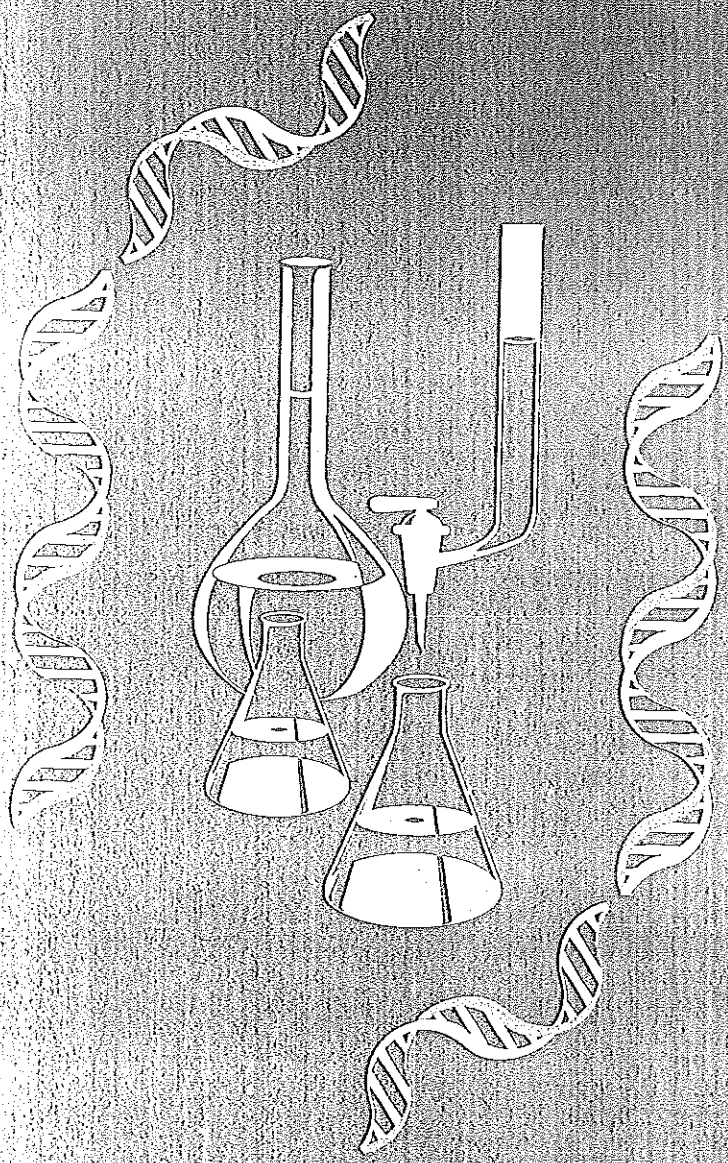

2002 – 2003

Annual Report

Arizona Disease Control Research Commission

January 2004

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
2002 – 2003

Janet Napolitano, Governor

Henry Reeves, Ph.D., Chairman

COMMISSION MEMBERS

General Public
C. Eileen Bond, J.D.
Lyra McCoy, M.P.H.
Steven Weinberg, J.D.

Medical Community
William Crisp, M.D.
Eladio Pereira, M.D.
Colleen Brophy, 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
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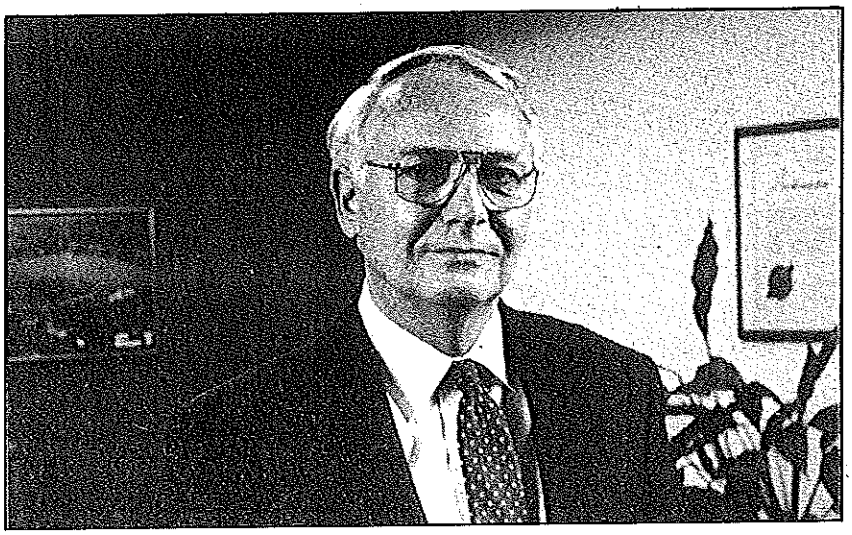
January 2004



Message from the Chairman

As Chairman of the Arizona Disease Control Research Commission (ADCRC), I am pleased to forward this agency's Fiscal Year 2003 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 Initiatives, passed by the voters in 1994 and 2002. The ADCRC receives five percent of the revenues collected from each of these taxes to fund medical 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 in FY 2001 and 2002. 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. These projects will end in early 2004.

The Translational Genomics Research Institute (TGen) selected Arizona for their new home, and the Commission provides $5,500,000 in State funds annually to support TGen. The presence of TGen has stimulated cooperative research efforts among the three universities, the public and the private sectors. Commissioner Steven Weinberg serves on the TGen Board of Directors and former Commissioner José Cardenas is the Board Chairman. The Commission will continue to encourage and support collaborative research efforts among the state's institutions, for-profit businesses and nonprofit organizations like Tgen.

The Commission has formed a strong partnership with the Flinn Foundation to move the biosciences forward in Arizona. The Flinn Foundation commissioned a study by the Battelle Institute to determine the steps that Arizona should take to become a leader in specific bioscience and medical research niches. The plan that is being developed is known as the Battelle Roadmap. Three Commissioners and the Executive Director are serving on committees that are

actively participating in mapping a new direction for Arizona's future in the biosciences. We look forward to joint projects with the Flinn Foundation in FY 2004.

The Commission continues to be involved in technology transfer and the patenting and licensing of discoveries funded with ADCRC monies. The Commission was awarded two patents in FY 2003 and two others are pending.

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 on the Commission look forward to another productive 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

Steven Weinberg, J.D. Phoenix

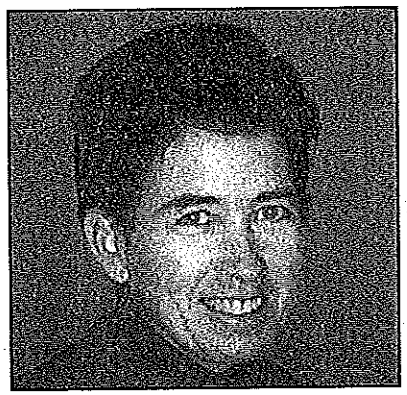
Greenberg Traurig, LLP

Commissioner Weinberg received his B.A. from State University of New York at Buffalo and his J.D. *cum laude* from St. John's University. He has been representing major corporations in trademark, copyright, software, and advertising litigation, principally in federal courts for over 22 years. He also represents clients in major IP and information technology transactions and oversees a global trademark prosecution practice. Commissioner Weinberg is the only Arizona lawyer included in the prestigious *International Who's Who of E-Commerce Lawyers* and the *International Who's Who of Trademark Lawyers*. He is listed in *Best Lawyers in America*. He has served as Editor In Chief of *The Trademark Reporter*, Editor of *The Journal of the Copyright Society of the USA*, and on the Board of the International Trademark Association. He serves on the Board of the Arizona Technology Council. Commissioner Weinberg was appointed in April of 2002 by Governor Hull. His term expires in May 2005.

Medical Community

Colleen M. Brophy, M.D. Scottsdale

Chief of Vascular Surgery, Carl T. Hayden VAMC

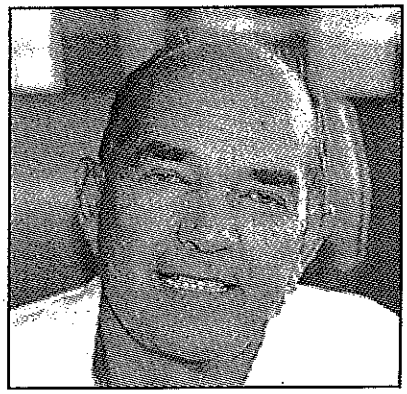


Commissioner Brophy received her undergraduate and medical degrees at the University of Utah. She completed her surgical residency at Yale University followed by a fellowship in vascular surgery at Harvard University. She is a Research Professor of Bioengineering at Arizona State University, a Clinical Professor of Surgery at the University of Arizona and the director of the Proteins and Peptides as Pharmaceuticals Center in the newly formed Arizona Biodesign Institute at ASU. She is a founder and president of a biotechnology start-up company, Arizona Engineered Therapeutics, which is developing proteomic based therapeutics. Dr. Brophy is an editor for the Journal of Surgical Research, sits on the Executive Committee of the Surgical Research Committee of the American College of Surgeons, Chairs the Committee on Women's Issues for the Society for Vascular Surgery, and is a member of the NIH Surgery and Bioengineering Study Section. Commissioner Brophy was appointed in 2002 by Governor Napolitano. Her term expires in May 2005.

Medical Community

William Crisp, M.D. Paradise Valley

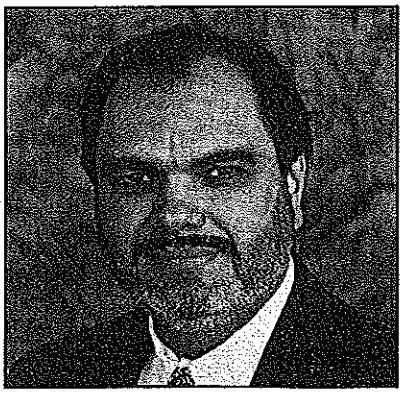
Resident Education Gynecology/Oncology,
Samaritan Regional Medical Center



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 reappointed to the Commission in 2001 by Governor Hull. His term expires in May 2004.

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.

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 2002-2003 Commission Activities

The Commission administers 64 contracts in five programs—tobacco-related research, anticnacer drug discovery, unrestricted medical research, brain research and Parkinson's disease research—with medical researchers in Arizona as of July 2002. In addition to the regular Commission programs, ADCRC will be supporting the Translational Genomics Research Institute with a $5,000,000 per year for a period of five years and a $500,000 annual award for a period of ten years. The section headings list each 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-E. 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 2003 can be found in Section F. The medical research projects began in July, FY 2003, while year one of the Parkinson's disease research projects began on April 1, FY 2002 and ended March 31, 2003.

Approximately 765 Requests for Proposals (RFPs) for 2003-2004 awards were mailed to potential applicants in September 2002. The amount available for new unrestricted medical research was approximately $1,700,000. In response to the RFPs, the Commission received 90 unrestricted medical research proposals. Section G lists the research proposals received in response to the 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 2003 the Commission selected 20 proposals for funding. During 2003-2004 the ADCRC will be managing 64 contracts.

SECTION A

CONTINUING CONTRACTS

TOBACCO-RELATED RESEARCH

YEAR THREE

FY 2003

Eugene W. Gerner, Ph.D.

University of Arizona
Award Amount FY03: $150,000

Interdisciplinary Basic Science Program in Colon Carcinogenesis

Colon cancer is associated with tobacco use and is the second leading cause of cancer deaths in Arizona and the United States. The hypothesis to be tested in this research was that tobacco-specific carcinogens interact with specific genetic risk factors to increase the likelihood of colon cancer incidence. This interaction could occur in colon epithelia or stroma. Specific aims were to determine if tobacco-specific carcinogens interact with genetic risk factors to influence colon cancer development in rodent models. It was found that the tobacco-specific carcinogen N-nitrosonornicotine (NNN) increased intestinal carcinogenesis caused by mutation of the adenomatous polyposis coli (APC) tumor suppressor gene in the Min (multiple intestinal neoplasia) mice, but not in these same mice lacking one or both alleles encoding the inducible nitric oxide synthase (NOS2). Loss of NOS2, by itself, was associated with increased intestinal and colon tumor numbers in Min mice. Mice lacking the NOS2 alleles displayed reduced levels of apoptosis in stem cell compartments of intestinal crypts. Treatment with NNN had no detectable effects on the expression of either NOS2 or NOS3, and only marginal effects on expression of angiogenic factors, in intestinal tissue. These data suggest that tobacco-specific carcinogens in cigarette smoke may increase the risk of colon cancer by inhibiting NOS2-dependent apoptosis in carcinogen-damaged colonic stem cells, thereby increasing the likelihood that these initiated cells will progress to neoplasia.

Publications:

Babbar N, Gerner EW. Polyamines as modifiers of genetic risk factors in human intestinal cancers. **Biochemical Society Transactions** 31:388-392, 2003.

Babbar N, *et al.* Cyclooxygenase-independent induction of apoptosis by NSAIDs is mediated by polyamines in colon cancer. **Journal of Biological Chemistry,** 2003 (Accepted).

Baines A, *et al.* Selenomethionine inhibits growth and suppresses cyclooxygenase-2 (COX-2) protein expression in human colon cancer cell lines. **Cancer Biology & Therapy** 1:370-374, 2002.

Boivin GP, *et al.* Pathology of mouse models of intestinal cancer: consensus report and recommendations. **Gastroenterol** 124:762-777, 2003.

Childs A, Mehta D, Gerner EW. Polyamine-dependent gene expression. **Cellular and Molecular Life Sciences** 60:1394-1406, 2003.

Gerner EW, Ignatenko NA, Besselsen DG. Preclinical models for chemoprevention of colon cancer. In: *Recent Results in Cancer Research.* Blue Springer Series. **Springer-Verlag,,** New York, New York, 2003.

Gerner EW, Ignatenko NA, Besselsen DG. Preclinical models for chemoprevention of colon cancer. *Recent Results in Cancer Research* 163:58-71, 2003.

Husbeck B, *et al.* Over expression of the redox protein thioredoxin-1 inhibits spermidine/spermine N^1-acetyl- transferase gene expression and enzyme activity in MCF-7 human breast cancer cells. Biochem & Biophys Research Communications 306:469-75, 2003.

Ignatenko NA, Gerner EW. Suppression of HIV1 LTR by a mutant heat shock factor 1. Experimental Cell Research 288:1-8, 2003.

Kramer DL, Gerner EW. Therapeutic strategies targeting polyamines. In: *Cancer Chemoprevention. Volume 1. Promising Cancer Chemopreventive Agents.* Kelloff, GJ *et al.* (eds.) Humana Press, Totowa, New Jersey. 2004, (In press).

Martinez JD, *et al.* The molecular biology of cancer. In: *Burger's Medicinal Chemistry and Drug Discovery, 6th edition,* Abraham D. (ed.), Wiley, Hoboken, New Jersey, 5:1-50, 2003.

Martinez ME, *et al.* Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proceedings of the National Academy of Sciences (USA) 100:7859-7864, 2003

Qu N, *et al.* Inhibition of human ornithine decarboxylase activity by enantiomers of difluoromethyl-ornithine. Biochem Journal, 2003, (In press).

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY03: $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 over-express c-myc, that down-regulation of this gene leads to slowing of tumor growth. During the second year of the award we have explored the structure-activity relationships 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:

Siddiqui-Jain A, *et al.*. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-myc transcription. Proc. Natl. Acad. Sci. USA 99:11593-11598, 2002.

Jean M. Schmidt, Ph.D.

Arizona State University
Award Amount FY03: $135,525

In Vivo Efficacy Evaluation of New Anticancer Drugs

Tests of seven anticancer drugs, all drug discoveries of the Arizona State University Cancer Research Institute, have gone forward in a SCID *in vivo* xenograft system, and in cell cycle experiments using flow cytometry and in HUVEC tube/cord assays *in vitro* to measure the inhibition of tube-forming ability--which can indicate possible anti-angiogenic activity for the drug. We have used several histological type of tumors, including H460 (human lung large cell carcinoma);THP-I, a human monocytic leukemia line, FADU,(another respiratory, tobacco-related tumor); and several others. The most promising drug, and thus the most extensively evaluated, is Auristatin PYE, a synthetic drug based on Dolastatin 10, which shows good activity against several tumors and has at least two mechanisms of cell cycle inhibition, both resulting in apoptosis (programmed cell death).

Publications:

Pettit GR, *et al.*. Antineoplastic Agents. 465. Structural Modifications of Resveratrol: Sodium Resverastatin Phosphate. **Journal of Medicinal Chemistry** 2002, (In press).

Pettit GR, *et al.*. Synthesis of 10b(R)-hydroxypancratistatin, 10b (s)-hydroxy-1-epipancratistatin, 10b(s)-hydroxy-1 ,2-diepipancratistin and related isocarbostyrils. **Heterocycles** 56:139-155, 2002.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY03: $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 three years of work of this project, drugs targeting histological cancer types afflicting many Arizonans have been developed namely leukemia, melanoma and non-small-cell lung cancer. Our approach to developing these drugs has been to exploit levels of the reducing enzyme DT-diaphorase in our drug designs. 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. A melanoma-specific drug has been developed based on phenylalanine uptake by these cells.

Molecular models of proteins have been used to develop new classes of drugs. Thus, a novel heat shock protein (Hsp 90) inhibitor has been developed that exploit high levels of this protein in certain cancers such as leukemia.

Topoisomerase II inhibitors have been designed based on marine natural products. These efforts have yielded active compounds by methods amendable to library synthesis.

The cyclopent[b]indole quinones, a rationally designed antitumor agent activated by DT diaphorase, has been selected for *in vivo* screening by the National Cancer Institute.

Henry I. Yamamura, Ph.D.

University of Arizona
Award Amount FY03: $49,522

Molecular Mechanism of Cannabinoid Action at the Human CB1 Receptor

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

We have been studying the effects of cannabinoids on nerve cell surfaces known as the cannabinoid CB 1 receptor. These receptors are found in the brain and the eye and interact with G-protein to produce their effects. We have previously demonstrated that a specific G-protein ($G_{\gamma z}$) appears to be involved in cannabinoid and opioid antinociception using antisense technology. However, the antisense technology appears to have some limitations. A new technique, "RNA-interference" appears to silence the synthesis of G-proteins up to 90%. We are investigating this new technology by synthesizing primers for the G-proteins and by measuring antinociception after cannabinoid treatment.

Robert A. Berg, M.D.

University of Arizona
Award Amount FY03: $50,000

Optimal Treatment of Prolonged Ventricular Fibrillation: CPR 1st versus Defibrillation 1st

Immediate defibrillation is clearly the treatment of choice for typical ventricular fibrillation (VF) sudden cardiac arrest, but the optimal treatment for prolonged ventricular fibrillation is not well established. This is important because most out-of hospital VF cardiac arrests are prolonged before emergency medical services are available. It was our objective to determine whether a brief period of cardiopulmonary resuscitation (CPR) prior to defibrillation would improve readiness of the myocardium for defibrillation and thereby improve outcome. During the first 2 years we demonstrated that provision of pre-countershock CPR improved the readiness for defibrillation, but did not improve ultimate outcome. In the last year, we repeated these studies after acute coronary obstruction. In this clinically relevant swine model of prolonged VF cardiac arrest after acute coronary obstruction, pre-countershock CPR did not improve readiness for defibrillation. In fact, pre-countershock CPR resulted in substantially worse 24-hour outcome.

Qin Mary Chen, Ph.D.

University of Arizona
Award Amount FY03: $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 hypertrophy marker gene ANF or βMHC. Microarray analyses have indicated that oxidants activate antioxidant/detoxification genes and decrease the expression of muscle genes. Three branches of MAP kinases are activated by oxidants: ERKs, p38 and SAPKs. Nicotine did not appear to activate these kinases or enhance the activation of these kinases by oxidants. Activation of calcineurin can only be observed with a brief treatment of H_2O_2. The reporter gene assay showed 2-3 fold induction of NF-AT3 promoter activity by oxidants and the data have been verified by gel shift assay. The ADCRC funding has contributed to 9 abstracts and 10 manuscripts.

Publications:

Chen QM, *et al.* Down regulation of p53 with HPY E6 expression inhibits apoptosis but induces oncotic cell death associated with mitosis in oxidant response of human diploid fibroblasts. Oncogene 21:5313-5324, 2002.

Purdom S, Chen OM p66Shc: At the crossroad of oxidative stress and genetics of aging. Trends Mol Med 9:206-210, 2003.

Tu YC, Bahl JJ , Chen QM. Distinct roles of ERKs and p38 MAPK in oxidant- induced AP-1 activation and cardiomyocyte hypertrophy. Cardiovascular Tax 3(2):119-133, 2003.

ABSTRACTS:

Purdom S, Wood J, Chen QM. Cell type dependent signaling web of oxidative stress. Toxicologist 72:158, 2003.

Coronella-Wood J, Sun H, Chen QM . Regulation of c-Fos phosphorylation and AP-1 activation by oxidants in cardiomyocytes. Toxicologist 72:389. 2003.

Chen, QM. Stress activated signal transduction pathways Toxicologist 72:561, 2003.

Sun H, Chen QM. Induction of cyclooxygenase-2 by H_2O_2 and corticosterone in cardiomyocytes Toxicologist, 2004, (In press).

Purdom S, Johnson J, Chen QM. Induction of antioxidant and detoxification response with H_2O_2. Toxicologist, 2004, (In press).

Chen QM, *et al.* Reprogramming gene expression with oxidative stress and stress hormones: A paradox of antioxidant responses. Toxicologist, 2004, (In press).

Janet L. Funk, M.D.

University of Arizona
Award Amount FY03: $103,698

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 the body's own defense systems might be harnessed to combat this injury. Our laboratories have identified a new substance that is produced by the brain in response to stroke. When brain arteries clog or blood flow is temporarily interrupted, as with cardiac arrest, this protein is produced by blood vessels at the site of injury and causes the blood vessels to dilate and allow more blood to flow to the injured area. Proof that this commercially available protein can be used to treat strokes comes from our animal studies demonstrating prevention of brain injury in strokes that can cause partial paralysis.

Publications:

Funk JL, *et al.* PTHrP induction in focal stroke: a neuroprotective vascular peptide. Amer. J. Physiol. Regul. Integr. Compo Physiol. 284:R1021-R1030, 2003.

Abstracts:

Ritter L, *et al.* PTHrP induction after permanent middle cerebral artery occlusion: a vasodilator with neuroprotective effects. Soc. Neuroscience , 28:392.16, 2002.

Tipton A, *et al.* Neutrophil function in aged *versus* young rats subjected to global cerebral ischemia and reperfusion. Experimental Biology, 2003.

Funk JL, *et al.* Increase in PTHrP immunoreactivity in rat brain at site of neuronal death following transient global ischemia. Endocrine Soc. Annual Meeting, 2003.

Ronald J. Lukas, Ph.D.

St. Joseph's Hospital
Award Amount FY03: $327,844

Stroke, the Blood Brain Barrier, Nicotine Acetylcholine Receptors

Three separate research projects are involved in this program of study seeking (i) to establish effects of nicotine on the blood brain barrier (8BB), 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; (ii) to establish whether nicotinic acetylcholine receptors (nAChR), which are targets of tobacco nicotine action and critically involved in natural chemical signaling throughout the body, are on micro vessels or other components of the BB8; and (iii) to establish some features of nAChR that are involved in BBB function and are likely to contribute to effects of stroke. The overarching hypothesis of the research program is that tobacco use and nicotine exposure affects the structure and transport characteristics of the BBB, and that these effects have deleterious consequences during stroke.

Work done in year three of the project has provided additional evidence that nAChR are expressed on or near brain blood vessels and are relevant to BBB function. We have extended studies using 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 radio ligand 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. Pharmacological studies indicating that effects of nicotine on brain blood vessel permeability can be blocked by systemic administration of either membrane-permeable or membrane-impermeant nAChR antagonists suggest either autonomic action of nicotine's effects or actions at lumenal nAChR on central vessels. Collectively, data obtained to date continues to be consistent with the project's central hypothesis that nicotine exposure alters BBB and brain micro vascular function. Our findings also indicate important roles for nAChR in these effects. These findings are relevant to an improved understanding of stroke and better strategies for stroke prevention and treatment. Furthermore, the results suggest that it might be possible to facilitate therapeutic drug access to the brain through manipulation of nAChR signaling regulating BBB function.

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

University of Arizona
Award Amount FY03 : $49,902

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 characterized the expression of FP_s in human tissues by Northern blot analysis, PCR and immunohistochemistry. FP_s appears to be expressed in human heart and placenta and possibly skeletal muscle and liver. In the placenta, FP_s was expressed specifically in endothelial cells, trophoblasts and decidual cells. The localization of FP_s in these cells and tissues suggests a role for FP s in cardiovascular function and/or in the reproductive functions of the human placenta. Further characterization of the physiological function of FP_s may provide a novel therapeutic target for the treatment of cardiovascular or reproductive disorders.

Publications:

Fujino H, Regan JW . Prostaglandin $F_{2\alpha}$ stimulation of cyclooxygenase-2 promoter activity by the FP_β prostanoid receptor. Eur J Pharmacal, 465:39-41, 2003.

Fujino H, Wei X, Regan JW. Prostaglandin E_2 induced functional expression of early growth response factor-l by EP_4, but not EP_2, prostanoid receptors via the phosphatidylinositol 3-kinase and extracellular signal-regulated kinases. J Bial Chem, 278:12151-12156, 2003.

Vielhauer GA, Fujino H, Regan JW. Cloning and localization of FP_s, a six-transmembrane mRNA splice variant of the human FP prostanoid receptor. Arch Biochem Biophys, 421:175-185, 2004.

Larry Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY03: $126,000

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

As per the amended protocol we have been investigating the relationship between water quality and cholesterol induced pathology. The basic observation of import to citizens of Arizona is that use of pure drinking water may reduce the pathology caused by increased circulating cholesterol levels, body-wide. The reason for the cost extension is to finish performing studies on collected tissue samples in the preliminary stages. These are highlighted below.

As a brief background we have found that distilled water attenuates the accumulation of the AD toxin, beta-amyloid, caused to be overproduced by increased circulating cholesterol (cholesterol-fed rabbits), and that some agent in tap water affected normal clearance of beta-amyloid to the blood from the brain. In addition, use of distilled water rather than tap water reduced the cholesterol-induced pathology of the spleen and liver in the rabbit. We have identified a very likely candidate, when added to distilled drinking water, increases pathology in the brains of cholesterol-fed rabbits but not pathology of the spleen or liver. The agent in the tap water that augments cholesterol-induced pathology of systemic organs remains a mystery.

The preliminary studies requiring completion include localization of the cholesterol transport protein, which may be used by beta-amyloid for natural clearance from the brain. Pilot studies suggest an increase in the transporter protein among animals on distilled water and a reduction among those cholesterol-fed animals on distilled water with the agent included.

Edward D. French, Ph.D.

University of Arizona
A ward Amount FY03: $127,288

Nicotine Dependence and Dopamine Neurons: Electrophysiological and Molecular Studies

The output of the ventral tegmental-mesolimbic dopamine pathway is critical in mediating the reinforcing effects of many drugs of abuse, including nicotine. Nicotine produces its reinforcing/motivational effects by altering the activity of dopamine neurons in the ventral tegmental area (VTA), often referred to as the brain's reward pathway. Although nicotine binds to nicotinic acetylcholine receptors to produce these responses, it is unknown which of the several different nicotinic acetylcholine receptor subtypes mediates its reinforcing effects. Paradoxically, we found that mouse VTA dopamine neurons are actually inhibited by nicotine. Moreover, with repeated exposure to nicotine the neuronal inhibitions are drastically reduced. Also, non-dopamine neurons within the vicinity of the dopamine neurons are excited by nicotine, an effect which may play an important role in the overall activity of the meso limbic reward pathway. Both effects appear to be mediated through the activation of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor. Since the $\alpha 4\beta 2$ subtype is the most ubiquitous nicotinic acetylcholine receptor in the CNS, it should be considered a good pharmacological target for developing novel drugs to treat addiction to nicotine containing products.

Publications:

French ED, Sandoval K. Determination of the ICR mouse as a nicotine-sensitive or nicotine-resistant strain of mice. Society for Neuroscience, 2003.

Sandoval K, French ED. An electrophysiological assessment of the ventral tegmental area (VTA) dopamine neuronal response to nicotine in mice chemically treated with nicotine. Society for Neuroscience, 2003.

Goerge AA, *et al.* Detection and cellular distribution of nicotinic acetylcholine receptors and their subunit transcripts in mouse midbrain nuclei. Society for Neuroscience, 2003.

Hugh Miller, M.D.

University of Arizona
Award Amount FY03: $133,872

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

The impact of smoking on women, children and their families remains a staggering problem for residents of the state of Arizona. We have suggested that postpartum relapse prevention is a unique opportunity to interrupt what is otherwise a self-perpetuating cycle of compounded healthcare consequences. Every women who achieves smoking cessation during pregnancy and extends abstinence into the future rescues herself and her child/family from direct and indirect tobacco exposure. We spent the last year completing enrollment and are now into our final 12-month follow-up of those last patients enrolled. The trial called for the randomization of postpartum women to standard of care, relapse prevention counseling with a placebo, or bupropion SR. The interim analysis of our baseline population characteristics has revealed important insight into the unique qualities of this group of women. We anticipate completing our final analysis once all 12-month follow-up is accomplished later this year.

James F. Collins, Ph.D.

University of Arizona
Award Amount FY03: $49,500

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

Surfactant lines the lungs and keeps the lungs from collapsing on themselves. Smoking adversely affects surfactant production and thus lung function. Surfactant producing cells in the lung intake phosphate via a phosphate transporting protein called the NaPi-IIb co-transporter. Our novel data have demonstrated that second-hand cigarette smoke exposure decreases expression of the NaPi-IIb gene in laboratory rodents. We surmise that this reduction in gene expression may be related to the smoking-induced perturbations in surfactant production. Other experiments have been initiated to develop a tissue-specific inactivation of this gene to assess the true physiological role that this gene plays in the lining of the lungs. 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 FY03: $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 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 progenitor cells isolated from infants of mothers who have been exposed to nicotine are severely limited in their ability to develop into immune T-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 FY03: $50,000

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

The goal of this research was to understand the effects of constituents of cigarette smoke on ovarian function. We determined that nicotine decreases production of androstenedione, an essential precursor for estrogen biosynthesis. Inhibition of androstenedione production by nicotine may contribute to lower estrogen levels 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 found that inhibition of oxidative stress did not influence the effects of nicotine on androstenedione production. Moreover, we found that several markers of oxidative stress are undetectable in ovarian tissues. Taken together, these results indicate that nicotine interferes with normal hormone production in the ovarian follicle. However, the mechanism underlying this deleterious effect of nicotine on ovarian function does not appear to involve oxidative stress.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY03: $150,000

Analysis of Autoantibodies to T-cell Receptor in Rheumatoid Arthritis

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are classic autoimmune diseases associated with elevated levels of autoantibodies. Both are prevalent in the State of Arizona, but RA has an abnormally high incidence of approximately 5% in the Tucson area due to the influx of individuals suffering from the disease and the high percentage of Native Americans. High levels of RA-associated autoantibodies termed rheumatoid factors (RFs) correlate with poor long term prognosis, and a correlation between levels of these autoantibodies and smoking has been documented in males. Furthermore, smoking causes a more rapid progression of RA associated lung disease and significant increases in need for clinical intervention. Under previous support of the ADCRC, we found that RA patients have significantly elevated levels of autoantibodies directed against recognition molecules of their own thymus derived lymphocytes (T-cell receptor). We have generated monoclonal autoantibodies from RA patients and determined that these are novel recognition molecules that have the potential to modulate the T-cell arm of the immune system. Autoantibodies of RA patients are usually of the IgM (immune macroglobulin class), whereas those of SLE patients tend to be of the IgG or major serum immunoglobulin class. Our ability to generate these monoclonal molecules gives us the unique opportunity to determine the gene usage in the generation of these in the two autoimmune diseases and to study their biological functions in interaction with T-cells and in modulating the immune response. It is essential to determine whether these disease-related molecules represent populations correlated with the rheumatic diseases or whether these are essentially the same ones expressed in low levels by healthy individuals in immunomodulation. Our combined molecular and genetic approach offers new possibilities for diagnosis and potential therapy.

Publications:

Schluter SF. *et al.* Natural autoantibodies to TCR public idiotopes: potential roles in immunomodulation. Cell Mol Bioi 49:193-207, 2003.

Marchalonis JJ, Jensen I, Schluter SF. Structural, antigenic and evolutionary analyses of immunoglobulins and T-cell receptors. J Mol Recog. 15:260-71, 2002.

Adelman MK, Marchalonis JJ. Endogenous retroviruses as etiological agents in systemic lupus erythematosus. In: Infection and Autoimmunity, Schoenfeld Y, Rose NR (eds.) Elsevier, Amsterdam, The Netherlands, 2003. (In press)

SECTION B

CONTRACTS

ANTICANCER DRUG DISCOVERY

YEAR TWO

FY 2003

Scot W. Ebbinghaus, Ph.D.

University of Arizona
Award Amount FY03: $50,000

Triplex DNA Based Gene Therapy of Lung Cancer

Our lab focus is cancer gene therapy. In this grant we proposed employing a unique system for delivering single-stranded DNA designed to shutdown an important gene called HER2/neu. The product of the HER2/neu gene is a known player in certain cancers such as lung and breast cancer.

To date, we have succeeded in genetically engineering a plasmid gene delivery system designed to produce single-stranded DNA inside cells. When produced in cells, the single-stranded DNA can then bind to the HER2/neu gene forming triplex DNA, a triple-stranded DNA structure. We have introduced this plasmid into a cancer cell line and are now attempting to verify production of single-stranded DNA inside these cells. Once that's established, we will test to see if our single-stranded DNA forms triplex DNA by binding its target gene (HER2/neu) and whether this alters certain cancerous characteristics of these cells such as growth and drug resistance.

Vince Guerriero, Ph.D.

University of Arizona
Award Amount FY03: $49,785

The Stress Protein Hsp70 as a Target for Anticancer Therapy

The purpose of this proposal was to provide preliminary information on a novel molecule that can be used to treat certain types of cancers. Cancers are a major health problem for the people of the State of Arizona and new methods to treat such diseases must be developed. It is well known that the stimulation of cell death in cancer cells is a target for the development of new therapies. This laboratory has recently identified a novel human gene that codes for a protein called HspBP1 that could be used to stimulate cell death in cancer cells while having a minimal effect on normal cells. The research accomplished here provided the first information about the structure of HspBP1 and how this relates to function. This information will be used to design new treatments for cancer.

Publications:

Kabani M, *et al.* HspBP1, a homologue of the yeast Fes1 and Sls1 proteins, is an Hsc70 nucleotide exchange factor. **FEBS Letters** 531:339-342, 2002.

McLellan CA, *et al.* HspBP1, an Hsp70 Cochaperone, has two structural domains and is capable of altering the conformation of the Hsp70 ATPase Domain. **J. Biol. Chem.** 278:19017 - 19022, 2003.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY03: $220,000

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

The overall goals of this project are to discover, evaluate and develop novel anticancer drugs from microorganisms associated with rhizospheres of desert plants growing under semi-extreme conditions. During the course of the second year of the project, we have prepared several derivatives of a fungal metabolite, terrecyclic acid A, which we have isolated previously and shown to be effective against the Lewis non-small cell lung cancer mouse model. Of these, terrecyclic acid A methyl ester was found to be more effective against lung cancer; whereas, dihydro-terrecyclic acid A was inactive. The data accumulated thus far on the mechanism of anticancer activity of terrecyclic acid A lead us to believe that it acts by causing oxidative stress and depletion of glutathione. Further studies to elucidate its mechanism of action are currently in progress. We have also evaluated 394 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 12 were active in the primary heat shock response activation assay. We have optimized the luciferase-refolding assay for screening extracts which are positive in the primary heat shock response activation assay. The extracts showing activity in this secondary assay and strong anti-angiogenic 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 and development of a novel anticancer agent active in Lewis non-small cell lung cancer 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 FY03: $137,500

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

Each year approximately 15,000 Americans die of malignant glioma (glioblastoma multiforme or GBM). Even with therapeutic intervention (surgery, radiotherapy and chemotherapy), the median survival is only 28 weeks. Immunotherapy is a novel treatment strategy designed to activate the human immune system to attack and eradicate cancer cells. Dendritic cells (DC) may prove the most potent in this line of intervention. DC are powerful antigen-presenting cells that stimulate the body's immune responses. When isolated DC are pulsed with (or fed) tumor proteins (antigens) and injected, the DC present antigen to T-cells that can then recognize and kill tumor. In Year 2 of our studies, we have investigated glioma cell products that could be used in the pulsing of DC for the generation of a DC-based vaccine for the treatment of glioma. Our results suggest that pulsing of DC with glioma cell lysate may prove best for rapid product development.

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY03: $149,677

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

Ecteinascidin 743 (ET743), a natural product derived from the Caribbean tunicate *Eteinascidia turbinata*, is a potent antitumor agent currently in phase II clinical trials. Although DNA is considered to be the macromolecular receptor for ET743, the precise mechanism by which ET743 exerts its remarkable antitumor activity has not yet been elucidated. Thus, the objective of this study was to provide a further rationale for the antitumor activity of ET743 and its analogs and for the drug resistance mechanisms of tumor cells against ET743 by identifying cellular proteins that recognize and bind to ET743–or its analogs–DNA adduct.

By developing a novel strategy for purifying ET743-DNA binding proteins, we were able to enrich ET743-DNA binding proteins by excluding other cellular proteins. ET743-DNA affinity column was used to further purify ET743-DNA binding proteins from other cellular proteins. To determine the identity of the ET743-DNA binding proteins, these proteins were separated on a SDS-polyacryamide gel, gel-purified and digested with trypsin to generate peptide fragments. The molecular weight of the resulting peptides was analyzed by MALDI-TOF. A search of MS-fit (algorithm by Peter Baker and Karl Clauser) with resulting peptides offered four human protein candidates (Ku70/80, DNA-PK, DDB-1, and RPA1). Those proteins were previously known to be involved in the DNA repair processes by recognizing the damaged region. This raises the possibility that the biological functions of those proteins might be conferring drug resistance or sensitivity to tumor cells.

Ronald Lukas, Ph.D.

Barrow Neurological Institute
Award Amount FY03: $27,500

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

Small cell carcinoma of the lung (SCCL) is a deadly lung tumor. Cerebellar medulloblastoma (CMED) is the most common brain tumor in children. 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 composed as unique combinations of building blocks (subunits). nAChR 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 SCCL or CMED 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 was that abnormal chemical signaling through nAChR contributes to formation of SCCL and/or CMED. The major goal of this pilot project was to determine whether drug therapy targeting nAChR, alone or in combination with other tumor cell treatments, has utility in controlling SCCL or CMED division or maturation. Identification and characterization of nAChR in these cells was an auxiliary aim.

Completed work indicates that nicotine exposure does not alter survival of human CMED cells from recurrent tumors or of SCCL cells from primary tumors either when applied alone or in the presence of known chemotherapeutic agents. Perhaps this is because recurrent CMED and primary SCCL cell lines do not express nAChR as functional or ligand binding entities, although they do express some forms of some nAChR subunits as messenger RNA. Although these studies succeeded in providing some definitive conclusion, additional work is needed to ascertain whether drugs targeting nAChR that are known to be expressed by SCCL cell lines derived from recurrent tumors could provide novel therapies to treat these secondary types of tumor, which show enhanced resistance to conventional chemotherapeutic agents.

Eugene A. Mash Jr., Ph.D.

University of Arizona
Award Amount FY03 : $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 the normal process by which abnormal cells die. Modulation of Akt is therefore regarded as a promising strategy for anticancer drug therapy. We are developing chemical inhibitors of Akt function for possible use as anticancer drugs. Compounds related to two lead compounds previously identified by us were synthesized and were tested for Akt binding activity. Three promising drug candidates have emerged and are currently in refinement.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY03: $400,000

Accelerated Discovery and Development to Clinical Trials of New Anticancer Drugs: Renewal

The past year we have made very exciting progress towards improving human cancer treatment.

Presently, the initial Phase I human cancer clinical trials of combretastatin A-1 phosphate prodrug (CA 1 P, now known as Oxi-4503) are planned for early next spring under the auspices of Cancer Research UK. We believe in certain circumstances CA1P may be superior to CA4P. Meanwhile, the clinical development of CA4P has progressed with initiation in the past few months of the following clinical trials: as a single agent (Phase II), with radiotherapy (Phase I/II), with carboplatin and paclitaxel (Phase I/II), with carboplatin (Phase Ib), and in another single agent (Phase I/II). Since fluorcomstatin, another key objective of this research contract, appears to have a somewhat different mechanism of action, we have

concentrated heavily on the scale-up syntheses over the past year and are ready for advanced animal studies. We are also advancing with the scale-up synthesis of pancratistatin prodrug. The synthetic production of hydroxyphenstatin prod rug has been completed and has provided some 12 grams for extended animal studies. All of that is exceptionally promising for improving future cancer treatments.

Publications:

Holwell SE, *et al.* Combretastatin a-1 phosphate a novel tubulin-binding agent with *in vivo* vascular effects 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.

Hill SA, *et al.* Preclinical evaluation of the antitumor activity of the novel vascular targeting agent Oxi 4503. **Anticancer Research** 22:1453-1458, 2002.

Nabha SM, *et al.* Combretastatin-A4 prodrug induces mitotic catastrpophe in chronic lymphocytic leukemia cell line independent of caspase activation and poly (ADP-ribose) polymerase cleavage. **Clinical Cancer Research** 8:2735-2741, 2002.

Holwell SE, *et al.* Anti-tumor and anti-vascular effects of the novel tubulin-binding agent Combretastatin A-1 phosphate. **Anticancer Research**, 22:3933-3940, 2002.

Pettit GR, *et al.* Antineoplastic agents 460. Synthesis of Combretastatin A-2 prodrugs. **Anti-Cancer Drug Design**, 16:185-193, 2001.

Luke Whitesell, M.D.

University of Arizona
Award Amount FY03: $185,637

Heat Shock Proteins as Targets for Drug Discovery

To achieve the goal of exploiting the molecular chaperone Hsp90 as a new anticancer drug target, we need to find better drugs with which to alter its function both in cells and in whole animals. Recently it was discovered that the readily available antibiotic novobiocin binds to Hsp90 at a different site than all the other Hsp90-active drugs that we and others have worked on in the past. Unfortunately, novobiocin's activity is weak and high concentrations must be used to see any effects on Hsp90's cellular function. With commission support, we have now synthesized and purified many new derivatives of novobiocin and the related compound coumermycin A1. We have also tested these new compounds for their ability to alter Hsp90 function in cells. The research accomplished has provided important new information about the chemistry of this class of drugs and their ability to target Hsp90 in cancer cells. Based on this information we are now combining the chemical features of our most potent derivatives into the design of new compounds that we hope will prove active enough to be useful in the treatment of cancer patients.

Publications:

Hargreaves R, *et al.* Design of Quinolinedione-based geldanamycin analogues. **Bioorganic & Med. Chem. Letters.** 13:3075-3078, 2003.

SECTION C

CONTRACTS

MEDICAL RESEARCH

YEAR TWO

FY 2003

Alyssa Panitch, Ph.D.

Arizona State University
Award Amount FY03: $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) The control of bleeding is not treated because these substitutes do not include factors that will help clot blood and stem bleeding. b) Tissue damaging inflammatory responses are not suppressed because the bodies 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 CD11b/CD18 binding pocket (A-domain) inhibits 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* or to prevent clotting as desired. Future work involves optimization of peptide domains.

Harris Bernstein, Ph.D.

University of Arizona
Award Amount FY03: $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. Success in these areas will depend on improved early detection. We studied the molecular events related to dietary factors (bile acids) and smoking that occur early in the progression to these cancers. Abnormal areas in the normal-appearing inner surfaces of the colon and esophagus (field defects) appear to predispose individuals to cancer of these organs. Our studies relating to these field defects have allowed identification of a number of early changes in specific proteins in the colon and esophagus that may serve as clinically practical biomarkers for assessing risk of further progression to cancer. In particular, we elucidated changes in the expression of genes involved in the key cellular processes of programmed cell death and DNA repair.

Publications:

Garewal H, *et al.* Perils of immunohistochemistry: variability in staining specificity of commercially available COX-2 antibodies on human colon tissue. Digestive Diseases and Sciences 48(1):97-202, 2003.

Crowley-Weber CL, *et al.* Development and molecular characterization of HCT -116 cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. Carcinogenesis 23:2063-2080, 2002.

Romagnolo DF, et al.. Deoxycholate, an endogenous tumor promoter and DNA damaging agent, modulates BRCA1 expression in apoptosis-sensitive epithelial cells: loss of BRCA1 expression in colonic adenocarcinomas. Nutr. & Cancer 46(1):82-92, 2003.

Crowley-Weber CL, *et al.* Nicotine increases oxidative stress, induces apoptosis, activates NF-κB and GRP78, and sensitizes cells to genotoxic/xenobiotic stresses by a multiple stress inducer, deoxycholate: relevance to carcinogenesis. Chem. Biol. Interact. 145:53-66, 2003.

Payne CM, *et al.* Caspase-6 mediated cleavage of the catalytic subunit of guanylate cyclase (Gcα1) during deoxycholate-induced apoptosis and role of the nitric oxide signaling module in apoptosis resistance. Cell Biology and Toxicology, (Accepted).

M. Donner Denton, Ph.D.

University of Arizona
Award Amount FY03: $64,812

A Unique Mass Spectrometer for Biomedical Studies

The past year has seen significant gains in characterizing and improving the hydrogen laser time of flight mass spectrometry instrument. Studies continue to demonstrate the ability of the instrument to easily detect drugs and compounds of interest from normally difficult matrices without any chromatographic separation or complex extraction procedure. Several classes of compounds were tested for their potential to be ionized by the hydrogen laser, but only those containing aromatic moieties or secondary or tertiary amines were detected. The use of simple extraction procedures demonstrated rapid detection of alkaloids from natural products such as tobacco and poppy seeds. Compounds of biomedical relevance such as tamoxifen (anti-cancer), erythromycin (antibiotic), ellipticine (anti-tumor), and ephedrine are detectable in their pure form and should thus be amenable to analysis in complex matrices such as blood or urine.

Bernard W. Futscher, Ph.D.

University of Arizona
Award Amount FY03: $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, the second leading cause of cancer death, and a significant health issue in Arizona and the United States. Understanding the molecular mechanisms that are responsible for the conversion of a normal breast cell into a malignant breast cancer cell will likely play a significant role in the diagnosis, monitoring and treatment of this disease. One mechanism that participates in this malignant conversion is the inappropriate silencing or turning off of the tumor suppressor gene named Maspin. When the Maspin gene is turned off, breast cells acquire malignant characteristics such as the ability to stimulate the growth of new blood vessels. The studies supported by the ADCRC allowed us to obtain conclusive evidence from clinical biopsy specimens that the Maspin gene is actually turned off even before the breast cancer cell becomes malignant. This information suggests that early treatment or prevention strategies designed to turn the Maspin gene back on in high risk women may prove to be a worthwhile therapeutic strategy. Complimentary studies in our lab have identified such potential approaches.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY03: $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 for 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 two has been on developing and evaluating a suitable catheter for the confocal instrument.

Publications:

Lean DC, Rouse AR, Gmitro AF. Development of a fiber-optic confocal microendoscope system for real-time, intraoperative (*in vivo*) imaging of brain tumors. BIOS 2003, SPIE Proc. 4957, 2003.

Rouse AR, *et al.* Fiber-optic confocal microendoscope as a daughter scope for clinical endoscopy. BIOS 2003, SPIE Proc. 4957, 2003.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY03: $180,514

Discovery, 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 second year of the project, roots of 55 species of desert plants representing 26 families and 51 genera have been sampled and 5070 bacteria and 950 fungi have been isolated adding to our library of Sonoran desert rhizosphere microorganisms. Of the new collection, 160 fungi have been cultured and their extracts were prepared. These extracts were screened for their potential anticancer activity using 45 cell-based [NCI-H460 (non-small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS cancer), MIA Pa Ca (pancreatic 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 three fungi which were found to inhibit the growth of at 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 5 compounds with significant activity against the cancer cell lines used. One of them was also found to be active in the primary heat shock response activation assay and has been identified as dehydrocurvularin. Structure elucidation of active compounds are currently in progress. If these compounds turn out to be active against solid tumors such as breast, prostate, colon, lung and pancreatic cancers, our results will have an impact on the more elderly and/or tobacco-dependent portion of Arizona's population since these cancers are prevalent in our state.

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

University of Arizona
Award Amount FY03: $131,520

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

During the last year, the Arizona Prostate Tumor Bank accomplished the following goals:

1) Protocols for the collection of blood and tissue samples were submitted and approved by the Scientific Review Committee of the Arizona Cancer Center and have been submitted for IRB approval.

2) Using existing tissue IRB protocols, we were able to collect 50 biopsies and 11 prostatectomies, bringing our total to 703 patient biopsies and 367 prostatectomy specimens. These have been entered into our web-based data system.

3) Using the archival tissue from the prostatectomies, we have constructed, in a single block, a tissue array representing 84 samples, including normal, PIN lesions and all grades of prostate cancer from specimens for which we have accurate pathologic staging. This array has been probed, thus far, for vimentin, cytokeratins 5 and 14, PSA, P504, PTEN, pAKT and Aurora-2 kinase proteins.

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Nagle RB. Role of the extracellular matrix in prostate carcinogenesis. **J Cellular Biochem,** 2003, (In press).

Irwin L. Flink, Ph.D.

University of Arizona
Award Amount FY03: $49,664

Mechanism of Cell Cycle Control: Axolotl Heart Regeneration

The long-term goal of this project is to develop a gene therapeutic approach for repairing damaged heart tissue following injury to the myocardium. The short-term aim is to elucidate the biochemical mechanism of cardiac regeneration of the salamander heart. As a first step to understand the process of cardiac muscle cell proliferation, a subtraction library of up-regulated genes has been constructed from the wounded salamander heart. Three major genes have been identified to date, which are known to play a role in cell proliferation: decorin, calmodulin-dependent kinase type II, and cyclin B. Full-length cyclin B cDNA contains an insertion of 17 amino acids near the NH_2-terminus and a deletion encoding a proteolytic degradation sequence commonly found among B cyclins from other species. The deleted region lacks a potential serine phosphorylation site found in human, rabbit, and mouse B-type cyclins. These unique features are novel for cyclin B and may be related to the regenerative capacity of the salamander heart. Studies are underway to examine how salamander cyclin B regulates cell division in yeast.

Karl B. Kern, M.D.

University of Arizona
Award Amount FY03: $50,000

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

After cardiac arrest and successful 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 an enzyme system within the heart, the nitric oxide synthase (NOS) enzymes, that if overly stimulated, could lead to such heart failure. Measurement of two forms of NOS enzymes following cardiac arrest shows the inducible form (iNOS) remains high at 5-6 hours after resuscitation, when the pumping function is especially poor, while the other form (eNOS) returns to its pre-arrest baseline level at 6 hours. This suggests that iNOS is more likely contributing to the poor heart function seen after resuscitation. We are now studying the effect of selective blockade post resuscitation of the iNOS enzyme system to examine whether such can improve heart pumping function. If proven effective, blockade of the iNOS system could improve outcome for thousands of Arizonans suffering cardiac arrest each year.

Marlys H. Witte, M.D.

University of Arizona
Award Amount FY03: $49,985

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

Lymphatics parallel blood vessels and return leaked plasma in tissues 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-C/D, 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 underdevelopment in LE mice lacking the Ang2 gene and the contrasting dysfunctional overdevelopment of lymphatics and lymph nodes 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:

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Fischer Verlag, Munchen, Germany (English text revised by Biotext, LLC, San Francisco), Chapter 16, 471-493, 2003.

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Kriederman B, *et al.* *Foxc2* haploinsufficient mice are a model for human autosomal dominant lymphedema-distichiasis syndrome. [ISL Presidential Prize] 19th International Congress of Lymphology, Freiburg, Germany, 9/1-6/03, p. 86.

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

University of Arizona
Award Amount FY03 : $48,877

Mechanism of Cigarette Smoking on Human Infertility

In Arizona, approximately one-third of the women of reproductive age smoke cigarettes. It has been demonstrated that cotinine, a metabolite of nicotine, is significantly increased in ovarian follicular fluids in smokers. It is unknown how cotinine damages the ovaries at the molecular level. We, for the first time, demonstrated that cotinine stimulates the nuclear transcription factor and induces the cell death in the ovarian granulosa cells. The results were confirmed at gene transcription level and protein level using different strategies. The damage for the ovarian cells will affect the oocyte development and luteal defect leading to early pregnancy loss. The outcome of this research will lead to better understanding the mechanisms of the toxicity of cigarette smoking, which should provide scientific information for the people in Arizona, especially the people at reproductive ages.

Thomas 0. Baldwin, Ph.D.

University of Arizona
Award Amount FY03: $50,000

Disruption of Cell - Cell Communication to Block Bacterial Pathogenicity

People with bacterial infections who would have been cured 30 to 40 years ago by antibiotics are dying today; antibiotics are no longer as reliable as they once were. During the past decade, there has been a major push to find non-antibiotic therapeutic treatments for bacterial diseases. One of the most promising approaches is the disruption of bacterial communication. Many bacteria utilize a quorum sensing system monitoring local population levels and inducing pathogenicity factors only when the cell density becomes sufficiently high to favor overcoming host defenses. The quorum sensing phenomenon is mediated by a small diffusible molecule, frequently an acyl homoserine lactone, which, as its concentration increases, interacts with a transcription factor and initiates a cascade of events that results in the expression of pathogenicity factors. Our work on quorum sensing is progressing towards potential therapeutic agents through a better understanding of quorum sensing. Our work has resulted in one manuscript and one NIH grant application.

Karl H. Schram, Ph.D.

University of Arizona
Award Amount FY03: $92,198

Biomarkers of Systemic Fungal Infections

Significant progress has been made in two important areas of this project. The first area involves the formation of a collaboration with Dr. Vladimir Havlicek of the Czech Academy of Sciences for the mass spectral analysis of our samples. While facilities are available for us to perform a preliminary mass spectral analysis of our samples, we do not have access to an instrument for mass spectrometry/mass spectrometry (MS/MS) studies. We have analyzed ethyl acetate and chloroform/methanol (1:1) extracts of *Aspergillus fumigatus*, *Aspergillus terrus* and *Candida albicans* fungal pellets; each of the three media used to grow the fungi/yeast and a medium blank. By comparing the medium blank and blank blood sample (analyzed earlier), peaks arising from the fungi in either the pellet or medium can be recognized. After analysis in our laboratory, the samples are then forwarded to Dr. Havlicek for analysis in his laboratory in Prague. A close correspondence in the data indicates both of the laboratories are producing the same results. The MS/MS analysis of peaks in the spectrum of these extracts which may provide biomarkers of these

fungal/yeast infections is currently in progress. In addition, Dr. Havlicek will be able to provide us with high resolution mass measurements of peaks which possess significant potential to serve as biomarkers of systemic fungal infections.

The second area of importance the continuing collaboration with Dr. Thomas Walsh of the NIH. Dr. Walsh is preparing a series of bronchial lavage samples from rabbits which are correlated with the degree of fungal infection by *Aspergillus fumigatus*. Dr. Walsh will send these samples to us for analysis. We will extract each of the samples with ethyl acetate and chloroform/methanol (1:1) and perform a preliminary mass spectral analysis of each extract. Comparison of the results should provide us with a definitive means for identifying peaks representing the production of biomarkers caused by Aspergillus fumigatus. Following identification of important peaks in our laboratory, the samples will be sent to Dr. Havlicek for MS/MS and high resolution studies. To our knowledge this will be the first time that a set of serial samples from *Aspergillus fumigatus* infected rabbits will have been analyzed by mass spectrometry.

Once the presence of biomarkers is established, further studies of rats infected with *A. fumigatus* will be analyzed to determine if the biomarkers can be detected in the blood of these animals and if the levels of the biomarkers indicate the degree of fungal infection. Similar analysis of blood samples from children undergoing bone marrow transplant operations at the University of Arizona Department of Pediatrics will be conducted as samples become available.

One abstract was presented at the American Society for Mass Spectrometry meeting in Montreal, Quebec, Canada in June describing the levels of detection of cyclic peptides in spiked blood samples and the peaks in ethyl acetate and chloroform/methanol extracts of blank blood samples.

Kemmons A. Tubbs, Ph.D.

Intrinsic Bioprobes, Inc.
Award Amount FY03: $138,977

Proteomic Analysis of Nicotine Receptor Structure and Composition

To date continued emphasis has been placed upon genetic engineering approaches targeting expressed nicotine acetylcholine receptor (nAChR) subunits that are both functional and novel as well as appropriate for biological mass spectrometry. These expression system preparations have been analyzed using traditional and newer proteomic approaches to discern identity, relative abundance and accuracy of the expressed protein. Additional work has also continued to refine native Torpedo preparations for nascent and associative protein targets.

During continuing work on expressed tagged protein systems, minimal binding when applied to traditional protein analysis approaches as well as mass spectrometric immunoassay with mass spectrometric readout delineated structure/function irregularity necessitating ligand or domain attachment augmentation. Expression system without tagged insertions show functionality when analyzed by ligand binding. Novel subunit fragments have been prepared for ligand binding and mass spectrometric analysis. Fragmented subunit expression has, so far, shown irregular mass assignments requiring further study.

Publication:

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Mary Kay O'Rourke, Ph.D.

University of Arizona
Award Amount FY03: $242,414

Integrated Epidemiological Study of Valley Fever

Breathing spores of *(Coccidioides immitis/posadasii)* or cocci causes Valley Fever. We have undertaken a survey recruiting almost 5,000 households and identifying 130 cases. We provided educational information about the disease to over 16,000 homes in both English and Spanish. We are near completion of the case/control study. Preliminary trends suggests greater risk to older people and local concentrations of the cases. Specific analyses assessing age, smoking, local soil type, race and ethnicity are underway. To assess the presence of C. *immitis* spores in soils, we use a method to detect specific DNA (Polymerase Chain Reaction or PCR). Our method detects 100-1000 spores per gram of soil. Independent confirmation in culture and live mice has been difficult. Recent work with mice shows promise. Data to evaluate the role of climate are being compiled. Good progress has been made on the study and results will provide better prevention strategies for Arizona residents.

Garth Powis, D. Phil.

University of Arizona
Award Amount FY03: $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. Paradoxically ROS production is also increased during hypoxia due to uncoupling of the mitochondrial electron transport chain. 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 mitochondrial Prdx-3 is an important cellular antioxidant that regulates physiological levels of H_2O_2 leading to decreased cell growth, while protecting cells from the apoptosis inducing effects of high levels ofH_2O_2. We have also shown that mice with both Trx-2 genes inactivated die early during embryo development at a time when mitochondrial respiration is just starting. The mouse model we have developed points to the importance of Trx-2 in protecting against ROS damage.

SECTION D

CONTRACTS

MEDICAL RESEARCH

YEAR ONE

FY 2003

Lokesh Joshi, Ph.D.

Arizona State University
Award Amount FY03: $175,000

Recombinant Protein Therapeutics

Cardiovascular disease and cancer are the two leading causes of death in the United States and in Arizona. There are two major challenges in combating any disease, discovering the right molecules that have the therapeutic affect and developing the method of manufacturing these molecules. Our group is investigating two molecules that are highly effective against vascular and cancer diseases. Until now, these two molecules and other such drugs have been obtained from mammalian sources (including humans). However, because of the inefficiency of the production method and the inherent threat of cross contamination such as prions, viruses and other adventitious agents, there is a strong need for alternative methods of production. We are developing plants as the source of these human proteins that are able to prevent and/or treat diseases. Plants are safe and can be scaled up to produce large quantities of medically-important proteins for patient care. This represents a novel approach that has significant therapeutic and biotechnology potential.

Molly A. Brewer, DVM

University of Arizona
Award Amount FY03: $167,748

Flourescence as a Biomaker for Prevention - Early Diagnosis of Ovarian Cancer

Two of our specific aims were:

1. Elucidate the molecular mechanisms and time course of 4-HPR (and later progesterone) induced apoptosis and/or growth inhibition on normal ovarian surface epithelial cells to understand the sequence of intermediate markers of cell quiescence.

2. Further develop fluorescence spectroscopy as a biomarker for drug activity of ovarian surface epithelial cells by analyzing the time course of emission changes and correlating them with the molecular changes occurring within the cells as described in Aim 1.

Our lab is now set up and producing data. We have investigated the effect of the retinoid (4-HPR), the chemopreventive agent that we are studying, on cells in cell culture. We have measured the effect on the fluorescence signal and on the redox ratio, showing that cancer cells have a consistent response to the retinoid that can be seen in the change in fluorescence intensity and redox ration (the energy potential of the cell). Thus, our progress to date is evidence that our technology of fluorescence spectroscopy can be used to measure the response to these agents. Even more importantly, we can extrapolate from this data to show that this technology can be used to identify an active ovary (predisposed to developing cancer) from a quiescent or inactive ovary.

Anna R. Giuliano, Ph.D.

University of Arizona
Award Amount FY03: $175,000

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

The goal of this research study is to further our understanding of Human Papillomavirus (HPV) infection in men. We will conduct a prospective cohort study of young men in Tucson, Arizona 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 socio-behavioral 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.

The progression of this project during the first fiscal year included finalizing the study protocol by 1) generating material for recruitment; 2) generating clinical instructions and checklists; 3) finalizing laboratory protocols; 4) completing HIP AA forms; 5) developing a tracking form and database; and 6) developing a web site.

Megan M. McEvoy, Ph.D.

University of Arizona
Award Amount FY03: $50,000

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

The goal of our research program is to try to understand asymmetric cell division by determining the structures of proteins that are involved in this process and characterizing their interactions. The primary methodology used to approach this goal is nuclear magnetic resonance spectroscopy. Two proteins that play an important role in this process, Bazooka and Inscuteable, were originally selected for structural characterization. The primary goals were to determine the NMR structures of the interacting regions of these proteins, and to determine the structures of the complex. The structures of these proteins will provide information on the ways that protein-protein interactions are made or excluded, and perhaps shed some light on the regulation of these complexes.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY03: $175,000

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

A high-fat diet is known to contribute to colon cancer, although the mechanism(s) by which this dietary modification contributes to colon cancer is poorly understood. In response to an intake of fat, bile acids are emptied into the gut where they are converted by bacteria to very damaging or cytotoxic detergents (*e.g.* deoxycholate). We have previously shown that deoxycholate induces apoptosis, a type of programmed cell death, in colon epithelial cells and that these cells can be made resistant to deoxycholate. We now show that a signaling pathway that is composed of 3 enzymes and 2 signaling molecules, including nitric oxide, contributes, in part, to the observed resistance of these cultured cells. Since colon cancer primarily affects individuals over the age of 50, it is anticipated that our findings should lead to the development of biomarkers to evaluate colon cancer risk for a significant number of Arizonans.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY03: $250,000

Development of New Anticancer Drugs for Improving Human Cancer Treatment

The following two structural modifications of dolastatin 10 and 15 that we isolated from a marine sea hare collected in the Indian Ocean, auristatin 15-DMO and 15-F, are now being used in our current research involving the new synthetic anticancer drug objectives. We expect to locate even more effective cancer vascular targeting for the purpose of shutting off the blood supply to metastatic cancer. In a related investigation involving dolastatin 16 and 17 where we isolated these quite unique cyclic depsipeptides with potent cancer cell growth inhibition from a sea hare collected in the Bismarck Sea north of Papua, New Guinea, we are very vigorously developing efficient total syntheses. Those syntheses will allow these two anticancer drug candidates to progress further into preclinical development. In short, overall progress has been exceptional and will be continued in the coming year and beyond.

Publications:

S. Ali, *et al.* Sensitization of human breast cancer cells to gemcitabine by protein kinase c modulator bryostatin 1, Cancer Chemother. Pharmacol (Accepted).

Pettit GR, *et al.* Synthesis and evaluation of 3' position structural modification of (2)- and (E)-combretastatin A-4, J Med. Chem. (In press).

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Danzhou Yang, Ph.D.

University of Arizona
Award Amount FY03: $49,940

DNA and Topoisomerase I Interactions of Novel Homocamptothecin Anticancer Drugs

Homocamptothecins 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. The main objective of the proposed project is to explore the molecular level details of the DNA and topoI interactions of the homocamptothecins. DNA oligonucleotides poly(GC) and poly(AT) have been synthesized and purified. Homocamptothecin interactions with synthetic DNA and genomic DNA have been studied by using high-field Nuclear Magnetic Resonance (NMR) spectroscopy, UV spectroscopy and High Performance Liquid Chromatography (HPLC) methods. The presence of DNA increased the stability of homocamptothecin, however, homocamptothecins didn't display strong sequence specificity when binding with DNA. Human topoisomerase I (htopoI) and its 6.3kD C-terminal fragment have been expressed and purified. The $^{13}C/^{15}N$-isotope-labeled 6.3kD C-terminal topoisomerase I has also been prepared for the further NMR studies. Better understanding the mechanism of action of homocamptothecins will help to develop improved drugs for cancer patients in Arizona.

Stephen Massia, Ph.D.

Arizona State University
Award Amount FY03: $169,929

Local Gene Therapy Targeting Vascular Graft Hyperplasia

Cardiovascular disease remains a major health problem in the nation and in Arizona. 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 the bypass can rapidly become obstructed following surgery. Another problem is that synthetic graft replacements have a higher tendency to occlude 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. This research focuses on developing gene therapy that can be placed in synthetic graft materials and delivered locally after surgery to inhibit processes leading to graft occlusion. During the first year of this project, we developed materials designed for sustained release of therapeutic genes from synthetic vascular grafts.

Rodney D. Adam, M.D.

University of Arizona
Award Amount FY03: $49,999

Gene Expression in *Giardia Lamblia*

When people are infected with Giardia, the symptoms may last for weeks or months, possibly because of antigenic variation of the VSP genes. The research done as part of this project has provided information on how gene expression is controlled differently for the VSP genes than for other Giardia genes. This expression is controlled by the DNA just upstream from the DNA encoding the VSP protein sequence. We have done experiments to determine whether VSP gene expression is affected by alterations in histones, the proteins that encircle the chromosomes, sometimes affecting the expression of genes. The best studied form of histone alteration is acetylation; so we tested inhibitors of histone deacetylation to see if they affected the rate of antigenic variation. They did not, but they did affect the ability of the cell to finish division. We are currently characterizing this affect by microscopic studies and by further molecular studies.

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY03 : $175,000

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

HIV-1 infection in women has increased by 63% in the United States in the past three years, and infants born to these mothers are at risk of acquiring HIV-1 infection and subsequently developing AIDS. In the State of Arizona, the number of AIDS cases is increasing at a significant pace, and Tucson, Arizona has been included in the top 50 metropolitan areas in the country with highest annual rates of AIDS cases. Moreover, a majority of HIV-1 infected neonates and infants develop AIDS faster compared with infected adults, including differences seen in clinical manifestations. However, the molecular mechanisms of HIV-l infection in immature hosts (neonates and infants) 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 and destroys immature (neonatal) mononuclear cells more rapidly compared with mature (adult) mononuclear cells, resulting in a more rapid disease progression in neonates and infants than in adults. We are using cord blood in place of neonatal blood because of its closeness to neonatal cells in terms of immaturity (high in CD45RA+ and low in CD45RO+) and adult blood mononuclear cells to investigate the mechanisms of HIV-1 infection. We have found that HIV-1 replicates better in cord blood lymphocytes and monocytes/macrophages compared with adult blood lymphocytes and monocytes/macrophages. There was no significant difference in the expression of HIV-1 receptor (CD4) and co-receptors CCR5 and CXCR4) between cord and adult blood mononuclear cells. In preliminary experiments, the enhanced HIV-1 replication in cord blood cells compared with adult blood cells could be attributed to higher rates of post entry events (reverse transcription and translocation of pre-integration complex into the nucleus) and up-regulation of HIV-1 gene expression as well as more rapid destruction of T-cells precursors activity. The insights generated from this study may be helpful in understanding the mechanisms of HIV-1 infection in immature (neonate and infant) target cells and aid in the development of strategies for the treatment and prevention of HIV-1 infection in children.

Jorge A. Giron, Ph..D.

University of Arizona
Award Amount FY03: $50,000

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

We have identified a novel pilus (hair-like) structure on enterohemorrhagic E. coli. We hypothesize that these pili contribute to the colonization of human tissues and, thus, to disease. Our data strongly supports this hypothesis. Namely, we have demonstrated that:1) rabbit antibodies raised against the pili block adherence of the bacteria to cultured human cells; 2) a pili-deficient mutant is less adherent than the parent strain; and 3) the pili mediate adherence to animal erythrocytes and bind to human proteins such as fibronectin and laminin. We believe our data contribute to the understanding of the pathogenic scheme of these bacteria.

Marlene P. Freeman, M.D.
Award Amount FY03 :$49,988

University of Arizona

Omega-3 Fatty Acids for Postpartum Depression

Ten to twenty percent of new mothers suffer from postpartum depression (PPD). Some are unwilling to accept antidepressant medications while breastfeeding. The goals of this study are: 1) to gather pilot data on the optimal dose of omega-3 fatty acids (0-3 FA) in PPD; 2) to characterize the amount of omega-3 fatty acids in red blood cells of women with PPD; and 3) to determine whether 0-3 FA supplementation increases red blood cell levels and correlates with treatment response. Over the course of 8 weeks, subjects are carefully monitored and complete depression rating scales. If efficacy is established, omega-3 fatty acids offer an inexpensive nonmedication alternative for PPD that is safe for nursing infants. We have enrolled N=11 subjects into this study. Of the two women that have completed the entire study protocol, both have experienced significant decreases in depression scores. The study is still in progress at this time.

Michael H. Ossipov, Ph.D.

University of Arizona
Award Amount FY03: $174,071

Fentanyl-induced Paradoxie 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 of Arizona. Chronic severe 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 socio-economic burden. Most often, chronic pain is managed by morphine and related opiate analgesic such as oxycodone or hydrocodone, but these analgesics are associated with tolerance and the development of abnormal pain. Fentanyl is a very powerful opiate analgesic that may produce less tolerance than morphine. The results of our investigation suggest that continuous administration of low doses of fentanyl produces abnormal pain after about 4 to 5 days of infusion, but higher doses that effectively block pain delay the onset of abnormal pain to 7 days after infusion. With morphine, it was shown that abnormal pain is associated with tolerance to the analgesic effect. However, fentanyl, although producing abnormal pain over time, does not show significant tolerance development over 7 days of infusion. This appears to be related to the fact that fentanyl, unlike morphine, does not lose its analgesic potency as pain intensity increases. We are currently conducting further experiments to understand the underlying mechanisms related to persistent exposure to fentanyl. The results seem to suggest that treatments such as a fentanyl patch would be appropriate for the long-term treatment of severe chronic pain.

Patricia E. Penn, Ph.D.

La Frontera Center, Inc.
Award Amount FY03: $109,946

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

Our study focuses on smoking cessation in persons with serious mental illness (PSMI) because national data suggests that:

1) PSMI smoke nearly half of the cigarettes in this country
2) Their smoking rate is 2-3 times higher than in the general population
3) Public funding provides most of PSMI support, cessation could mean health care savings
4) Cessation interventions for this population are understudied
5) Most cessation studies exclude PSMI

This study compares two interventions with the potential to help PSMI quit smoking. The interventions are contingent reinforcement alone and contingent reinforcement plus 21 mg. nicotine patches for 16 weeks. Participants in both contingent reinforcement groups will earn progressively more money for each visit where they demonstrate abstinence as measured by self-report and breath carbon monoxide (CO) levels. The intervention groups will be compared with a self-quit group who complete three assessments and receive brief smoking cessation literature. Measures (over 36 weeks) include: saliva cotinine; breath CO; health, smoking and other substance history; and current status, psychiatric status, craving, and quality of life.

Thus far we have hired and trained staff in study procedures and cessation skills; purchased equipment; designed and distributed recruitment materials; attended case management and medical team meetings and establishing ongoing contact. We have also initiated three smoking cessation clinics, recruited 81, enrolled 42, and intervened with 28 PSMI. Very preliminary data suggests that 53% of our active participants have quit or are smoking much less.

Barry M. Pryor, Ph.D.

University of Arizona
Award Amount FY03: $49,986

Characterization of Alternaria Isolates Associated with Allergenic Asthma

Soil and plant debris samples were collected from 6 locations in native desert ecosystems and 16 locations in urban landscape environments. 505 isolates of *Alternaria* species were recovered using a newly developed medium (acidified weak PDA with thiabendazole), which was very efficient in enumeration and isolation of *Alternaria* sp. A method for coding conidiation patterns was developed and applied to 20 representative species and 40 Tucson isolates. From morphological examination, it was found that the Tucson isolates differed from representative species. AFLP fingerprinting methods were applied to analyze relationships among isolates and representative species. From analysis of 27 representative species and 85 Tucson isolates, it was found that most of the Tucson isolates were included in alternata species-group and divided into four subgroups. None of them showed close relationships with representative species of alternata species-group.

Bradley S. Moore, Ph.D. University of Arizona
Award Amount FY03: $50,000

Engineered Biosynthesis of "Unnatural" Natural Products for Drug Discovery

The biosynthesis of the anticancer-antibiotic natural products marinone and neomarinone in a marine bacterium was evaluated with isotopically labeled precursors and shown for the first time to involve polyketide and terpenoid metabolic pathways. This study, which has been submitted for publication, further resulted in the overall structural revision of neomarinone that was shown to exhibit modest antibiotic activity against methicillin-sensitive *Staphylococcus aureus* (4 μg/ml) and *Enterococcus faecalis* (8 μg/ml). Based on the proposed biosynthetic pathway to these biologically active meroterpenoids, we cloned and sequenced a putative biosynthetic gene cluster and developed a genetics system in this bacterium for gene disruption. While we have not yet identified the genes that are responsible for encoding the marinone biosynthesis enzymes, we have made substantial progress in developing the genetic tools for manipulating this microorganism for future genetic engineering and drug discovery efforts in this new class of antibiotics.

SECTION E

CONTRACTS

PARKINSON'S DISEASE RESEARCH

YEAR ONE

FY 2003

Jeffrey N. Joyce, Ph.D.

Sun Health Research Institute
Award Amount FY02: $505,000

Arizona Parkinson's Disease Center

All three research cores made progress in their aims, including enrollment of Parkinson's disease (PD) patients into the brain bank, motor and neuropsychological examination of brain donor patients, and completion of neuropathological findings in cases coming to autopsy. This will allow for future testing of mechanisms underlying the pathogenic (disease-causing) mechanisms of PD. There was significant progress towards understanding the causes of inflammatory responses and dopamine (DA) neuron attack in PD (Project 3). Progress was made towards determining if the increased frequency of Marinesco bodies and ubiquitinated nuclei in DA neurons of old as compared to young patients and in Diffuse Lewy Body disease can lead to PD (Projects 2). There was also progress towards elucidating mechanisms of neuro-protection against death of DA neurons afforded by DA agonists (Project 1). With continued progress it will be possible to come up with neuro-protective agents that offer lasting benefits for PD patients.

Publications:

Rogers J, Kovelowski C. Inflammatory mechanisms in Parkinson's disease. In: *Neuroinflammation: Mechanisms and Management*. Vol.2, Wood. (ed). Humana Press, Totowa, NJ, 387-399, 2002.

Beach TG, *et al.* Hippocampal sclerosis dementia with tauopathy. Brain Pathol. (In Press).

Yong Shen, M.D., Ph.D.

Sun Health Research Institute
Award Amount FY02: $147,500

Gene Targeting TNF Receptors Reveals Dopamine Cell Death

Parkinson's disease (PD) is a chronic and progressive degenerative brain disorder. It is mainly characterized 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 environmental and inherited factors, as well as gender and aging, are thought to contribute to the development of the disease. In the past year, we and our collaborators found that tumor necrosis factor alpha (TNFα) is the most abundant cytokine (a protein within the body that can chronically promote inflammation) among many cytokines in the brain with PD. This is consistent with other reports. Moreover, we found similar results in mouse brains treated with MPTP. In order to study the mechanisms at the cellular level, we have developed neuron culture from the midbrain. Because PD is an aging-related neurodegenerative disorder, we have established a neuron culture system from aged mice. Now we are characterizing the percentage of dopaminergic neurons in our aged mouse neuron cultures compared to fetal mouse neuron cultures. In order to examine whether estrogen affects dopaminergic neuron survival, we have recently developed two strains of mice, one with a depletion of the estrogen synthesizing enzyme, aromatase, and the other with a depletion of the estrogen receptor. In our preliminary data we found that neurons expressing tyrosine hydroxylase, a dopamine marker, are decreased in the striatum of mice with estrogen deletion as well as estrogen receptor deletion. We are actively characterizing whether estrogen deleted mouse brain dopaminergic neurons have an alteration of TNF receptor type I expression, which relays signals into the cell to die. Also, we will examine whether estrogen can protect dopaminergic neuron death from TNFα and MPTP.

Publications:

Yang LB, *et al.* Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease. Nature Medicine 9(1): 3-4, 2003.

Yang LB, *et al.* Targeting deletion of TNF receptor subtypes reveals hippocampal neuron survival and death through distinct signal transduction pathways. Journal of Neuroscience 22(8):3025-32, 2002.

Michael Sierks, Ph.D.

Arizona State University
Award Amount FY02: $147,500

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

During the past year we have isolated several antibodies to different regions of α-synuclein, and have importantly shown that these antibodies can inhibit potentially toxic aggregation of α-synuclein *in vitro*. We have also been developing the protocols necessary to isolate antibodies to specific morphologies of α-synuclein using phage display antibody libraries and Atomic Force Microscopy. We have developed techniques where we can produce and purify the phage displayed antibody for AFM studies. We have also developed immobilization techniques that allow us to not only image but also recover bacteriophage from the AFM surface. We have found that by decreasing the concentration of Mg^{2+} cations, we can go from conditions where the phage displayed antibodies are firmly bound to the mica surface, to conditions where they can be easily removed from the surface. These protocols will enable us to begin isolating antibodies to specific morphologies of α-synuclein.

SECTION F

NEW CONTRACT AWARDS

BEGINNING IN FY 2004

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY04: $50,000

Molecular and Biological Characterization of HIV-1 Associated with Pathogenesis and Disease Progression in Children

AIDS in children is a grave concern in the United States and a catastrophe worldwide. In the past 3 years, new cases of HIV-1 infection in women of childbearing age have increased by 63% in the United States. 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, with Tucson and Phoenix being included in the top 50 metropolitan areas in the country with highest annual rates of AIDS. Furthermore, with the lower success rates of HAART and development of multi-drug 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. This problem is becoming more complex due to presence of several subtypes/clades of HIV-1 worldwide and emergence of new recombinant subtypes that may infect people in the U. S. and Arizona. Therefore, there is a need to develop new lines of better and effective drugs and preventive measures, which will only be possible if the molecular mechanisms of HIV-1 infection and disease progression in children are elucidated. The population of Arizona is on rise and so is AIDS; therefore, infected infants receiving HAART may develop resistance and rapidly progress to AIDS. 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 pathogenesis would be helpful in obtaining information to develop new strategies in combating the growth and development of AIDS.

HIV-1 infected children develop AIDS faster compared with infected adults, including differences seen in clinical manifestations. The reasons for these observed differences are not clearly understood but most probably depend on the dynamic interplay between HIV-1 and the relative immaturity of the immune system in early infancy. It is also likely that increased susceptibility of neonatal mononuclear cells to HIV-1 may contribute to high plasma viremia leading to an accelerated disease progression in infants. However, the molecular mechanisms of HIV-1 pathogenesis and disease progression in infected infants are not known, making it difficult to define better and effective strategies for prevention and treatment of HIV-1 infection. Better characterization of HIV-1 from infected infants during the course of infection and disease progression should provide relevant information for the development of strategies for prevention and treatment of HIV-1 infection in infants. Our hypothesis is that there are specific molecular and biological properties of HIV-1 that are critical determinants of HIV-1 pathogenesis and disease progression in infected infants. In the proposed studies, the molecular and biological characterization of HIV-1 during the course of infection and disease progression in 30 infected infants every 4 months for 3 years will be performed. The specific aims are to 1) characterize HIV-1 quasi-species in infected infants during the course of infection and disease progression, and 2) determine the biological properties and co-receptors utilization of HIV-1 from infected infants during the course of infection and disease progression. The insights generated may contribute to a better understanding of the molecular mechanisms of pathogenesis of several HIV-1 subtypes/clades and clinical aspects of disease progression

in infants. The understanding of HIV-1 pathogenesis of several subtypes in infants is critical because of the emergence of several recombinant subtypes that may infect people in the United States. These results may be helpful in developing better, more effective and new strategies for the treatment and prevention of HIV-1 infection in children.

Nathan Cherrington, Ph.D.

University of Arizona
Award Amount FY04: $50,000

New Drug Target in Liver Disease

Thousands of Arizonans suffer from liver diseases such as biliary cirrhosis, cholangitis, or what is commonly referred to as liver failure. During the early stages of these diseases, or when environmental contaminants cause what should be only minor damage, the normal functions of the liver slow down. Normally, the liver metabolizes toxic compounds and excretes them into the feces. However, in diseased patients, these toxic compounds can't be eliminated fast enough and begin to accumulate in the liver and cause considerable toxicity. This in turn compounds the original condition leading to a progression of the disease and eventually liver failure. Smoking has been implicated as a major risk factor in a predominant form of chronic liver disease. The cause of this increased risk is not known but may well involve activation of the same inflammatory pathway that is the focus of one of the aims in this proposal.

Mrp3 is a transporter protein in the liver which moves toxic compounds from the liver, back into the blood (to be excreted in urine), thereby decreasing the exposure and toxicity to the liver. Certain chemicals can cause an increase in Mrp3 activity. If the exact mechanism of increased Mrp3 protection can be discovered, then newer and safer drugs can be developed that could reduce liver toxicity in patients and potentially decrease the number of Arizonans in liver failure.

Along with the tremendous implications that Mrp3 transport has on the health effects of environmental pollutants, this proposal is unique in the potential for pharmacologic intervention of chronic liver diseases. It is hypothesized that Mrp3 is transcriptionally activated through multiple, distinct pathways that can be used as a means of liver protection. These pathways include CAR activation, which is a nuclear receptor and a potential drug target, as well as oxidative stress and/or inflammatory signaling. Aims 1 and 2 are each designed to determine suitable molecular targets within these pathways in order to develop new drug therapies that could utilize the unique protective effects of Mrp3 activity. Aim 3 specifically examines the molecular mechanisms of Mrp3 gene expression in the best model of short-term liver disease. The end goal of this research would be to develop new drug therapies that could artificially increase the capacity of diseased livers to excrete toxic substances and reduce liver failure by pharmacologically increasing Mrp3 activity. For this reason, pharmacologic intervention by targeting these new molecular pathways to relieve toxicity may prove to be vitally important to the long-term health of many Arizonans.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY04: $70,000

Effect of Nicotine on T-cell Development

The immune system is the body's natural system of defense. Thus, it is very important to understand how the immune system develops and functions. However, our current understanding indicates that these processes are incredibly complex. In addition, it appears that there is significant cross-talk between the immune and nervous systems. An emerging concept is that nervous and immune systems share signaling strategies and molecules. For example, evidence is accumulating that the natural neurotransmitter, acetylcholine, and the tobacco alkaloid, nicotine, can affect immune system function. This also opens the possibility that tobacco use could affect immune system development. Remarkably, little is known about effects of physiological signaling by acetylcholine, tobacco use, and nicotine exposure on immune system development. The mediators of any of these effects would be the family of signaling molecules called nicotinic acetylcholine receptors (nAChR). Our preliminary evidence suggests that nAChR are found on several cell types in the immune system at different stages of development and can influence immune system development. However, yet to be elucidated are the specific effects (enhancement or suppression of T cell maturation) of acetylcholine or nicotine on immune system development, the nAChR subtypes that mediate these effects, and the gene expression or chemical signaling pathways that are affected. Knowledge of these entities and effects is needed to illuminate whether and how tobacco use affects immune system function and to aid possible immunotherapies based on nicotinic cholinergic signaling.

The broad goal of this project is to establish effects of nicotine exposure and roles of nicotinic cholinergic signaling on development and function of the immune system. The broad thesis of the project is that immune system development is sensitive to such signals carried naturally by acetylcholine or affected environmentally by exogenous nicotine from tobacco and other products. Aim 1 is to determine effects of nicotinic drug exposure on development of human thymic T-cells and murine thymic stromal cells in an organ culture model. The hypothesis to be tested is that specific nicotinic ligands block or mimic nicotine's effects on T-cell development and reveal influences of endogenous signaling by acetylcholine on T-cell development. Aim 2 is to identify and characterize nAChR expressed by human T-cells and murine thymic stromal cells. The hypothesis to be tested is that nAChR involved in immune system development are expressed by T-cells, their progenitors, and thymic stomal cells in developmentally-relevant patterns. Aim 3 is to establish mechanisms involved in nicotinic and cholinergic modulation of immune system function and development. The hypothesis to be tested is that immune system nAChR acting as ion channels or via novel signaling cascades mediate their effects on T-cell development by altering expression and/or secretion of cytokines and/or by altering expression genes involved in T-cell development. These studiers will be conducted using a variety of proven techniques well known to the investigators in molecular and cellular biology and pharmacology. Consequences of this research could include an improved perspective on roles of nAChR and acetylcholine signaling on immune system development and on effects of nicotine in the immune system and their relevance to immune system defects and susceptibility to tobacco-related diseases. Also possible is development of new therapies to affect immune system function in autoimmunity or immunodeficiencies.

Eugene W. Gerner, Ph.D.

University of Arizona
Award Amount FY04: $55,000

Interdisciplinary Basic Science Program in Intestinal Carcinogenesis

Gastrointestinal (GI) cancers pose a significant health care problem for citizens of Arizona and the United States. The American Cancer Society estimates that cancers of the colon, rectum, pancreas and esophagus will account for 15% of all cancers, and 18% of all cancer deaths, in the United States in 2002. Adenocarcinomas of the esophagus and pancreatic cancers are especially lethal, with numbers of cancer deaths nearly equaling the numbers of new cancers of these types. Colon cancer is the number two cause of cancer deaths in Arizona and the United States. Treatment is effective for early, localized colon cancers, with five-year survival rates exceeding 90%. However, treatment is ineffective for colon cancers that have spread beyond the colon, with five-year survival rates of less than 10% for advanced colon cancers.

The overall goal of this research is to prevent or cure GI cancers. Since advanced GI cancers have proven very difficult to treat, our overall objectives are to identify individuals at risk for these cancers and then to treat these at-risk individuals with drugs, or other therapies, that will prevent GI cancers from occurring. Alternatively, we hope to use risk assessment as a means to identify cancers at earlier stages of disease when current therapies are most effective.

The adenomatous polyposis coli (APC) tumor suppressor gene is the cause of the Familial adenomatous polyposis (FAP) syndrome, which is a genetic form of colon cancer. Essentially all sporadic, or non-inherited, forms of colon cancer also acquire somatic, or non-germ cell, mutations in the APC gene. We have used animal models to identify polyamine metabolism as a mediator of the APC gene and APC-dependent intestinal carcinogenesis. Further, we have recently identified a specific type of genetic variability, a single nucleotide polymorphism (SNP), which affects the expression of a specific polyamine metabolic gene. This SNP is highly associated with a decreased risk of colon polyp recurrence in aspirin users in a colon cancer prevention trial.

The specific goal of this research is to establish the rationale for development of genetic tests for risk of GI cancers and identification of novel strategies for intestinal cancer prevention and treatment. The specific objectives of the proposed research are to use genetically altered rodent and human cell models of colon cancer to determine if polyamine metabolism is a mediator of APC-dependent intestinal cancers. We will cross genetically altered mice that do not express ornithine decarboxylase (ODC) or the spermidine/spermine N1-acetyltransferase (SSAT), or SSAT over-expressing mice, with mice expressing a mutant APC tumor suppressor gene. We will also use small interference RNA (siRNA) techniques to suppress the expression of these genes in human cancer cells. These studies may lead to a genetic test for colon cancer risk in humans that could be used to identify high risk individuals for subsequent colon cancer prevention strategies. Sequential risk assessment, followed by chemoprevention for high risk individuals, could be useful additions to current methods of colon cancer detection and more effective alternatives to current colon cancer chemotherapies, especially those for advanced stages of disease.

Indraneel Ghosh, Ph.D.

University of Arizona
Award Amount FY04: $49,500

Inhibiting Protein-Protein Interactions Involved in Cancer

The American Cancer Society estimates that in the year 2002, there will be 1,284,900 new cancer cases diagnosed in the United States, of which 22,100 will be in Arizona. The prevention of cancer is the long-term goal of our proposal.

The unregulated growth of new cells is the hallmark of cancer. The growth of new cells is controlled at the level of proteins that interact with one another. However, there have been very few cancer therapeutics that are targeted towards preventing protein-protein interactions. In this proposal we outline a general strategy for developing leads for potent cancer therapeutics utilizing novel technological advances that target the disruption of protein-protein interactions. We believe that our technological platform for drug design will be simple and powerful and will not only aid in designing cancer therapeutics but also impact other diseases, such as diabetes and Alzheimer's.

We outline the details of how to create a library of over 1 billion drug-candidates from which we can identify individual protein-drugs that are capable of preventing cancer progression. We will specifically target two proteins that are widely implicated in cancer, the vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6). VEGF is implicated in solid tumor development and IL-6 in multiple myeloma.

We have made excellent initial progress towards our goals in the last year, having designed and created over 1 billion drug candidates. We are currently in the process of targeting VEGF. We will also develop the necessary biological assays to fully characterize our protein drugs, and we will begin targeting IL-6 in the near future.

Elizabeth Glisky, Ph.D.

University of Arizona
Award Amount FY04: $49,977

Identifying Early Neuropathologic Markers in Alzheimer's Using Diffusion Weighted MRI

Considerable progress has been made over the past few years in understanding Alzheimer's disease. In particular, several new approaches to treatment have been developed recently that hold promise for slowing or even halting the progression of the disease. Identifying Alzheimer's disease in its earliest stages, however, remains an important and difficult challenge. A diagnosis of Alzheimer's disease is currently made through evaluations of cognitive problems such as forgetfulness and assessment of personality changes. However, by the time these changes are obvious, extensive damage to the brain has already occurred. Identifying Alzheimer's disease early, even before the onset of memory or other cognitive problems, is essential if drug treatments are to be effective. Finding methods that are sensitive to the very early brain changes that occur in Alzheimer's disease is also critical for the evaluation of new therapies. Such methods should provide a way to monitor the progression of the disease in affected individuals and measure the effectiveness of drug treatments that are designed to delay the onset, slow the progression, or even halt the disease.

In the present study, we plan to explore a new method of imaging the brain called diffusion weighted MRI, which is particularly sensitive to inflammation in the white matter of the brain. Recent evidence suggests that inflammation in the brain is implicated in Alzheimer's disease and may be an early contributor to the overall damage in the brain. If that is the case, then this new method of imaging may be able to detect inflammation in the brain early in the disease process, perhaps before any other symptoms have appeared. The present study compares four groups of adults over the age of 65: those who have already been diagnosed with Alzheimer's disease those who are cognitively normal and have no known risks for Alzheimer's disease, one at-risk group of older adults who have begun to show declines in memory that are abnormal but do not show other signs of Alzheimer's disease, and a second at-risk group who have a family history of Alzheimer's disease, but are cognitively normal. We expect to see clear differences in inflammation in brain regions affected in Alzheimer's disease between the Alzheimer's group and the cognitively normal older adults. Of particular interest is whether the imaging technique will detect inflammation in our at risk groups. If so, we will have identified a method that is capable of very early detection of the effects of the disease, which will be critically important for early treatment.

Haiyong Han, Ph.D.

University of Arizona
Award Amount FY04: $49,881

Development of New Anti-renal Cell Carcinoma Agents using Pharmacological Synthetic Lethal Screening

Renal cell carcinoma (RCC) is the most common malignant lesion of the kidney, comprising approximately 3% of all adult cancers. It is estimated by the American Cancer Society that this year about 31,000 new cases of RCC will be diagnosed and 11,000 patients will die from RCC in the United States. In Arizona the latest statistics from the Department of Health showed that there were about 500 new RCC cases and 160 patients died from this disease in 1998. The risk factors for RCC include cigarette smoking and exposure to asbestos.

The primary treatment for clinically localized RCC is surgical removal of the lesion. The biologic agent IL-2 can help a very small percentage of patients (10%). There is no effective chemotherapy for the disease. Therefore, new therapy is urgently needed, particularly for those patients with unresectable disease. Like other types of cancer, RCC is also a genetic disease. It is caused by the accumulation of mutations in genes. The most significant advance in the past decade in our understanding of RCC is the identification of the von Hippel-Lindau disease (VHL) gene. Carriers of the VHL gene mutations are predisposed to develop tumors in multiple organs including retina, brain, spinal cord and kidney. The VHL gene is a tumor suppressor gene that has been found to be disabled either through mutations or through hyper-methylation in over 75% of all patients with RCC. In this proposal we describe a novel approach that allows us to develop new agents that specifically target cancer cells with VHL deficiency.

It has been reported that VHL suppression plays a critical role in the development and progression of RCC, and that suppression of VHL expression will result in the alteration of expression of other genes. The hypothesis to be examined in this proposal is that small molecular weight agents can target these alterations and, consequently, selectively kill cells with mutated/inactivated VHL.

The specific aims for this proposal are to 1) establish matched-pair cell lines that include (a) normal kidney cell lines and (b) cell lines that have VHL gene expression specifically suppressed by siRNA, 2) identify lead compounds that selectively kill VHL deficient renal cancer cells using high throughput screen of chemical libraries, and 3) design and synthesize new generations of compounds based on the quantitative structure-activity relationship (QSAR) analysis of compounds identified in specific aim 2 and test them *in vivo* using SCID mouse models. The best compound identified at the end of this grant period will be brought forward for development as a clinical candidate.

Nancy Horton, Ph.D.

University of Arizona
Award Amount FY04: $44,825

Recognition of Damaged DNA by Human XPC- A Glimpse into an Early Step in DNA Repair

Damage to genomic DNA is an unavoidable consequence of exposure to UV radiation, oxidation, and exposure to mutagenic chemicals including those found in tobacco smoke. Repair of damaged DNA is critical to the maintenance of normal cellular function and to the prevention of mutation. Large amounts of mutation can lead to either cell death or uncontrolled proliferation, as occurs in cancer; therefore, the proper functioning of DNA repair pathways is necessary to prevent the proliferation of cancerous cells. A better understanding of the functions of the DNA repair components can lead to new therapeutic strategies for treatment and prevention of cancer. For example, many types of cancer develop initially by mutations in some of the DNA repair functions. Further mutation in these cells is then sped up since one of the pathways by which the cell can prevent mutation has been removed. This is the reason cancer cells can rapidly develop resistance to chemotherapeutics. But multiple pathways of DNA repair exist in a healthy cell, and drugs selectively targeting the remaining functional DNA repair pathways of the cancer cell can completely abolish repair in that cell. Combining this type of repair inhibition drug with the DNA damaging drugs currently in use to treat cancer can selectively kill cancer cells by damaging their DNA so severely that they can no longer survive. Meanwhile, healthy cells will be able to use the back up repair systems to limit and repair any DNA damage that might occur from the drugs, and maintain normal function. In addition, lower amounts of the DNA damaging drugs will be effective since the cancer cells will be selectively sensitized as a result of the repair inhibition drug.

The overall goal of this proposal is the determination of the three dimensional structure of human DNA damage recognition protein XPC bound to damaged DNA. This structure will provide invaluable information for the design of new chemotherapeutics for treatment and prevention of cancer. Since the method of x-ray crystallography will be used for the structure determination, high quality crystals of the complex between XPC and damaged DNA are required. Therefore, experiments have been designed to first determine the smallest, most stable, functional complex of XPC bound to damaged, or adduct containing, DNA, since such complexes tend to form better crystals more easily. Then, these complexes will be subjected to an extensive crystallization screen, and the best crystals will be used for the structure determination.

The goals of this proposal are summarized below.

- Determine the binding affinity of XPC for different lengths of duplex DNA, with and without chemical adducts, and with and without mismatched base pairs around the site of adduction.
- Measure the modulation of I above by the hetero-dimer partner protein hHR23B.
- Identify smaller DNA binding domains in XPc.
- Determine the three dimensional structure of XPC bound to damaged or adduct containing DNA by x-ray crystallography.

Ian Nils Jongewaard, Ph.D.

University of Arizona
Award Amount FY04: $49,999

Gene Expression Profiling of Early Cardiac Development

The proper formation of a 4-chambered heart requires precise gene programs and interactions among multiple cell types. The high frequency of congenital heart defects (CHD) reflects the complexity of these events. Among live born, CHD are the largest class of birth defects and account for one of the three most common causes of death in the first year of life. The burden and cost of CHD care are substantial for families and society. In Arizona, patients with CHD consume one-fourth of all pediatric health resources (J. Keagy, CRS Southern Arizona, personal communication). Increased understanding of the molecular mechanisms that regulate early heart development is required to better understand the causes of CHD and improve the diagnosis and treatment of this large public health problem.

Traditionally, the approach taken to identify and understand genes involved in this process has been to look at single, or at most, a handful of candidate genes at a time. The recent development of DNA microarray technology now provides us with the ability to examine the genes that are involved in specific diseases on a much larger scale. The experiments outlined in this proposal are designed to increase our understanding of the gene programs that are needed for proper heart valve formation by identifying, through microarray analysis, those programs that are used during the very early stages of this complex organ formation.

Our hypothesis is that this approach will identify both known and novel gene programs used by cells during early heart valve formation. The specific aims of this proposal are as follows:

> We will profile gene expression during normal early heart valve formation. We have developed the reagents and expertise to conduct these experiments using cDNA microarray analysis. We will also examine gene expression profiles using an established model of early heart valve formation. A comparison of both of these types of gene expression profiles will be made to clarify and confirm the applicability of this assay for *in vivo* events. Additionally, gene expression profiles in early heart development directed by specific biochemicals (TGFBeta 2 and TGFBeta 3) will be examined.

The experiments described in this project will greatly enhance our knowledge of the way in which a large number of genes are used during early heart valve formation. Analysis of gene data sets from both *in vivo* and *in vitro* sources will provide a more complete understanding of the biological process of heart valve formation and allow optimal utilization of *in vitro* modeling and experimentation. The proposed experiments will provide novel gene profile correlations and new avenues of experimental direction.

Emmanuel Katsanis, M.D.

University of Arizona
Award Amount FY04: $96,492

Chaperone Rich Cell Lysate (CRCL) Vaccine for Ovarian Cancer

Ovarian cancer is the leading cause of death from gynecologic malignancies. Despite dose escalation of cancer chemotherapy drugs and increasingly radical surgery, the overall survival in ovarian cancer has not changed. Thus, if adjuvant treatment (such as effective vaccines) could be developed for ovarian cancer that would prevent the extremely high rate of recurrence, survival of these patients could be improved. Although the concept of stimulating the body's immune system to fight cancer has existed for many decades, recent discoveries in how the body recognizes foreign proteins offer promising new approaches to cancer immunotherapy. The immune system has cells called dendritic cells whose role is to pick up foreign proteins such as bacterial or viral products and present them to T-cells, the immune cells that carry out the response against the invading organisms. These foreign protein products presented to T-cells by the dendritic cells can thus stimulate an immune or a T-cell response. Studies have shown that cancers also express unique foreign proteins, which means that cancers should also be able to stimulate T-cells to react against them. However, the body appears to be unable to present cancer proteins to T-cells and educate them to attack. To overcome the immune system's apathy toward cancer and apply immunotherapy more widely, one would need 1) to have accessible and effective proteins from every cancer and 2) to improve the presentation of these proteins to T-cells in a recognizable form so that T-cells can react against them. Special types of proteins called heat shock proteins (HSPs) or chaperone proteins can be extracted from cancer cells. These HSPs have been found to carry cancer proteins and, therefore, can function as unique anti-cancer vaccines. We have developed a relatively simple, rapid, and efficient procedure that generates multiple HSPs called Chaperone Rich Cell Lysates or CRCL. Our efficient CRCL enrichment technique has enabled us to overcome the lack of cancer protein material that may often be encountered by purifying individual HSPs using the other methods. We have found that when CRCL is used to immunize mice with different cancers, they provide significant protection against all cancers. Moreover, we have demonstrated that CRCL vaccines can be improved further by loading them onto dendritic cells. In fact, dendritic cells loaded with CRCL are very effective vaccines that can cure up to 75% of mice with pre-existing tumors.

Our hypothesis is that several types of HSPs together (such as found in CRCL) induce the maximally effective anti-cancer responses by providing more cancer proteins for the dendritic cells to present to T-cells, and by improving the function of dendritic cells. Our goals are to prove that this is true in the human system as we have shown in mice. In summary, CRCL loaded dendritic cell vaccine may prove to be a promising form of immunotherapy for ovarian cancer (and other cancers) and, therefore, warrants further investigation. Our studies will examine the effects of ovarian cancer derived CRCL on human dendritic and T-cells to establish efficacy and safety before embarking into clinical trials.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY04: $166,250

Immunomodulatory Autoantibodies to T-Cell Receptor in Autoimmune Disease

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are classic autoimmune diseases associated with elevated levels of autoantibodies and autodestructive thymus-derived lymphocytes (T-cells). Both diseases are prevalent in Arizona, with RA having an abnormally high incidence of approximately 5% due to the influx of individuals suffering from the disease and the high percentage of Native Americans who have increased susceptibility. The incidence of SLE in women is about 10 times greater than in men. Under previous ADCRC support, we generated monoclonal autoantibodies to recognition portions of T-cell receptors from RA patients, characterized them genetically, and obtained evidence that these antibodies have an anti-inflammatory effect on potentially auto-destructive T-cells. Thus, it appears that these particular autoantibodies are immunomodulatory, rather than pathogenic, and arise early in disease in an attempt by the body to protect itself against destructive inflammation. We propose to characterize further the functional and molecular properties of the RA-derived monoclonal autoantibodies to T-cell receptors including an investigation of therapeutic potential in a murine model where animals transfected with human HLA genes are susceptible to an experimental form of arthritis resembling RA. Because of the generality of the anti- TCR autoantibody recognition mechanism proposed, we will extend these studies to SLE and to multiple sclerosis. We have already generated a few monoclonal IgG autoantibodies from SLE patients. T-cells of restricted specificity play major pathogenic roles in multiple sclerosis, and although autoantibodies are common, their functions are not established. Our combined molecular and genetic approaches offer new possibilities for diagnosis and potential therapy in these T-cell-mediated autoimmune diseases.

Our central hypothesis is that autoantibodies (AAbs) to public idiotopes of T-cell receptor variable domains function in immuno modulation to protect against potentially destructive reactivity and arise in response to overproduction of destructive T-cells bearing particular Vβ gene products. Normal healthy individuals express low levels of AAbs directed against TCR-Vβ CDR1 and FR3 public epitopes. In support of this hypothesis, the levels of the AAbs increase in patients with the autoimmune diseases RA, SLE and also in MS. Significantly, AAb activity in MS is skewed towards particular Vβ gene products, notably Vβ 5.2 which is known to be expressed by auto destructive T-cells. Preliminary studies with monoclonal IGM AAbs derived from RA patients show that these molecules bind to T-cells and can suppress *in vitro* the capacity of antigen-activated T-cells to generate the pro-inflammatory cytokine interleukin-2 (Il-2). Monoclonal IgG AAbs of comparable specificities have been generated from SLE patients. We will isolate monoclonal anti-TCR AAbs from RA, SLE and MS patients, and characterize these for specificity, gene usage and binding properties. We will conduct *in vitro* and *in vivo* assays to define and characterize the immunomodulatory mechanisms initiated by these autoantibodies. Two transgenic mouse models will be used to determine *in vivo* the capacity of human monoclonal anti-TCR AAbs to modulate immune reactions. The first model, a well-established murine model for human RA, is collagen-induced arthritis in mice transgenic for human HLA-DQ8, which will serve as a model for therapy. The AAb responses in the murine model parallel findings in human RA. The second model is Balb/C- TgN (DO 11.10) mice transgenic for TCR of DO 11.10 monoclonal T-cells that are specific for ovalbumin. We have established the feasibility of using human AAbs in the murine models. We will test *in vitro* appropriately selected monoclonal Abs for their ability to modulate parameters of activation or function of T-cells in order to address the cellular and molecular mechanisms by which these AAbs modulate immune function. These studies should lead to the generation of human monoclonal AAbs or constructs of potential therapeutic use in inflammatory autoimmune diseases.

Paul F. McDonagh, B.S., M.S., Ph.D.

University of Arizona
Award Amount FY04: $164,350

Mechanisms By Which Air Pollution Increases the Severity of Heart Attacks

In Phoenix and Tucson, Arizona, as well as in many other metropolitan areas around the world, inhalation of air pollution is associated with a significant increase in hospital admissions and deaths from respiratory and cardiovascular diseases. It is not too surprising that the admissions from respiratory diseases are increased, but an increase in admissions and deaths due to cardiovascular diseases, particularly heart attacks, is puzzling. The particulate component of air pollution, especially the very small particles, (< 10 micrometers) correlates strongest with the increased incidence of heart attacks. It is not known exactly how inhaled particulate matter (PM) causes severe complications in organs distant from the lungs. It is our primary aim to find out. Also, it appears that certain populations are especially susceptible to heart attacks following PM inhalation. For example, a recent report indicates that diabetics are twice as likely to suffer a heart attack following a bad air day compared to non-diabetics. Based on our earlier finding that the inflammatory response amplifies heart disease, we suspect that inhalation of PM induces an inflammatory response, first in the lungs, then the immune cells in the circulating blood. Blood changes then may help initiate the heart attack as well as increase the severity of the heart attack. This theory (hypothesis) is quite consistent with the observation that diabetics are more susceptible because diabetics are vulnerable to inflammatory (oxidative) complications (Preliminary Studies). If this hypothesis proves to be correct, we can then design more effective pharmacological treatments to protect susceptible populations, such as the elderly and diabetics. In a study of the twenty largest U.S. cities, Phoenix ranked fourth in airborne PM. Thus, the results will likely help many Arizonans exposed to air pollution as well as many other people in metropolitan and industrial areas around the world.

The aim of this research project is to determine how inhalation of airborne particulate matter (PM) increases the severity of heart attacks. Our working hypothesis is that the increased severity of myocardial infarctions is due to a multi-stepped etiology involving the lungs, the blood and the heart. Inhalation of fine fraction PM causes a pulmonary inflammatory response. We hypothesize that cytokines released from the inflamed lung prime or activate the circulating phagocytic blood cells (PMNs) as part of an acute phase (inflammatory) response. Systemic inflammation can exacerbate cardiovascular disorders in several ways. Because of the acute nature and the severity of the response to PM inhalation, we suspect that, under reduced flow conditions, the activated PMNs accumulate in the heart by sequestering in the coronary microvasculature. The sequestered cells cause an acute oxidative injury. The PMN-oxidant injury combined with the ischemic injury increases the total injury and the severity of the heart attack. The injury may be amplified in populations with depleted anti-oxidant reserves, such as patients with established cardiovascular disease and type 2 diabetes. A cardiovascular physiologist, a pulmonologist and a combustion engineer will collaborate to test this working hypothesis. Pharmacologic agents targeting specific steps in the etiology will be used to block the cardiovascular effects of PM inhalation in normal and diabetic animals. The results of this interdisciplinary project will improve our understanding of the specific mechanisms underlying the morbidity and mortality associated with myocardial infarctions and PM inhalation. The results will also determine if a population low in anti-oxidant defense mechanisms is particularly susceptible to infarctions following PM inhalation. These findings will help in the design of more effective pharmacological treatments to protect susceptible populations. In a study of the twenty largest U.S. cities, Phoenix ranked fourth in airborne PM. Thus, the results will help Arizonans exposed to air pollution as well as many other people in metropolitan and industrial areas around the world.

Huge Miller, M.D.

Tucson VA Medical Center
Award Amount FY04: $165,137

A Family Alliance Intervention for Pregnant Smokers

There are few health behaviors as profoundly dangerous as smoking during pregnancy. The magnitude of this problem is vastly unrealized since only 6.8% of Arizona's pregnant women admit to smoking during pregnancy. If twice as many women smoke as our data suggests, we have a serious problem that has not been adequately addressed. Tobacco use during pregnancy is responsible for serious pregnancy complications including low birth weight (LBW), intrauterine growth restriction (IUGR), antepartum hemorrhage and preterm birth (PTB). Even when newborns initially appear healthy, researchers have discovered that children born to mothers who smoked are at higher risk of developing asthma, suffering from other respiratory diseases, experiencing cognitive delays, and they succumb in significantly greater numbers to sudden infant death syndrome (SIDS).

The last 10 years has brought few innovations in the area of counseling pregnant smokers. The current gold standard for counseling women who smoke during pregnancy neglects the powerful influence of her social environment, namely her significant other. In so doing, we have also limited the effectiveness of any specific cessation intervention to address the impact of environmental or second hand smoke exposure. The best evidence suggests that the success of individual directed cessation counseling may be capped at 10-15%.

The co-investigators have developed an intensive family-consultation (FAMCON) approach to promote cessation in couples where one or both partners continues to smoke despite having heart or lung disease. In this multi-investigator collaboration we propose to test a brief, family-alliance intervention that includes the significant other in the counseling process. A guiding assumption, based partly on our previous research, is that a pregnant smoker's close relationships play a key role in whether she continues to smoke and, most importantly, provide a powerful resource for change. Accordingly, key components of the proposed intervention are a family ultrasound session designed to enhance motivation for cessation counseling and conjoint (significant other) counseling to achieve it.

Our goal is to improve the health of Arizona's women and infants by reducing the number of pregnancy complications and consequent illness resulting from smoking during pregnancy. Given the large numbers of pregnant smokers, it is notable that even slight changes in smoking rates would have a profound impact on health care in Arizona in lives and dollars saved. Because there have been few innovations in recent years in this research area, new efforts are desperately needed.

We are proposing a clinical trial in which smoking pregnant women will be randomized to either 1) standard cessation counseling, 2) family-alliance cessation counseling and 3) family-alliance cessation counseling + ultrasound. We hypothesize that family-alliance cessation counseling will achieve higher rates of smoking cessation and 50% reduction, thereby improving maternal-child health in Arizona. Our first objective is to develop and manualize a brief family-alliance intervention for pregnant smokers and their significant others. Specific aims are 1) to establish the efficacy of a family alliance intervention to increase smoking cessation and 50% smoking reduction during pregnancy; 2) to evaluate sustained abstinence throughout the pregnancy and immediate postpartum period (6 weeks postpartum); 3) to

evaluate the intervention's impact on collateral outcomes such as reduction in environmental tobacco smoke exposure (e.g., whether the significant other quits as well) and measures of neonatal health; and 4) to identify other psychosocial factors (e.g., depression, anxiety and the significant other's smoking status) that may predict successful *vs.* unsuccessful cessation among pregnant women assigned to each treatment group.

Patients will be surveyed to determine the important demographic factors, psychosocial variables, concurrent illness, and psychiatric disease such as depression. Maternal outcome measures will include smoking cessation and 50% reduction by self-report and biochemical analysis. Postpartum relapse will be assessed to determine sustained abstinence for each treatment group. Neonatal outcome measures will include fetal complications, birth weight, and utilization of neonatal hospitalization. Comparison of the three treatments should determine whether the significant other's involvement in cessation counseling holds promise for smoking cessation and improves maternal-child health and, if so, whether the family-ultrasound component adds appreciably to the intervention's effect.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY04: $166,250

Anticancer Drug Preclinical Development

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

The development in Arizona of new anticancer drugs to clinical trials forms the overall objective and sharp focus of this research proposal. Specifically, four new anticancer drugs discovered in the past two years in the ASU Cancer Research Institute and now at various levels of development have been selected for scale-up syntheses and preclinical research leading to clinical trials. As these very promising anticancer drug candidates are moved toward the clinic, resources (as available) will also be devoted to the scale-up, procurement, and processing of other very promising plant, marine organism, and microorganism anticancer constituents. The four anticancer drug candidates selected for vigorous research directed at development to clinical trials comprise structurally new and very potent anticancer substances that we have discovered based on original leads from terrestrial plants or marine animals. These comprise phenpanstatin, pancratistatin 3,4-0 cyclic phosphate prodrug, iodocomstatin phosphate prodrug, and auristatin MO. The rapid introduction of these promising anticancer drugs into human cancer clinical trials should lead to a series of very important advances in improving human cancer treatment.

Donato F. Romagnolo, Ph.D.

University of Arizona
Award Amount FY04: $49,424

Tobacco Metabolites Induce Negative Regulation of BRCA-l Through a p53-Dependent Mechanism

In year 2000, 2,800 new cases of breast cancer were reported for the State of Arizona. However, only 5-10% of these cases (140-280) are attributable to BRCA-l mutations, whereas the remaining portion 90-95% (2,520-2,660) is sporadic. To date, no mutations in the BRCA-l gene have been identified in sporadic breast cancers, whereas the expression levels of BRCA-l in breast tumors are lower than those observed in normal mammary tissue. Polycyclic aromatic hydrocarbons (PAHs) are found in foods, coal tar, tobacco smoke, and industrial pollution. Work from our laboratory suggests that PAHs may repress the levels of the BRCA-l protein in breast epithelial cells. Because BRCA-l is involved in DNA repair, loss of BRCA-l protein may favor the accumulation of DNA damage and the onset of sporadic breast cancer. The loss of BRCA-l in women exposed to these environmental pollutants may increase the susceptibility to developing mammary neoplasia. The -knowledge gained through the proposed studies will help in understanding the mechanisms of repression of BRCA-l by PAHs and their contribution to environmental carcinogenesis of the breast.

The overall goal of this proposal is to dissect the mechanisms through which polycyclic aromatic hydrocarbons present in tobacco smoke, foods, coal tar, and environmental pollution may repress the levels of the BRCA-l protein, a tumor suppressor involved in the maintenance of genomic integrity. The primary objective of this proposal is to characterize one of the mechanisms through which metabolites of PAHs may repress normal BRCA-l levels. The central hypothesis is that metabolites of PAHs increase the cellular content of sentinel proteins, which ultimately are responsible for transcriptional repression of the BRCA-l gene.

Andrej A. Romanovsky, M.D., Ph.D.

St. Joseph's Hospital
Award Amount FY04: $155,321

Vagal Anti-inflammatory System

Systemic inflammation / sepsis is a leading cause of morbidity and mortality both nationwide and in Arizona. The incidence of sepsis is especially high in trauma and burn patients. Patients suffering from diseases that compromise the immune system (cancer, diabetes, AIDS) also have a high risk of developing sepsis and septic shock. With a mortality rate of ~80%, septic shock is responsible for the death of 200,000 Americans each year. The success of the current therapeutic modalities for sepsis is limited. This proposal is aimed at exploring a new treatment for systemic inflammation. It has been recently demonstrated that the vagus nerve (the largest nerve in the body servicing internal organs, including the liver) plays a key role in natural defense against systemic inflammation. The protective action of the vagus is mediated by nicotinic receptors, possibly in the liver. We propose to better define the vagal anti-inflammatory system by determining which branches and fibers of the vagus nerve, which cells in the liver, and which types of nicotinic receptors are involved. Next, we will study the effects of nicotine-like drugs on this natural defense system and establish whether these drugs have the potential to treat systemic inflammation/sepsis successfully.

Working hypotheses are: 1) the anti-inflammatory system involves vagal branches servicing the liver (*i.e.* the hepatic and the anterior and posterior celiac branches), 2) this system primarily uses efferent (motor, descending) vagal fibers, 3) suppression of macrophages (protective white-blood cells) residing in the liver plays a key role in anti-inflammatory responses, 4) liver's macrophages bear nicotinic receptors, perhaps of a type containing a specific building block called alpha-5, 5) drugs stimulating or blocking nicotinic receptors can, respectively, attenuate or exaggerate inflammatory responses, both in macrophage culture and in the whole body and 6) in a clinically relevant animal model of septic shock, drugs that activate a specific nicotinic receptor suppress production of pro-inflammatory substances by macrophages and block systemic
inflammation.

These hypotheses will be tested in whole animal (rat) and cell culture models by applying a wide range of surgical, physiological, biochemical, molecular biological, and histological techniques. The applicants are proficient with all the methods involved and currently using all these methods in their laboratories.

The proposed research will generate new knowledge about the recently discovered vagal anti-inflammatory system. The long-term goal of this research is to find a way to enhance the activity of this natural defense system in patients with systemic inflammation/sepsis.

Seth Rose, Ph.D.

Arizona State University
Award Amount FY04: $50,000

Eluding Drug Resistance in Cancer Chemotherapy

The importance of the cancer chemotherapy is still in its infancy. When perfected, it will be much more effective and desirable than surgery or radiation treatment, as the cancer drugs will search out and destroy even isolated groups of cancer cells as, or even before, they spread through the body by metastasis. Unfortunately, chemotherapy fails when cancer cells develop resistance to the administered drugs. A principal mode of drug resistance is the expulsion of the drug from cancer cells by a cellular biochemical pump. Expulsion of the drug negates the effectiveness of classical anticancer agents because the drug can no longer block the process of cancer cell division. Our strategy is to prevent the expulsion of the drug from negating the drug's effectiveness. We plan to use chemical bond formation between the drug and its target site in the cancer cell to keep the drug from being removed from the cell, allowing the anticancer effect to be maintained. One way we propose to do this is to supply the cancer cells with faulty substitutes for what the cells need to divide. This produces faulty biomolecules that thwart the cell division process. Another way we propose to evade drug resistance pathways is by knocking out a key enzyme in cancer cell division by chemically bonding an inhibitor to the enzyme, preventing its removal from the cell by cancer cell drug pumps.

Our goal is to evade the process of drug resistance in cancer cells so that chemotherapy will not lose its effectiveness during the course of treatment of patients with anticancer drugs, which is a major and heretofore unavoidable failing of drug regimens. Our hypothesis is that cancer cell pumps that expel drugs from the cancer cell will be unable to do this if the drug has formed chemical bonds to its target in the cancer cell. Our objectives are to test this hypothesis in two ways. One is to prepare and test potential drugs that are faulty substitutes for the enzyme farnesyl transferase. This enzyme in the cancer cells will then put the faulty group on biomolecules in the cell that are supposed to cause cancer cell division, but the biomolecules will fail to do so because of the built-in structural flaw. Our other approach will be to prepare and test substances designed to chemically bond to the enzyme farnesyl transferase and block the enzyme from carrying out its function in cancer cell division. The cellular mechanisms for drug resistance will undoubtedly try to remove the drugs from the cell, but it will be too late when the chemical bonding has occurred: Faulty substitutes will have been chemically bonded into key biomolecules to render them functionless, or the enzyme farnesyl transferase will have been rendered inactive by the substance chemically bonded to it. We will test the new compounds with the enzyme and also for activity against human cancer cells in culture.

Cancer deaths were estimated at nearly 10,000 in Arizona in 2001, with an estimated 22,000 new cases diagnosed. A strategy for evading cancer cell drug resistance could have a tremendous impact on cancer survivability and enhancement in the quality of life of Arizonans.

D. Larry Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY04: $166,250

Water quality and cholesterol-induced pathology

In our cholesterol-fed rabbit model of Alzheimer's disease (AD), we have discovered that the increased circulating cholesterol-induced accumulation of the toxin β-amyloid in neurons of the brain is greatly reduced among animals administered distilled drinking water compared to animals receiving local tap water. Deposition of β-amyloid, after neuronal production, in the extra-neuronal spaces of the brain as senile plaques (SP) is generally considered to be linked to the etiology of AD. We have also observed that use of distilled water significantly alters the severity of pathology in other organs due to increased circulating cholesterol levels (aorta, liver and spleen). We had the water tested from the three separate locales where this differential pathologic effect due to water quality had been observed. An EPA certified laboratory performed a Standard A Analysis of water quality, and in special studies determined the levels of Arsenic, Mercury, and Aluminum. This evaluation suggested that a common agent in the tap water, previously reported to be implicated in the etiology of AD, was copper. In very pilot data we have shown that among cholesterol-fed rabbits, administration of distilled water supplemented with 0.12 ppm copper (one-tenth the EPA limits) induced significant accumulations of β-amyloid in the brain compared to animals allowed unaltered distilled water. This level of dietary copper produced even more severe AD-like neuropathology than had been observed with tap water alone. There is overwhelming data emerging that clearly identifies increased circulating cholesterol as a risk factor for developing AD in addition to increased risk of coronary heart disease, myocardial infarction, and premature death. Increased blood cholesterol level is a problem for Arizonans and Americans in general. Coupling this known problem with our observations suggests that water quality could influence 1) the pathology produced in organs of the body including the heart and the brain, 2) the risk of developing devastating diseases, and 3) premature death due to increased circulating cholesterol levels. We believe that it is imperative to establish the agent in tap water that produces this effect, whether it is copper or not, to possibly protect the health of all that might be susceptible, and therefore at risk.

As noted above our overall goal is to disclose the agent in tap water that augments the pathology caused by increased circulating cholesterol levels in cholesterol-fed New Zealand white rabbits as a small animal model of both Alzheimer's disease and coronary heart disease. Based entirely on the presented pilot data (1.3), we hypothesize the presence of copper in tap water causes the observed difference in pathology induced by cholesterol. We will test this hypothesis by adding copper in various forms (sulfate, nitrate, chloride) to distilled water and quantify the pathology produced in the brain, spleen, liver and aorta by dietary cholesterol compared to animals allowed distilled water without copper added. Pilot data is based on addition of only copper-sulfate to distilled drinking water. Using this approach, because trace levels of sulfate, nitrate and chloride are also found in tap water, we can effectively rule-in copper and rule-out sulfate, nitrate and chloride if the biologic effect occurs in all animals on copper. Because of the potential importance of the results from these studies, we will require that we consistently observe the altered biologic effect at least four times (4 separate experiments) for any specific additive, with a minimum of 20 animals per group. As an alternative approach, if we find the pathologic effect occurs in only the copper-sulfate animals, we will test sulfate compounds for a consistent biologic effect. Using such a process of elimination, we expect to identify the agent(s) in tap water that may be harmful to those with elevated circulating cholesterol levels. In addition to grossly observing and histologically quantifying

pathologic alterations in organs of the body, we will assess a number of blood-borne markers. Such indices will include establishing the levels of β-amyloid, copper and ceruloplasmin (a main copper carrying protein), and markers of free radical activity (increased in activity with increased cholesterol levels). We will also establish the level or activity of each of these markers in the brain. We have also found that adding copper to the drinking water augments cholesterol-induced AD-like neuropathology but not pathology of the liver in excess of that found in animals on distilled water without copper. Based on this we will also attempt to disclose the agent in tap water eliciting this effect on pathology of the liver. In coordination with the primary goals, because we have tentatively ruled out some trace metals that have been linked to AD, we will first test such trace metals (i.e., aluminum, zinc, iron) to confirm that there is no induction of cholesterol-induced AD-like pathology in the brain while testing each agent for the capability of augmenting cholesterol-induced pathology of the liver.

Daekyu Sun, Ph.D.

University of Arizona
Award Amount FY04: $89,183

Development of Human Telomerase Inhibitors as New Anticancer Drugs

Cancer related deaths account for about 20% of all deaths in both men and women. The main problem with standard anticancer chemotherapy lies in their toxic side effects that result from interaction with the drug target in normal tissues. Thus, there have been many efforts to find new treatments that may be more specific to killing or stopping the growth of cancer cells. Many genetic validation experiments indicate that telomere maintenance by the enzyme telomerase is a key event during the immortalization and tumorigenesis of human cancers. Furthermore, the discovery of telomerase expression in a majority of tumors has significantly raised its profile as a target for therapeutic intervention. However, no telomerase inhibitor has been tested yet in clinical trials mainly due to the absence of highly selective telomerase inhibitors. We have recently identified several agents that inhibit telomerase activity. With our expertise in drug design, synthesis and evaluation, new agents based on telomerase inhibition with desirable therapeutic properties would emerge from the planned study in this proposal. The long-range goals of this project are to develop novel anticancer agents based on telomerase inhibition with fewer undesirable side effects in normal tissues than the standard anticancer drugs. We will generate new telomerase inhibitors based on our lead compounds that have structural and mechanistic diversities, and we will then evaluate their antitumor activities against human tumors using *in vitro* cell culture model system. The specific aims of the research project submitted for this ADCRC grant are to 1) design novel telomerase inhibitors with improved selectivity and potency through the organic synthesis of new analogs based on lead compounds and 2) evaluate the antitumor activities of new telomerase inhibitors against human tumors using *in vitro* cell culture model system.

Paul Torrence, Ph.D.

Northern Arizona University
Award Amount FY04: $49,940

Nucleic Acid Therapeutics for West Nile Virus Infections

West Nile Virus invaded the United States from the Mid-East in the summer of 1999 when it infected people in New York. Since then the virus has spread quickly throughout the U.S. into 37 states and the District of Columbia. Ths virus is here to stay, and it is highly probably West Nile virus will be resident in the rest of the lower 48 states, including Arizona, by summer of 2003. Although usually transmitted by a mosquito bite, it can also be transmitted in blood transfusions, organ transplants, and by breast feeding of infants. Older individuals, and immuno-suppressed populations are very susceptible to serious West Nile virus infection sequelae. There is no drug that can be used to combat such West Nile Virus infections. In this proposal, I outline a strategy to discover a lead agent to defeat West Nile Virus infection.

This approach to a therapeutic for West Nile virus infections is based on a considerable understanding of the genes and proteins of the West Nile Virus, and it uses an approach based upon nucleic acids - the components
of RNA and DNA. In addition, this approach is grounded in aspects of the natural interferon antiviral defense mechanism. I have applied this novel approach to another virus that is somewhat similar to West Nile; namely, respiratory syncytial virus (RSV). This latter research has led to a drug that is active against RSV infection in primates and is now under preclinical development by a commercial entity. I want to use these same principles to design and synthesize potential drugs that would work to block West Nile virus infections I have selected specific "Achilles Heels" of the West Nile virus genes and proteins. The agents I design are targeted to these weak areas of the West Nile virus. I hope to identify lead antiviral agents that will generate the interest of pharmaceutical companies and other granting agencies to fully fund development of these"leads" into clinically applicable therapeutics.

SECTION G

PROPOSALS RECEIVED

FY 2003

Adams	Arizona State University	Essential Fatty Acid Treatment Study in Autism	$89429 66360
Ahmad	University of Arizona	Molecular and Biological Characterization of HIV-1 Associated with Pathogenesis and Disease Progression in Children	$50000 50000 50000
Allen	Arizona State University	Structures and Functions of Nicotinic Acetylcholine Receptor Cytoplasmic Domains	$175000 175000 175000
Allen	Canyonlands Community Health Care	Rural Impementation of Chronic Care Model for Treatment of Diabetes in Medically Underserved Communities	$50000 50000 50000
Allen	Arizona State University	Chemical Composition of Aerosols Deposited in the Lung	$50000 50000
Bagatell	University of Arizona	A Phase I Study of 17-Allylamino-Geldanamycin in Pediatric Patients with Refractory Solid Tumors	$173133 173133 133533
Bagatell	University of Arizona	Effects of a Heart Shock Protein 90 Binding Drug on Molecular Endpoints in Tumor Cells from Pediatric Patients	$49374 49374 49099
Bayles	University of Arizona	Impact of Amplification on Performance of Hearing Impaired Alzheimer's Patients	$168300 165527 167718
Bielski	University of Arizona	The Coagulation Cascade Response to Plasma Transfusion	$137924 145873 145235
Bosco	University of Arizona	A Genetic Dominant Synthetic Lethal Screen in Drosophila: A Model System for Identifying Novel Molecular Targets for Chemotherapy	$50000 50000 50000
Brooks	University of Arizona	Dissection of Age Related Kidney Disorders *via* Analysis of Cell Specific Gene Expression	$50000 50000 50000

Brown	University of Arizona	Membrane Lipid Rafts in Disease Transmission	$50000 50000 50000
Burd	University of Arizona	Isolation of Adult Bone Marrow-derived Stem Cells Capable of Neuronal Replacement in Neurodegenerative Disease	$50000 50000 50000
Cherrington	University of Arizona	New Drug Targets in Liver Disease	$50000 50000 50000
Clark	Arizona State University	Development of a Plant-Based Adjunct Therapy for the Treatment of Brain Cancer	$175000 175000 175000
Clark	Arizona State University	Effects of a Plant-Based Medicine on Malignant Brain Tumor Cells	$50000 50000 50000
Cone-Wesson	University of Arizona	Speech Feature Discrimination and It's Electrophysiological Correlates in Human Infants	$49871 49943 49635
Cress	University of Arizona	Epigentic Alteration of Cell Adhesion in Cancer	$175000 175000 175000
Dehdashti	University of Arizona	Myocardial Angiogenesis in Ischemic Heart: Development of Cardiac Bioreactor Models & Novel Therapies	$49944 49880 49957
DeLuca	University of Arizona	Effect of Nicotine on T-Cell Development	$70000 70000 70000
DeLuca	University of Arizona	Effect of Antenatal Tobacco Smoke Exposure on Human T Cell Development	$175000 175000 175000

Erickson	University of Arizona	The Role of Multiple Drug Resistant P-Glycoprotein, and Other Genes, in Doxin Caused Cleft Palate	$49803 49990
Funk	University of Arizona	Investigation of the Use of Statins for the Treatment of Rheumatoid Arthritis	$49995 49995 49995
Gaballa	University of Arizona	Stem Cell-Based Therapy of Heart Failure	$175000 175000 175000
Galgiani	University of Arizona	Diagnosis of Early Valley Fever	$156292 157909 158258
Garcia	University of Arizona	Human Papilloma Virus as a Predictor of Recurrent/Persistent Cervical Dysplasia after LEEP	$173720 173417 168600
Gerner	University of Arizona	Interdisciplinary Basic Science Program in Intestinal Carcinogenesis	$55000 55000 55000
Ghosh	University of Arizona	Inhibiting Protein-Protein Interactions Involved in Cancer	$49500 49500 49500
Glass	University of Arizona	Structure and Oxidation of Selenium Cancer Preventive Agents	$50000 50000 50000
Glisky	University of Arizona	Identifying Early Neuropathologic Markers in Alzheimer's Disease Using Diffusion-Weighted MRI	$49977 49771 49504
Graner	University of Arizona	Chaperone-rich Cell Lysates: Antigen and Adjuvant in an Anti-cancer Vaccine	$50000 50000 50000
Guerriero	University of Arizona	Novel Anti-cancer Activity Related to the Structure of HspBP1	$49884 49867 49647

Han	University of Arizona	Development of New Anti-renal Cell Carcinoma Agents Using Pharmacological Synthetic Lethal Screening	$49881 49630 49822
Harman	Kronos Longevity Research Institute	Effects of Smoking Cessation with and without Antioxidant Supplementation on Oxidative Stress, Inflammatory Factors and Insulin Resistance	$174954 174954 174954
Harris	University of Arizona	Strategies to Reduce Skin Cancer in Arizona	$168892 174446 173418
Hoffman	Arizona State University	Autoimmune Disease and CNS Manifestations	$50000 50000 50000
Horton	University of Arizona	Recognition of Damaged DNA by Human XPC-a Glimpse into an Early Step in DNA Repair	$44825 49431 49005
Hoying	University of Arizona	Tissue-Vessel Interactions in Tissue Regeneration	$175000 175000 175000
Huang	University of Arizona	Functional Characterization of Intrinsic Cardiac Adrenergic Cells	$49998 49998 49998
Huntoon	University of Arizona	Promoting Healthy Behaviors: The Role of the Built Environment in Facilitating and Hindering Physical Activity	$134482 135278 74322
Joens	University of Arizona	Campylobacter Jejuni Infection of Cultured Cells	$166122 173987 172789

Jongewaard	University of Arizona	Gene Expression Profiling of Early Cardiac Development	$49999 49999 49999
Joyce	Sun Health Research Institute	Molecular Events of Long-Term Memory Formation in Aging Hippocampus	$50000 50000 50000
Katsanis	University of Arizona	Chaperone Rich Cell Lysate (CRCL) Vaccine for Ovarian Cancer	$96492 100352 104367
Koss	Sun Health Research Institute	An Integrated Approach to Driver Assessment in Maricopa County	$174872 173266 174883
Kuo	Barrow Neurological Institute	Regulation of Nicotinic Receptor α6 and α9 Genes in Immune and Neuronal Stem Cells	$164480 163590 168978
Lake	University of Arizona	Dendritic Cell Immunity to Coccidioides Immitis	$50000 50000 50000
Lance	University of Arizona	Role of Fibroblasts in Colorectal Carcinogenesis	$50000 50000 50000
Li	Sun Health Research Institute	Estrogen and Alzheimer's Disease: Novel Transgenic and Knockout Animal and Cellular Models	$174861 174941 174943
Lorton	Sun Health Research Institute	The Sympathetic Nervous System, Bone Destruction and Arthritis	$175000 175000 175000
Lukas	Barrow Neurological Institute	Receptors for Nicotine Involved in Reward and Mood	$174967 174959 174950

Lukas	Barrow Neurological Institute	Brain Microvasculature, Blood-Brain Barrier, Nicotine, and Nicotinic Receptors	$174838 175000 174974
Lukas	Barrow Neurological Institute	Molecular Bases for Nicotine Dependence	$174996 174910 174939
Lyubchenko	Arizona State University	Morphology Specific Antibodies for Alzheimer's	$174999 174950 174999
Macia	Arizona State University, East	A Study of the Effect of Controlled Oxygen Delivery on Retinopathy of Prematurity	$49735 49745 49909
Marchalonis	University of Arizona	Immunomodulatory Autoantibodies to T-Cell Receptor in Rheumatoid Arthritis	$166250 166250 166250
McDonagh	University of Arizona	Mechanisms by Which Air Pollution Increases the Severity of Heart Attacks	$164350 159163 163939
Meuillet-May	University of Arizona	Inhibition of EGF Receptor Tyrosine Kinase to Enhance the Radiosensitivity of Human Brain Tumors	$50000 50000 50000
Miketova	University of Arizona	Early Detection of Fungal Infections in Immunocompromised Patients	$113280 99759 103189
Miller	University of Arizona	A Family Alliance Intervention for Pregnant Smokers	$165137 165622 151244
Morkin	University of Arizona	Molecular Mechanisms of DITPA Actions	$153366 156771 160252

Osterhout	University of Arizona	Development of Anti-microbial and Anti-viral Peptide Drugs by Combinatorial Chemistry	$49999 49999 49999
Pettit	Arizona State University	Anticancer Drug Preclinical Development	$166250 166250 166250
Poznanski	Midwestern University	Nicotine Effects on Neural Expression of Nicotinic Acetylcholine Receptors in the Zebrafish, *Danio rerio*	$135727 139373 143167
Quan	University of Arizona	Pervalence and Correlates of Childhood Sleep Apnea	$175000 175000 175000
Radford	Phoenix Children's Hospital	Improving Care for Patients with Cystic Fibrosis	$174691 170548 170398
Ramakumar	University of Arizona	Applications of Tissue Sealants with Therapeutic Renoprotective Genes in Minimally Invasive Surgery	$145225 130024
Rensing	University of Arizona	Pathways of Iron Uptake in Uropathogenic Escherichia Coli Strains	$45727 46872
Romagnolo	University of Arizona	Tobacco Metabolites Induce Negative Regulation on BRCA-1 Through a p53-Dependent Mechanism	$49424 49424 49424
Romanovsky	St. Joseph's Hospital	Vagal Anti-Inflammatory System	$155321 160630 166166
Rose	Arizona State University	Eluding Drug Resistance in Cancer Chemotherapy	$50000 50000 50000
Schmidt	Arizona State University	*In Vivo* Efficacy Studies of New Anticancer Drugs for Preclinical Development	$156069 151795 157482

Segal	University of Arizona	The SEER Method of Detecting Carcinogenic Mutations in Living Cells	$50000 50000 50000
Selmin	University of Arizona	Function and Regulation of Expression of the mPR	$49245 49468 49471
Singh	Arizona State University	Novel Biosensors for Monitoring Tumor Progression	$50000 50000 50000
Skibo	Arizona State University	Inhibitors of Tyrosine Kinases- Design and Study of New Cancer Drugs	$169842 162815 166948
Sparks	Sun Health Research Institute	Water Quality and Cholesterol-Induced Pathology	$166250 160289 164667
St. John	University of Arizona	Beta-Amyloid and Acetylcholine Receptor Internalization in Muscle Cells	$49997 49999 49999
Stevens	Northern Arizona University	Electro-cortical Alterations During Hypnotically-Suggested Cardiovascular Responses	$47850 46200 47630
Sun	University of Arizona	Development of Human Telomerase Inhibitors as New Anticancer Drugs	$89183 94683
Taren	University of Arizona	Cycling to Avoid Diabetes in Youth - The CADY Study	$49821 49828 49081
Torrence	Northern Arizona University	Nucleic Acid Therapeutics for West Nile Virus Infections	$49940 49940 49940
Vail	Northern Arizona University	Paramagnetic Nanoparticle Immunoassay for Food Pathogen Detection	$38225 32725

Vakeski	University of Arizona	Why Do Some Venules Leak and Not Others?	$50000 47581 46100
Varga	University of Arizona	Development of an *In Vitro* Cardiomyocyte Mode of O-opioid Agonist Mediated Cardioprotection	$173800 173800 173800
Willott	University of Arizona	Facing West Nile Virus in Arizona	$80381 64761 64761
Wilson	University of Arizona	Neurophysiology of Motor Sequence Learning	$49254 49254 49254
Winzerling	University of Arizona	Lung Cell Growth and Differential: Iron Control of Early Growth Factor-1	$50000 50000 50000
Witten	University of Arizona	Role of Neurokinin Substance-P in Smoke-induced Acute Lung Injury	$134134 129190 130015
Wu	Barrow Neurological Institute	Function of α4-Nicotinic Acetylcholine Receptors	$174603 174603 174822

SECTION H

INDEX

Principal Investigators, New Awards and Continuing Contracts