotoxicity of Hydrazines: Mechanisms and Modulation by N-Acetyltransferases	$49995 49995 49995
Merkle	University of Arizona	Aged Endothelial Cell Interactions with Breast Cancer Cells	$43733 47322 45582
Meuillet-May	University of Arizona	Regulation of PTEN Activity in Cancer Cells	$50000 50000 50000
Miesfeld	University of Arizona	Molecular Genetic Analysis of Steroid-Regulated Thymocyte Apoptosis	$50000 50000 50000
Miller	University of Arizona	Infant and Early Childhood Outcome Measures in a Postpartum Relapse Prevention Study	$49696 49344 48881
Miller	University of Arizona	Smoking in Pregnancy: The Role of Apoptosis	$48209 47793 46936
Misra	Arizona State University	Antibiotic Resistance in Bacteria and the Role of TolC in Antibiotic Efflux	$49995 49995 49995

Monroy	Northern Arizona University	Neuro-Intestinal Interactions in Murine Toxoplasmosis	$38493 32102 33708
Montfort	University of Arizona	Nitric Oxide Signaling Through Soluble Guanylate Cyclase	$50000 50000 50000
Moore	University of Arizona	Engineered Biosynthesis of "Unnatural" Natural Products for Drug Discovery	$50000 50000 50000
Morkin	University of Arizona	Molecular Mechanisms of DITPA Actions	$174900 174900 174900
Nakazato	University of Arizona	Development of an Organ Donor Protocol for Use of Dimethyl Sulfoxide to Improve Organ Function in Liver Transplantation	$168515 151340 153819
Nieman	Arizona State University	Specific Binding Interactions of SMN that Result in Spinal Muscular Atrophy	$48978 49974 49896
O'Brien	University of Arizona	Anti-HIV Liposomes	$173909 173909 173909
Ossipov	University of Arizona	Fentanyl-Induced Paradoxic Pain: Antinociceptive Tolerance and Receptor Down-Regulation	$174071 173026 169261
Payne	University of Arizona	Role of cGMP-Dependent Protein Kinase (PKG) in Apoptosis Resistance and Colon Cancer Biomarker Development	$175000 175000 175000
Penn	La Frontera Center, Inc.	Comparing Smoking Cessation Treatments for Persons with Schizophrenia and Other Psychotic Disorders	$109946 108131 111356
Pettit	Arizona State University	Discovery and Development of Novel Anti-infective Agents	$169242 161572 166065

Pettit	Arizona State University	Development of New Anticancer Drugs for Improving Human Cancer Treatment	$250000 250000 250000
Phillips	University of Arizona	Identifying Parameters of Care-giving Crisis	$49580 48292
Pomatto	Cranial Technology Inc.	Efficacy of Cranial Banding Following Endoscopic Assisted Craniectomy for Sagittal Synostosis	$25221 25221
Propper	Northern Arizona University	The Effect of Prenatal and Adult Pesticide Exposure on Stress Responsivity	$173647 154908
Pryor	University of Arizona	Characterization of Alternaria Isolates Associated with Allergenic Asthma	$49986 49982 49918
Rakela	Mayo Clinic Scottsdale	Transmission and Evolution of Hepatitis C Virus Variants in Natural Infection	$104500 108680 113027
Roeske	University of Arizona	Agonist Specific Regulation of the Human Delta Opioid Receptor	$172700 172700 172700
Romagnolo	University of Arizona	Repression of BRCA-1 Transcription by Tobacco Metabolites Mediated by Functional p53	$49500 49500 49500
Romanovsky	St. Joseph's Hospital & Medical Center	Vagal Anti-Inflammatory System	$121298 125407 129690
Romero	University of Arizona	Culturally Appropriate Tobacco Prevention for Youth	$158175 169146 170878
Rose	Arizona State University	Anticancer Agents Based on Irreversible Inhibition of Protein Farnesylation	$50000 50000 50000

Rousseau	University of Arizona	Theoretical Development of Family-Based Elder Abuse Models	$48790
Sadrzadeh	University of Arizona	Tobacco Smoke Activates Neutrophils and Increases Oxidative Damage in Maternal and Fetal Blood	$132729 119470 121197
St. John	University of Arizona	Molecular Determinants of Agonist-Induced Endocytosis of Nicotinic Acetylcholine Receptors	$171002 170122 174722
Scheck	Barrow Neurological Institute	Molecular Analysis and Magnetic Resonance Spectroscopy for the Diagnosis and Prognostication of Human Meningiomas	$175000 175000 175000
Schneider	Southwest Autism Research Center	Environmental Factors in the Etiology of Autism	$50000 50000 50000
Selmin	University of Arizona	Characterization and Function of the mPR in Estrogen Responsive Cell Lines	$49341 49341 49341
Shapiro	University of Arizona	Smoking Cessation in Pregnancy: Motivational Counseling	$149714 141555 135813
Sherman	University of Arizona	Developing New Treatment Strategies for Parkinson's Disease: Selective Control of the Basal Ganglia Pathways	$399798 290687
Silva	University of Arizona	Risedronate Therapy for Osteoporosis In Severe Chronic Renal Insufficiency	$50000 50000 50000
Stamer	University of Arizona	Molecular Mechanisms of Cannabinoid Effects in Glaucoma	$167577 146952 140352
Sterling	University of Arizona	Assessing the Zoonotic Potential of Giardia Duodenalis and Trypanosoma Cruzi in Arizona	$19496 21504 20216

Trevor	University of Arizona	Treatment of Adenomatous Polyps and Colon Cancer Using Antigen-Specific Immunotherapy	$174321 174651 174838
Trouard	University of Arizona	3D Diffusion - Weighted Radial MRI of the Brain	$50000 50000 50000
Valeski	University of Arizona	Why Do Some Venules Leak and Not Others?	$48793 48867 46534
Wilson	University of Arizona	Neurophysiological Analysis of Motor Sequence Learning	$49764 49764 49764
Witten	University of Arizona	Role of Lung Afferent Nerves in Smoke-Induced Acute Lung Injury	$120387 115443 115443
Wu	Barrow Neurological Institute	Role of GABAa Receptor in MPP+ - Induced Dopamine Neuron Degeneration	$49861 49899 49538
Wu	Barrow Neurological Institute	Molecular Bases of Functional a4 Nicotinic Acetylcholine Receptors	$148928 146876 148379
Yamamura	University of Arizona	The Role of Calcium Calmodulin Dependent Kinase in Adenylyl Cyclase Superactivation upon Chronic Delta Opioid Treatment	$172700 172700 172700
Yang	University of Arizona	DNA and Topoisomerase I Interactions of Novel Homocamptothecin Anticancer Drugs	$49940 49940 49940
Zhang	University of Arizona	The Role of Catalase in Skin Cancer Prevention	$49995 49995 49995
Zunkel	Arizona State University	Integrating Primary Care and Behavioral Health Care Services to Promote the Identification and Treatment of Depression, Anxiety, and Substance Abuse	$50000 50000

Joyce	Sun Health Research Institute	Arizona Parkinson's Disease Center	$505000 505000
Shen	Sun Health Research Institute	Gene Targeting TNF Receptors Reveals Dopamine Cell Death and Protection by Estrogen Therapy: Implication for Parkinson's Disease	$147500 147500
Sherman	University of Arizona	Developing New Treatment Strategies for Parkinson's Disease: Selective Control of the Basal Ganglia Pathways	$748382 518482
Sierks	Arizona State University	Controlling a-Synuclein Aggregation as a Tool for Studying Parkinson's	$147500 147500
Yamamura	University of Arizona	Functional Desensitization of D2 Receptor Signaling Upon Chronic Dopamine Treatment	$148500 148500

SECTION G

INDEX

Principal Investigators, New Awards and Continuing Contracts