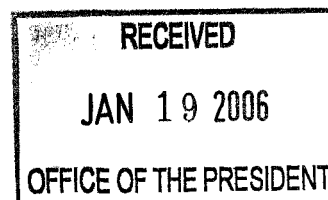




ARIZONA
BIOMEDICAL
RESEARCH
COMMISSION



January 17, 2006

Janet Napolitano
GOVERNOR

The Honorable Ken Bennett, President
Arizona State Senate
Senate Wing
1700 West Washington
Phoenix, Arizona 85007

COMMISSIONERS:

Dear Sir:

C. Eileen Bond, JD
David Landrith, MPA
Steven Weinberg, JD

A.R.S. §36-273 requires that the Arizona Biomedical Research Commission [ADCRC] prepare and submit a report in January each year to the Governor, the President of the Senate and the Speaker of the House of Representatives. The improvement of the health of Arizona's citizens through research by Arizona scientists and clinicians is the mission of the Arizona Biomedical Research Commission.

Gary S. Krahenbuhl, EdD
Henry C. Reeves, PhD
Walter H. Williams,
PhD, MD

As Deputy Director of the Commission, I am pleased to transmit to you the copy of the 2004-05 Annual Report.

Eladio Pereira, MD
Colleen Brophy, MD

Sincerely,

Dawn C. Schroeder,
DDS, MA
EXECUTIVE DIRECTOR

James Matthews, MPA, HR-CP
Deputy Director

James Matthews,
MPA, HR-CP
DEPUTY DIRECTOR

JM:iq
Enclosure

15 SOUTH 15TH AVENUE
SUITE 103-A
PHOENIX, AZ 85007
PHONE: 602.542.1028
FAX: 602.542.6380

ARIZONA BIOMEDICAL RESEARCH COMMISSION



2004 – 2005
ANNUAL REPORT

January 2006

ARIZONA BIOMEDICAL RESEARCH COMMISSION

ANNUAL REPORT

2004 -2005

Janet Napolitano, Governor

Henry Reeves, Ph.D., Chairman

COMMISSION MEMBERS

General Public

C. Eileen Bond, J.D.

David Landrith, M.A.

Steven Weinberg, J.D.

Medical Community

Eliadio Pereira, M.D.

Colleen Brophy, M.D.

Scientific Community

Henry C. Reeves, Ph.D.

Walter H. Williams, Ph.D., M.D.

Gary S. Krahenbuhl, Ed.D.

Staff

Executive Director: Dawn C. Schroeder, D.D.S., M.A.

Deputy Director: James Matthews, M.P.A.

Executive Staff Assistant: Damika D. Brock

Accountant II: Ismene Quintanilla

Program Project Specialist II: Daniel J. Powell

Administrative Assistant: Cecelia Tosie

15 South 15th Street, Suite 103A

Phoenix, Arizona 85007

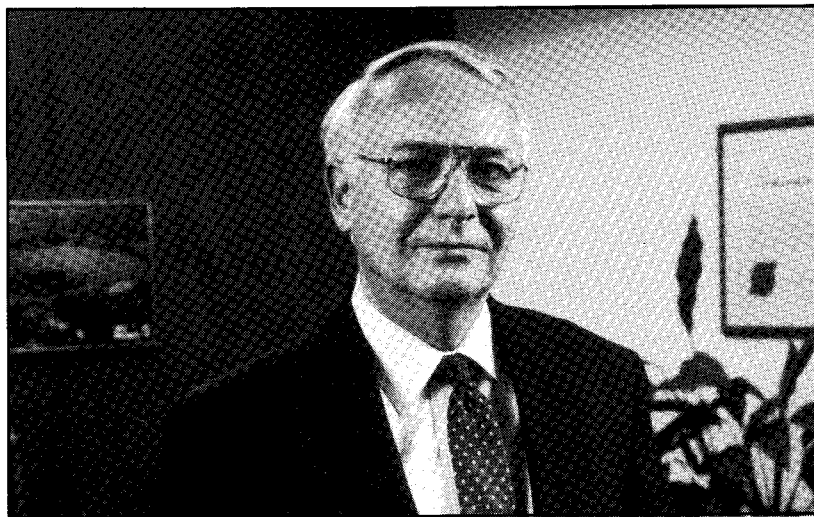
Telephone: 602.542.1028

Fax: 602.542.6380

January 2006

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Message from the Chairman

Fiscal year 2005 was the year that the Commission adopted its new name, the Arizona Biomedical Research Commission. Our new name more accurately reflects the mission of the Commission to support Arizona investigators in becoming world class biomedical researchers.

Twenty-seven new scientific research contracts were awarded this year. The Annual Report contains abstracts for all of the projects along with information on funding levels and institutional involvement. The abstracts demonstrate the wide breadth of inquiry being undertaken by Arizona investigators. Commission contract awards continue to enable many Arizona researchers to prove their investigative concepts and go on to obtain additional funding at the national level. The Commission is continuing its technology transfer efforts.

A significant effort by the Commission has been to promote the development of translational research. An on-going alliance among the Flinn Foundation, Battelle Memorial Institute, and the Commission has aggressively pushed an agenda of translational research and reformation of the public policy supporting translational research.

Contracts pursuing the translational research areas outlined in the Arizona Bioscience Roadmap (a link to the Roadmap may be found at www.adcrc.com) have been awarded. A significant number of collaborative multi-institutional investigative projects are underway in the neurosciences including a Parkinson's Disease Center, on an Alzheimer's disease collaboration, cancer, bioengineering, and imaging. Commission funding supports Translational Research Project Advisory Committee and

processes and harmonizing approaches to key business practices such as intellectual property agreements. Activities are underway to reach out and effectively collaborate with Arizona special populations.

As I complete ten years of activity with the Commission, I take this opportunity to thank every Commissioner that I have had the pleasure of working with. The Commission is especially fortunate to have a dedicated support staff. The work of the Commission is a team effort that continues to advance research and researchers in the pursuit of scientific excellence.

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. It is the hope of all the members of the Arizona Biomedical Research Commission that encouraging both new researchers and large scale multi-institutional/multi-disciplinary investigations will advance scientific discovery in the search for better health and lives for Arizona citizens.

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 2004 – 2005 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 expired 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 UCLA. 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. Commissioner Bond was recently appointed as a Judge Pro Tem in Yavapai County. 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 and reappointed by Governor Napolitano in May 2003. Her term expires in May 2006.



David Landrith

Mesa

**Vice President for Policy and Political Affairs
Arizona Medical Association**

Commissioner Landrith received his undergraduate degree in philosophy and history from Arizona State University. He received a Masters of Public Administration from Harvard University John F. Kennedy School of Government. He was a Dougherty Foundation Fellow. Commissioner Landrith is co-chairman of the ASU Dean's Advisory Council, a member of the Arizona Town Hall Board of Directors, member of the St. Vincent De Paul Free Medical and Dental Clinic Endowment Committee, Director of the Arizona Bioethics Network, and past chairman and executive secretary of the Arizona Council of Governments Directors' Association. He has received the Partnership Award from the Arizona Chapter of the American Academy of Pediatrics, and the Presidential Award for the Arizona State Association of Physician's Assistants. Commissioner Landrith was appointed by Governor Napolitano in 2004. His term expires in 2007.

Photo Not Available

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. He was reappointed by Governor Napolitano in 2005. His term expires in 2008.



Medical Community

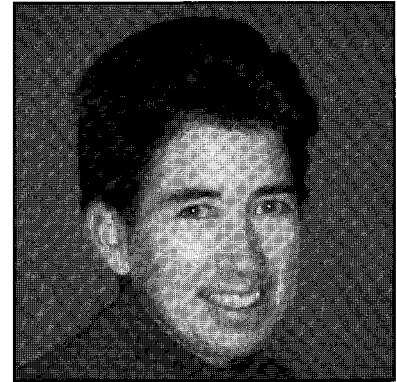
Colleen M. Brophy, M.D.

Tempe

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, Arizona Biodesign Institute at ASU. She is a founder and president of a biotechnology start-up company developing proteomic based therapeutics, Arizona Engineered 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 Bio-engineering Study Section. She was appointed in 2002 by Governor Napolitano. Her term expires in May 2006.



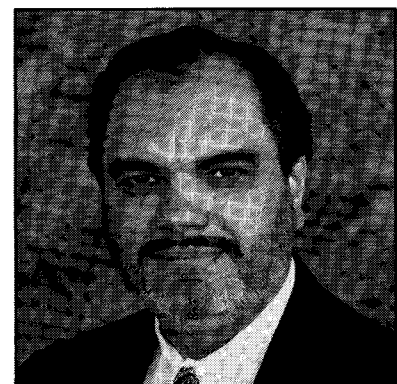
Eladio Pereira, M.D., F.A.C.P.

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 expired in May 2005.



Scientific Research Community

Gary S. Krahenbuhl

Tempe

Senior Vice President and Deputy Provost - Retired

Arizona State University

Commissioner Krahenbuhl received his B.S.Ed. and M.S.Ed. from Northern Illinois University and his Ed.D. from the University of Northern Colorado in 1969. He joined Arizona State University in 1973 and served as the Director of the Human Performance Laboratory, Associate Dean and Dean of the College of Liberal Arts and Sciences, Senior Vice President and Deputy Provost of the University, and retired from the University in 2003. Commissioner Krahenbuhl's major fields of interest include biogenic amines and acute stress and the physiology of distance running. He has published extensively on human stress response, the effects of catecholamine excretion, and the physiological effects of training regimes in adults and children. Commissioner Krahenbuhl was appointed to the Commission by Governor Napolitano in 2004. His term expires in 2007.

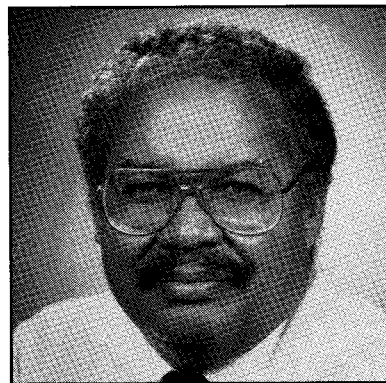
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Walter Williams, Ph.D., M.D.

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. Dr. 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 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 reappointed by Governor Hull in May 2000 and by Governor Napolitano in May 2003. His term expires in May 2006.



Summary of 2004 – 2005 Commission Activities

The Commission administers 63 contracts worth over \$6 million with medical researchers in Arizona. The Commission continues its commitment to individual investigators as well as expanding into translational research. 17 new contracts and \$846,000 were directed toward assisting individual investigators in developing proof of their research concepts and collecting preliminary data. In addition, the Arizona Biomedical Research Commission has broadened its support of translational research with 11 new translational contracts totalling over \$1.6 million.

A special effort is being made to ensure the success of the Translational Genomics Research Institute. TGen receives \$5,000,000 per year for a period of five years plus a \$500,000 annual award for a period of ten years. An additional \$3,000,000 is directed toward Alzheimer's research at TGen.

Section headings in this report 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.

Nearly 1,000 Requests for Proposals (RFPs) for 2005-2006 awards were mailed to potential applicants in September 2004. The amount of funding available for new unrestricted medical research was approximately \$2.8 million. In response to the RFP, the Commission received 147 unrestricted medical research proposals.

ABRC Projects Submitted/Accepted FY 2005

Institution	Submitted	Accepted	Percent Accepted	Dollar Amount	Percent of Total
Arizona State University	26	2	7	400,000	14
Northern Arizona University	7	1	14	149,920	5
University of Arizona	93	17	18	1,376,323	49
Sun Health Research Institute.	5	2	3	275,000	10
Barrow's Neurological Institute	3	2	67	99,694	4
Others	13	4	31	499,985	18
Total	147	28	19	2,800,922	100

In November and December the medical research proposals received 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 170 out-of-state peer reviewers. Three reviews were sought for each proposal. Translational proposals were also sent to three member peer reviewer panels covering neuroscience, cancer, and bioengineering/bioimaging. The translational peer review panels met in Phoenix to interview and discuss the translational proposals with the principle investigators. All proposals and evaluations were distributed to Commission members. In the spring and summer of 2005 the Commission selected 28 proposals for funding. During 2005-2006 the ABRC will be managing a total of 63 contracts.

ABRC Total New and Continuing Project Contracts 2005

Institution	Award	Percent Awarded	Dollar Amount	Percent of Total
Arizona State University	8	13	\$ 1,055,694	17
Northern Arizona University	3	5	\$ 239,352	4
University of Arizona	37	60	\$ 3,291,461	53
Sun Health Research Institute	4	6	\$ 489,667	8
Barrow's Neurological/St. Joseph's	3	5	\$ 265,860	4
TGen	3	5	\$ 149,822	2
Mayo Clinic	1	2	\$ 250,000	4
5AM Solutions	1	2	\$ 100,000	2
Watching Over Mothers and Babies Foundation	1	2	\$ 151,244	3
Molecular Profiling Institute	1	2	\$ 149,985	3
Total	63	100	\$ 6,093,085	100

SECTION A

CONTINUING CONTRACTS

Medical Research

Year Three

FY 2005

Lokesh Joshi, Ph.D.

Arizona State University
Award Amount FY05: \$175,000

Recombinant Protein Therapeutics

Cardiovascular disease and cancer are the two leading causes of death in the United States and in the state of Arizona. Our group is investigating two molecules that are highly effective against vascular and cancer diseases. Until now, recombinant proteins of therapeutic value have largely been obtained from microbial and mammalian sources. However, because of the costly production methods and the inherent threat of cross-contamination, such as prions, viruses and other adventitious agents, there is a strong need for alternative technologies of production. We are developing plants as the source of these human proteins that are able to prevent and/or treat disease. Plants are safe and can be scaled up to produce large quantities of medically-important proteins for patient care. In the past year we were able to introduce human genes encoding the proteins of interest in plants. We have bioengineered plants that contain a protein which modulates our immune system to combat cancer growth and infectious microorganisms. These plant-derived proteins will be characterized and tested for their efficacy in biological assays. This represents a novel approach that has significant therapeutic and biotechnology potential.

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY05: \$50,000

Sodium Narcistatin in Treatment of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is characterized by inflammation and destruction of the articular joints and affects 5% of the population of the United States including Arizona residents. Historically, treatments for RA have been developed from anti-cancer therapies targeting cell invasion and proliferation. These compound are usually very toxic to immune cells, leaving the patient immuno-compromised. Four novel nontoxic anti-cancer drugs (sodium narcistatin (SNS), Combrestatin-4-phosphate (CA4P), Dolastatin 10 and Auristatin PYE) were evaluated for their ability to prevent joint destruction and inflammation in an animal model of RA. Additionally, blood metabolic parameters, liver/kidney enzymes, and white and red blood cell counts were determined to assess toxicity. SNS and CA4P were the most efficacious. Additionally, metabolic parameters, blood cell counts and liver/kidney toxicity measures were unaffected. In conclusion, SNS and CA4P are highly effective in reducing disease parameters and hold great promise for development as safe alternatives to current immuno-compromising treatments for RA.

Molly A. Brewer, DVM, MD, MS

University of Arizona
Award Amount FY05: \$149,048

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

A total of twenty patients were recruited and measured for this study. It was shown that fluorescence measurements can provide accurate and non-destructive measurements of cells in suspension, as well as in cultured cells, and that information derived from these measurements are affected by chemopreventive drug treatments and the genetic make up of cells.

Biochemical changes occur as tissue progresses from normal to pre-neoplastic to cancer. The areas that offered the most promising distinctions were in the UV excitation range at 270 and 340 nm. The preliminary analysis supports the need for further studies at the UV-B excitation wavelengths, which would be possible with an experimental design limiting tissue exposure.

Fluorescence spectroscopy has the ability to distinguish ovarian cancers from normal ovarian structures and benign neoplasms and to differentiate between normal variations and metaplastic structures. This will be further explored as a device for the early detection of ovarian cancer.

Publications:

Kirkpatrick N, Zou C, Brands W, Brewer MA, Utzinger U. Endogenous fluorescence spectroscopy of cell suspensions for chemopreventive drug monitoring. **Photochemistry and Photobiology**, 81:125-134, 2005.

Robin Harris, Ph.D.

University of Arizona
Award Amount FY05: \$175,000

HPV Infection In Men: A Prospective Cohort Study

The goal of this research is to further our understanding of human papillomavirus (HPV) infection in men. We are conducting 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 37 different genotypes of HPV, and the development of HPV antibodies. We are also identifying 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 provides an educational forum about the most commonly acquired sexually transmitted infection of which few men are aware. The progression of this project during the third fiscal year includes finalizing recruitment of 378 men, enrollment of 337 men, and conducting 2-week and 6-month follow-up clinical visits.

Megan M. McEvoy, Ph.D.

University of Arizona
Award Amount FY05: \$50,000

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

Asymmetric cell division is a process which generates cellular diversity during development. The goal of this research was to determine the structures of several proteins important to the formation of asymmetric protein complexes in neural stem cells. The atomic level features of these proteins will shed light on how particular proteins are recruited to these complexes and how the interactions are regulated. Central to this process are the scaffolding protein Inscuteable and the RNA-binding protein Stauf. In order to obtain the large amounts of protein needed for structural studies, various constructs of Inscuteable and Stauf were created. Two constructs of Inscuteable and one of Stauf were successfully made with the appropriate properties. Screens to obtain crystals suitable for structure determination are presently underway with one of the constructs of Inscuteable.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY05: \$175,000

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

Deoxycholic acid (DOC) is a bile acid and natural detergent that is released into the gut after a high-fat meal and is responsible for inducing many stresses (e.g. oxidative stress, nitrosative stress) including oxidative DNA damage. We have previously determined that DOC induces a form of cell death called apoptosis. Persistent exposure of cells to DOC, however, leads to the development of apoptosis resistance, a process that increases more damage to DNA and contributes to the development of cancer. We determined that the up-regulation of the Nitric oxide (NO) signaling pathway is responsible, in part, for this apoptosis resistance. One of the mechanisms by which NO can contribute to apoptosis resistance is to modify pre-made proteins so that they cannot induce apoptosis, a process called S-nitrosylation. The importance of these findings to residents of Arizona is to emphasize the importance of diet in the prevention of cancer.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY05: \$250,000

Development of New Anticancer Drugs for Improving Human Cancer Treatment

Outstanding progress has been realized during this past year toward completing the first total syntheses of dolastatin 16 and 17 that we originally isolated and characterized from an Indian Ocean sea hare. Both and especially dolastatin 16 have given exciting results in initial anticancer evaluations, and further preclinical development is urgently needed but depends on availability only practical by total syntheses. The syntheses of auristatin 15 - DMO and 15 - F are also proceeding well, and completion will also allow these two promising new anticancer drugs to advance in preclinical development.

Publications:

Pettit GR, Minardi HJ, Rosenberg E Hamel, Bibby MC, Martin SW, Jung MK, Pettit RK, Cuthbertson TJ, and Chapuis JC, Antineoplastic Agents 509, Synthesis of Sodium Fluorcombstatin Phosphate and Related 3-Halo-Stilbenes. *J. Nat. Prod.*, 68: 1450-1458, 2005.

Pettit RK, Weber CA, Kean MJ, Hoffmann H, Pettit GR, Tan R, Franks KS, and Horton, ML, Microplate Alamar Blue Assay for *Staphylococcus epidermidis*, Biofilm Susceptibility Testing. *Antimicrobial Agents and Chemotherapy*, 49: 2612-2617, 2005.

Pinney KG, Jelinek, C, Edvardsen K, Chaplin DJ, and Pettit GR, The Discovery and Development of the Combretastins, in **Anticancer Agents from Natural Products** edited by Cragg GM, Kingston DGI, and Newman DJ, 2005.

Pettit RK, Woyke T, Pon S, Zbigniew AC, Pettit GR, and Herald CL, *In vitro* and *in vivo* Antifungal Activities of the Marine Sponge Constituent Spongistatin. **Medical Mycology**, 43: 453-463, August 2005.

Ali MA, Bates RB, Crane Z, Dicus CW, Gramme MR, Hamel E, Marcischak J, Martinez DS, McClure KJ, Nakkiew P, Pettit GR, Stessman CC, Sufi BA, and Yarick GV, Dolastatin 11 Conformations, Analogues, and Pharmacophore. **Bioorganic & Medicinal Chemistry**, 13: 4138-4152, 2005.

Muller IM, Dirsch VM, Rudy A, Lopez-Anton N, Pettit GR, and Vollmar AM, Cephalostatin 1 Inactivates Bcl-2 by Hyperphosphorylation Independent of M-Phase Arrest and DNA Damage. **Molecular Pharmacology**, 67:1684-1689, 2005.

Pettit GR, Zhang Q, Pinilla V, Hoffman H, Knight JC, Doubek DL, Chapuis JC, Pettit RK, and Schmidt JM, Antineoplastic Agents. 534. Isolation and Structure of Sansevistatins 1 and 2 from the African *Sansevieria ehrenbergii*. **J. Of Nat. Prod.**, 68: 729-733, 2005.

Pettit GR, Meng Y, Herald DL, Stevens AM, Pettit RK, and Doubek, DL, Antineoplastic Agents 540. The Indian *Gynandropsis gynandra* (Capparidaceae). **Oncology Research**, 15: 59-68, 2005.

Pettit GR, Ducki S, Herald DL, Doubek DL, Schmidt JM, and Chapuis JC, Antineoplastic Agents 470. Absolute Configuration of the Marine Sponge Bromopyrrole Agelastatin A. **Oncology Research**, 15: 11-20, 2005.

Pettit GR and Tan R, Isolation and Structure of Phakellistatin 14 from the Western Pacific Marine Sponge *Phakellia* sp. **J. Nat. Prod.**, 68: 60-63, 2005.

**DNA and Topoisomerase I Interactions of Novel
Homocamptothecin Anticancer Drugs**

The camptothecin (CPT) derivatives are among the most promising anticancer drugs recently introduced in the clinic. Camptothecin derivatives are renowned for their unique mechanism of action, inhibition of DNA topoisomerase I (topo I). Homocamptothecins (hCPTs) 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. hCPT fully conserves the topoisomerase I inhibiting activity and stimulates high levels of DNA cleavage. Novel 20-fluorocamptothecin has for the first time been successfully synthesized and has been shown to be biologically active. The 20-fluorocamptothecin analogs represent a novel system for NMR to probe the lactone conformation and dynamics as well as the drug binding with Topo I/DNA.

Human topoisomerase I (topo I) is an essential cellular enzyme and is the sole molecular target for homocamptothecins. Topo I contains two highly conserved globular domains, the C-terminal and core domains. The 6.3 kDa C-terminal domain of topo I contains the active site tyrosine (Tyr723) and is the only region highly conserved for the type IB topo I family. Even though several crystal structures of topo I/DNA complexes are available, structural information for the free topo I protein(s) or fragments has not been successfully obtained. Using a variety of biophysical techniques, we have shown that the topo I C-terminal domain protein (topo6.3) appears to be in a molten globule state of native-like tertiary fold. Topo6.3 protein can be readily activated by associating with the core domain, which can be considered as an initial step on the path of the formation of a completely productive topo I complex. The molten globule state of topo6.3 is highly competent to fold into a functionally active conformation and may be an energy-favorable conformation for the free topo6.3. The structural fluctuation and plasticity may represent an efficient mechanism in the topo I functional pathway, where the extent of the flexibility directs the formation of a fully productive topo I complex and provides an exquisite control of topo I function and specificity.

Rodney D. Adam, M.D.

University of Arizona
Award Amount FY05: \$49,999

Gene Expression in *Giardia Lamblia*

Giardia lamblia trophozoites undergo antigenic variation of the variant specific surface proteins (VSPs), a process which is probably controlled epigenetically. Our studies of two vsp genes have shown that the transcription and translation start sites are synonymous. We have identified a 60 bp core promoter that includes an initiator element (Inr) surrounding the start codon. In addition, the region up to 3 kb preceding the start codon is also essential for maximal expression. These observations provide the framework for understanding how VSP expression is controlled and for how to proceed with further studies. As part of our evaluation of potential epigenetic mechanisms of control of gene expression in *Giardia*, we identified the histone modifying genes, then used inhibitors to determine that although there was no identifiable effect on the rate of antigenic variation, there was a marked effect on the completion of cytokinesis. These observations could lead to the development of novel chemotherapeutic agents for *Giardia*.

Jorge A. Giron, Ph.D.

University of Arizona
Award Amount FY05: \$50,000

Molecular Characterization of Type IV Pili Produced by Enterohemorrhagic *E. coli* O157:H7: The Etiologic Agent of the Hemolytic Uremic Syndrome

This study begun with the characterization of a novel hair-like structure (called pili) produced by the food-borne pathogen enterohemorrhagic *E. coli* O157:H7. We hypothesized that these pili that we call Hcp contribute to bacterial adherence and colonization of the human (accidental host) or bovine (natural host) intestines. Several important findings arise from the data generated in this study supported by the ABRC: 1) that EHEC does produce type IV pili; 2) antibodies against Hcp block adherence; 3) Hcp agglutinates animal erythrocytes; 4) Hcp binds to human matrix proteins such as fibronectin and laminin; and 5) Hcp contributes to biofilm formation in EHEC. These lines of evidence indicate that the Hcp may play a role in the adherence of EPEC to epithelial cells. We believe our data contribute to the understanding of the pathogenic scheme of these bacteria. The funding provided by the ABRC was central to obtain extramural funding from the NIH.

NEUROLOGICAL, MENTAL, AND BEHAVIORAL DISEASES AND DISORDERS

Marlene P. Freeman, M.D.

University of Arizona
Award Amount FY05: \$49,658

Omega-3 Fatty Acids for Postpartum Depression

Postpartum depression (PPD) affects 10-15% of mothers. Omega-3 fatty acids are an intriguing potential treatment for PPD. The efficacy of omega-3 fatty acids for PPD was assessed in an 8-week dose-ranging trial. Subjects were randomized to 0.5 g/d (N=6), 1.4 g/d (N=3), or 2.8 g/d (N=7). Across groups, pre-treatment Edinburgh Postnatal Depression Scale (EPDS) and Hamilton Rating Scale for Depression (HRSD) mean scores were 18.1 and 19.1 respectively. Post-treatment mean scores were 9.3 and 10.0. Percent decreases on the EPDS and HRSD were 51.5% and 48.8%, respectively. Changes from baseline were significant within each group and when groups were combined. Groups did not significantly differ in pretest or posttest scores. The treatment was well tolerated.

These results support further study of omega-3 fatty acids as a treatment for PPD. Health benefits of omega-3 fatty acids make this intervention attractive for women and their babies.

Michael H. Ossipov, Ph.D.

University of Arizona
Award Amount FY05: \$173,814

Fentanyl-Induced Paradoxical Pain: Antinociceptive Tolerance and Receptor Down-Regulation

The treatment of chronic pain with morphine often causes abnormal pain and tolerance. We found that opioid-induced abnormal pain and analgesic tolerance develop substantially more slowly with fentanyl when compared to morphine. Consequently, we expect that long-term use of fentanyl would require fewer increases in fentanyl dosage over long periods of time, therefore maintaining its efficacy. Like morphine, prolonged fentanyl use causes changes in the nervous system that promote an increase in pain perception. Blocking these changes with a proteasome inhibitor (epoxomicin) prevents development of opioid-induced abnormal pain and analgesic tolerance. Because fentanyl is a potent, highly effective opioid analgesic, it appears to produce less abnormal pain and tolerance and is well suited for the long-term treatment of chronic pain states. The combination of fentanyl with other drugs that block dynorphin production or spinal sensitization of the spinal cord may produce an even more effective treatment for chronic pain states such as those related to nerve injury and cancer.

**Comparing Smoking Cessation Treatments for Persons with
Schizophrenia and Other Serious Mental Illness**

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

- Persons with mental illness smoke nearly half of the cigarettes in this country
- PSMI smoking rate is 2-3 times higher than in the general population
- As public funding provides most of PSMI support; cessation could mean cost savings
- Cessation interventions for this population are very understudied.

This study compared two interventions to help PSMI quit smoking: contingent reinforcement (monetary rewards; CR) alone and CR plus 21 mg nicotine patches (CR+NRT). Participants in both CT groups earned progressively more money for each visit where they demonstrated abstinence. The two intervention groups were compared with a third self-quit group. Measures (over 36 weeks) included saliva cotinine, self-report of smoking, breath carbon monoxide (CO), psychiatric status, craving, nicotine withdrawal symptoms, and quality of life. Qualitative data were also collected from the clients at each assessment.

Participants were from three La Frontera Center, Inc. clinics. We recruited 231, enrolled 181, and intervened with 121 PSMI in the two treatment groups. Contrary to popular impressions about PSMI, participants were quite motivated to quit (48%) or reduce (50%) their smoking. Based on a conservative estimate (cotinine level < 15), quit rates for the CR and CR+NRT groups respectively were 19% and 7% at week 20 (end of active intervention) and 6% and 11% by week 36 (final follow-up). The CR+NRT quit rates were actually higher (15% at week 20, 18% at week 36) when only participants who were adherent to NRT were included in the analyses. Thus, a follow-up, nicotine replacement via transdermal patches improved quit rates over contingent reinforcement alone *when used as directed*. It was evident from both our quantitative results and intervention staff experiences that these participants were able to control their smoking (i.e., quit) for short periods (24-36 hrs) in order to exhale CO below 10 ppm to receive the contingent reinforcement. There was also clear evidence that participants were able to reduce the amount they were smoking by about 2/3 overall. Perceived life stress was reported by our participants to be an important moderator of outcome, provoking relapses. However, there was no change in psychiatric symptoms in any group over time. Research intervention staff found that time devoted to interacting with individual participants during study appointments represented a unique and beneficial experience for the participants.

The quit rates we found with CR and CR+NRT compare favorably to other studies (Addington, 1998; Ziedonis, Williams, & Smelson, 2003), suggesting that this highly addicted and vulnerable population can experience successful smoking cessation using common treatment methods. Also as in other studies, combination methods (e.g., adding NRT) produce better quit rates than single methods. The results suggest that when using a contingent reinforcement paradigm, raising the frequency of breath CO measurements and reinforcements to every day or every other day would enhance efficacy. Many

participants who didn't quit did reduce the amount smoked. Reduced smoking would yield health and economic benefits, according to studies that have looked at the relationship of dose to disease. In addition, allowing participants to commit to reducing cigarette consumption (v. quitting) was a critical strategy to maintain engagement in the interventions. Although treatment workers often express concern that encouraging PSMI to quit smoking will exacerbate their psychiatric symptoms, in this study psychiatric symptoms did not change for any group over time, an important finding. Qualitative data indicate that the effects of stressful events should be quantified in future studies with this population and interventions should include a stress management component. Developing therapeutic alliance was a critical element in this population; this is a known predictor of positive outcome in behavioral treatment and should be controlled for any future intervention. Further qualitative studies could help illuminate unique issues for persons with a serious mental illness and whether they discovered different and useful quitting strategies. Those who successfully quit and those who significantly reduce smoking will experience significant health and economic benefits, both personal and for their families. Because smoking leads to increased physical and mental health care costs, reducing or eliminating smoking in this population (persons supported by public systems) would result in reduced costs for the state of Arizona.

Barry M. Pryor, Ph.D.

University of Arizona
Award Amount FY05: \$49,918

Characterization of *Alternaria* Isolates Associated with Allergenic Asthma

Soil and plant debris samples were collected from 22 locations around Tucson, AZ, and *Alternaria* isolates were recovered. Recovered isolates were analyzed for variation in morphology, genetics, and allergen diversity. Morphological examination revealed that Tucson isolates encompassed the range of variation exhibited by 10 different *Alternaria* species (AFLP fingerprinting of fungal DNA also genetic variation in Tucson isolates that encompassed 8 different *Alternaria* species.) Immunoblot analysis of allergen diversity revealed more diverse allergens in Tucson isolates compared to standard (type) *Alternaria* isolates, including those used in commercial allergen preparation. Immunoblot analysis revealed that blood sera previously characterized as *Alternaria* negative by skin prick tests actually had immunoglobulin E (IgE) specific to similar allergens as sera characterized as *Alternaria* positive. This reveals that skin prick tests do not reflect the presence or absence of *Alternaria*-specific IgE. One allergen-specific IgE was present only in the *Alternaria* positive sera, and the allergen was present in all Tucson fungal extracts but not in standard *Alternaria* isolates.

SECTION B
CONTINUING CONTRACTS

Medical Research

Year Two

FY 2005

Paul McDonagh, Ph.D.

University of Arizona
Award Amount FY05: \$159,163

Mechanisms by Which Air Pollution Increases the Severity of Heart Attacks

In major metropolitan areas such as Phoenix, hospital admissions for ischemic events (heart attacks) increase significantly following a "Bad Air Day". The particulate matter (PM) component of air pollution correlates most strongly with the increase in ischemic events. The aim of this project is to uncover the mechanisms underlying the relationship between pulmonary intake of particulate matter and heart attacks. Ambient particulate matter with an aerodynamic diameter less than 10 μ m (PM₁₀) is strongly associated with cardiovascular complications from air pollution. PM₁₀ causes pulmonary injury and inflammation in the lower airways. Lipopolysaccharide (LPS), is a pro-inflammatory molecule associated with the coarse fraction (PM_{>2.5}) of PM₁₀. We hypothesize the PM_{>2.5} and LPS induced pulmonary inflammation cause the release of pro-inflammatory cytokines into the systemic circulation. To test this hypothesis we instilled mice with one of four treatments: saline, PM (200 μ g), PM+LPS₁₀ (PM + 10 μ g LPS), or PM+LPS₁₀₀ (PM + 100 μ g LPS). Histologic analysis was performed on two lungs from each group. To qualify pulmonary and systemic inflammation, an ELISA was performed on the pulmonary supernatant and plasma for the proinflammatory cytokines TNF- α was elevated with PM+LPS₁₀ and significantly increased with PM+LPS₁₀₀ treatment. PM and PM+LPS₁₀ pulmonary IL-6 nearly doubled compared to saline. Pulmonary IL-6 in PM +LPS₁₀₀ treated mice significantly increased compared to saline. Plasma TNF- α significantly increased with PM+LPS₁₀ and PM+LPS₁₀₀ treatments. Regarding IL-6, all groups increased relative to saline with a significant increase with PM+LPS₁₀₀ treatment. Histologic analysis revealed a dose dependent increase in pulmonary inflammation with all treatments. These results suggest that coal fly ash PM_{>2.5} and LPS cause pulmonary inflammation and the release of significant quantities of pro-inflammatory cytokines into the systemic circulation. Systemic inflammation induced by inhalation of PM₁₀ might therefore be a plausible mechanism by which cardiovascular ischemic events are exacerbated. In year 3, we plan to assess the effect of a finer fraction of particulate matter (PM_{<2.5} μ m) on cytokine production and activation of circulating neutrophils. We also plan to test a particle derived from the combustion of diesel fuel which is a more representative source of PM inhaled in major metropolitan areas following a 'Bad Air' Day.

Vagal Anti-Inflammatory System

Consistent with data showing that tobacco smoking decreases (whereas smoking cessation increases) the risk of certain inflammatory gastrointestinal diseases, we hypothesized that stimulation of nicotine receptors is protective in sepsis. Mice were made nicotine-dependent (modeling such a dependence in smokers), subjected to microbial sepsis (by intestinal puncture), and then either received no nicotine (mimicking what happens to ICU patients) or continued receiving nicotine. The outcome of the experimental sepsis was found to be independent of nicotinic stimulation. We proposed that stimulation of nicotinic receptors limits harmful effects of systemic inflammation but blocks its anti-microbial effects, and that the two actions cancel each other in microbial sepsis. To test this hypothesis, we initiated a study in a mouse model of aseptic (without live bacteria) systemic inflammation (caused by lipopolysaccharide). During year 2, we also continued characterizing the nicotinic system of the gastrointestinal tract. Three original papers and a review were published.

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Steiner AA, Chakravarty S, Robbins JR, Dragic AS, Pan J, Herkenham M, and Romanovsky AA. All Thermoregulatory Responses to Conventional Preparations of Lipopolysaccharide are Triggered by Lipopolysaccharide per se and Not by Lipoprotein Contaminants. *Am. J. Physiol. Regul. Integr Comp. Physiol.*, 289: R348-R352, 2005.

Rudaya AY, Steiner AA, Robbins JR, Dragic AS, and Romanovsky AA. Thermoregulatory Responses to Lipopolysaccharide in the Mouse: Dependence on the Dose and Ambient Temperature. *Am. J Physiol. Regul. Integr. Comp. Physiol.*, 289: R1244-R1252, 2005.

Indraneel Ghosh, Ph.D.

University of Arizona
Award Amount FY05: \$49,500

Inhibiting Protein-Protein Interactions Involved in Cancer

The American Cancer Society estimated that over 9,700 lives have been lost in Arizona in 2004 from cancer. The prevention of cancer is the long-term goal of our proposal. We have developed a general strategy for developing leads for potent cancer therapeutics utilizing novel technological advances that target the disruption of protein-protein interactions leading to cancer progression. We believe that our technological platform for drug design is simple and powerful and will not only aid in designing cancer therapeutics but also impact other diseases, such as Alzheimer's. We have made excellent initial progress towards our goals in the last two years, having designed and created over 1 billion drug candidates and have demonstrated proof of concept against a cardiovascular target. We are currently in the process of evaluating the activity of our compounds against the protein, vascular endothelial growth factor, widely implicated in cancer progression.

Haiyong Han, Ph.D.

Translational Genomics Research Institute
Award Amount FY05: \$49,630

Development of New Anti-Renal Cancer Carcinoma Agents Using Pharmacological Synthetic Lethal Screening

The overall goal of this project is to identify novel antitumor agents that selectively kill renal cell carcinoma cells with loss-of-function mutations in the von Hippel-Lindau (VHL) tumor suppressor gene. In the past year our research has been focused on the identification of compounds that selectively kill renal cancer cells with deficient VHL function but not cells with intact VHL. We first obtained a pair of isogenic cell lines of which one line has intact VHL and the other has a mutated (therefore inactive) VHL. By screening a chemical library against this pair of cell lines, we identified 5 potential lead compounds that showed differential killing of the cells. Further evaluation of these compounds demonstrated that they were highly active in killing renal cancer cells that were lacking VHL function. In the coming fiscal year, we will continue to screen library compounds to identify new leads and evaluate their antitumor activities in renal cancer cell line models as well as animal models.

Nancy Horton, Ph.D.

University of Arizona
Award Amount FY05: \$49,431

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

Humans and yeast share the same dilemma living in daylight, the issue being how to repair DNA damage caused by UV radiation. Both use the same method to repair UV damaged DNA. In addition to UV, many other environmental compounds also cause DNA damage. The same factors required for repair of UV damage are also involved in the repair of these lesions. We have been working on both the human form of the DNA damage detection protein, XPC, and its yeast counterpart, Rad4. We are currently creating DNA with various types of damage in order to investigate damaged DNA recognition by these factors. In addition, we have initiated experiments to determine the three dimensional structure of Rad4. The structure of Rad4 bound to damaged DNA will be invaluable to efforts to design drugs to treat various types of cancer.

Natalia A. Ignatenko, Ph.D.

University of Arizona
Award Amount FY05: \$55,000

**Effect of Spermidine/Spermine N1-Acetyltransferase and Ornithine Decarboxylase on Intestinal
Tumorigenesis in Genetically Altered Mice**

Colorectal cancer is the third most common cause of cancer death in both men and women. Human colon cancer is influenced by specific genetic and intestinal luminal risk factors. The adenomatous polyposis coli (APC) tumor suppressor gene acts as a “gatekeeper” for colorectal adenoma formation. All patients with Familial Adenomatous Polyposis (FAP) and the majority with sporadic colon cancers have APC mutations. Polyamine levels are significantly higher in cancers with APC mutations than in normal tissue.

Animal models have provided valuable systems for studying tumor initiation and progression, target issues of carcinogenic agents and preclinical evaluation of potential chemopreventive and therapeutic drugs. In this study we used the multiple intestinal neoplasia (MIN) mice which have the APC gene mutation, similarly to the FAP patients.

We evaluate the effect of the NSAID sulindac on the polyamine pathway. Sulindac (167 ppm in the AIN93G diet) increased steady state levels and the enzymatic activity of the polyamine catabolic enzyme spermidine/spermine N1-acetyltransferase (SSAT) in the small intestine and in the colon of treated mice. The sulindac effect on SSAT was independent of the mutation in the APC gene.

We developed a new mouse model of colon carcinogenesis for study the role of the transcriptional regulator of the ODC gene, the c-myc protooncogene, in colon carcinogenesis. Cre expression and

recombination in the transgenic c-myc null mouse (*Fabpl4^{xat-132} Cre* x *c-Myc*^{-/-}) resulted in mosaic loss of the expression of the c-MYC protein in ileum, cecum and colon tissues, in which the *Fabpl4^{xat-132}* promoter is active. Min c-Myc mice had normal intestinal crypts. We observed a statistically significant decrease in the tumor number per mouse in the small intestine of Min c-Myc compared with Min c-Myc +/- mice or littermates with wild type *c-Myc* gene ($P < 3.03$). The c-MYC expression in the intestinal adenomas of Min c-Myc^{-/-} mice was statistically significantly lower than in Min c-Myc^{+/+} mice ($P = 0.035$). These data demonstrate that *c-Myc* directly impacts Apc-dependent intestinal tumorigenesis in mice.

Ian N. Jongewaard, Ph.D.

University of Arizona
Award Amount FY05: \$49,999

Gene Expression Profiling of Early Cardiac Development

During the last two years, we have developed experiments that give us high levels of new information. This information provides us with a large number of genes that are used in early heart development. On a simple basis, we are now able to determine those genes that have turned on, and those that have turned off. Additionally, we have collected this data and aligned specific groups of genes that behave the same way over several time periods during early heart development. We are gaining knowledge of the specific order, over time, in which these genes interact with each other. This experimentation has allowed us to learn how a number of chemicals treat specific groups of genes in developing hearts. With this information, we can aid in the development of drugs that counteract problems in early heart development. Along with these experiments, we have found several novel genes that we are currently analyzing. Novel genes will give us a large range of targets for unique drug treatment in early heart development.

Emmanuel Katsanis, M.D.

University of Arizona
Award Amount FY05: \$100,352

Chaperone Rich Cell Lysate (CRCL) Vaccine for Ovarian Cancer

Heat shock proteins (HSPs) that are found in all cancer cells carry specific cancer proteins and are currently being studied as vaccines in human trials. We have developed a vaccine that contains multiple HSPs extracted from cancer cells. The advantage of our vaccine, which we have named CRCL, over the ones in clinical trials is that with a simple and relatively rapid procedure, we can generate multiple HSPs in the same vaccine. We hypothesize that several types of HSPs together will induce more effective anti-cancer responses by providing more cancer proteins that can stimulate cancer killing T-cells.

We have successfully generated CRCL from ovarian cancers. In each case the amount we got would be enough to vaccinate a patient. We have performed multiple experiments in cells from patients with ovarian cancer and found that CRCL can be used to generate specific cancer killing T cells that kill the particular cancer cells that the vaccine was prepared from. CRCL, therefore, appears to have excellent qualities in stimulating the immune system to fight against ovarian cancer.

George Pettit, Ph.D.

Arizona State University
Award Amount FY05: \$166,250

Anticancer Drug Preclinical Development

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 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 five 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.

Publications:

Pettit G. R., Meng Y., Herald D. L. Knight J. C., and Day J. F., Antineoplastic Agents 553. The Texas Grasshopper *Brachystola magna*. *J. Nat. Prod.* 68: 1256-1258, 2005.

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Pettit G. R., Rhodes M. R., Herald D. L., Hamel E., Schmidt, J. M., and Pettit, R. K., Antineoplastic Agents 445. Synthesis and Evaluation of Structural Modifications of (Z)-and (E)- Combretastatin A-4, *J. Med. Chem.*, 48: 4087-4099, 2005.

Pettit G. R. and Melody N., Antineoplastic Agents 527. Synthesis of 7-Deoxy-narcistatin, 7-deoxy-trans-dihydronarcistatin, and Trans-dihydro-narcistatin 1. *J. Nat. Prod.*, 68: 207-211 2005.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY05: \$50,000

Eluding Drug Resistance in Cancer Chemotherapy

Cancer cells can become resistant to drugs by pumping the drugs out of the cell so the drugs cannot achieve their biomedical purpose of killing the cancer cells. To prevent cancer cells from countering the effects of drugs in this way, we have made and tested chemical agents that were designed to chemically bond to targets in the cancer cell so that the effects of the agents cannot be reversed by the pump. We have also designed agents to trick cancer cells into chemically bonding the agent to cellular machinery to prevent cell division. More than three dozen compounds in six different chemical categories were designed (some by computer simulation methods), prepared, and/or tested. Several new compounds were found to inhibit the growth of human cancer cells grown in culture. This work may lead to new, more effective anticancer agents for the benefit of Arizona residents.

Development of Human Telomerase Inhibitors as New Anticancer Drugs

Telomerase is a potential molecular target of anti-cancer agents because it is highly specific and essential for the survival and growth of cancer cells. To identify the effective ways of targeting human telomerase or maintenance mechanisms, a number of novel, structurally diverse telomerase inhibitors have been identified as potent human telomerase inhibitors, and their biological activities have been evaluated using MiaPaCa human pancreatic cancer cell line as a model system. In our study, telomerase inhibitors based on G-quadruplex-interactive agents were found to be most effective among various types of telomerase inhibitors in inducing MiaPaCa cell growth arrest, senescence, apoptosis, and telomere length shortening. Our data also suggested that the different biological effects of G-quadruplex-interactive agents could be attributed to their selectivity for interaction with G-quadruplex structures associated with human telomerase. NSC647133 and NSC635488, semicarbazone derivatives complexed with metal ions, were also identified as potent telomerase inhibitors with no interaction with G-quadruplex structures, showing a potent inhibitory effect against human telomerase with 50% inhibition at $\sim 5 \mu\text{M}$. However, they also inhibit DNA polymerase alpha and retroviral reverse transcriptase, which share some homology to telomerase reverse transcriptase. A 2-3 week cultivation of the hTERT-RPE cell line with both drugs at a concentration that did not cause acute cytotoxicity resulted in growth arrest with the appearance of gigantic cells with irregular shapes. These results indicate that telomerase inhibitors with a unique mode of interaction or multiple mechanisms of action might have some advantage over pure telomerase catalytic inhibitors for further development of telomerase inhibitors as a promising anti-cancer agent. Our results also indicate that telomerase inhibitors with the ability to interact with G-quadruplex structures have a better potential for the further development as a promising anti-cancer agent than pure telomerase catalytic inhibitors such as BIRD1532.

Publications:

Liu W., Hurley L. H. and Sun D., Binding of G-quadruplex-interactive Agents to distinct G-quadruplexes Induces Different Biological Effects in MiaPaCa Cells. *Nucleosides, Nucleotides, & Nucleic Acids*, 2005 (In press).

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY05: \$50,000

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

HIV-1 infected infants develop AIDS faster than adults but the molecular mechanisms of HIV-1 mother to infant transmission and HIV-1 pathogenesis in infants are not known, making it difficult to define better and effective strategies for prevention and treatment of HIV-1 infection. We have shown that a minor genotype of HIV-1 with R5 phenotype from infected mothers is transmitted to their infants and initially maintained in the infants with the same properties. Moreover, we found that HIV-1 sequences from transmitting mothers were more heterogeneous than non-transmitting mothers, suggesting that viral heterogeneity may play a role in transmission. The human immunodeficiency virus type 1 (HIV-1) nucleocapsid (NC) plays a pivotal role in the viral lifecycle including encapsulating the viral genome, aiding in strand transfer during reverse transcription, and packaging two copies of the viral genome into progeny virions. Another gene product, p6, plays an integral role in successful viral budding from the plasma membrane and inclusion of the accessory protein Vpr within newly budding virions. In this study, we have characterized the gag NC and p6 genes from six mother-infant pairs following vertical transmission by performing phylogenetic analysis and by analyzing the degree of genetic diversity, evolutionary dynamics, and conservation of functional domains. Phylogenetic analysis revealed six separate clusters that corresponded to each mother-infant pair, suggesting that epidemiologically linked patients were closer to each other than epidemiologically unlinked patients. Nucleotide and amino acid distances showed a lower degree of viral heterogeneity. A low degree of genetic diversity was also estimated. Positive selection pressure was found to be acting on the NC sequences motifs. The two important motifs within NC, the zinc-finger motifs, were highly conserved in most of the sequences, as were the late Vpr binding and AIP1 binding domains in p6. These data suggest that the NC and p6 open reading frames and functional domains are conserved in mother-infant pairs' sequences following vertical transmission, which confirms the critical role of these gene products in the viral lifecycle. This may result in development of new strategies for an HIV-1 vaccine that may help Arizonans and others.

Publications:

Ahmad N. The Vertical Transmission of Human Immunodeficiency Virus Type 1: Molecular and Biological Properties of the Virus. **Critical Reviews in Clinical Laboratory Sciences**, 42:1-34, 2005.

Sundaravardan V, Hahn T, and Ahmad N. Conservation of Functional Domains and Limited Heterogeneity of HIV-1 Reverse Transcripts Gene Following Vertical Transmission. **Retrovirology**, 236:1-17, 2005.

Immunomodulatory 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. Under ADCRC support, 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 (mABs) from RA and SLE patients and determined that these are novel recognition molecules that have the potential to modulate the T cell arm of the immune system. Our findings to date indicate that these monoclonal autoantibodies can suppress the inflammatory (TH1) arm of the T cell response. Moreover, we have obtained preliminary evidence for a previously-unknown immunomodulatory system in which the TCR-peptides to which the mABS are directed enhance TH1-immunity, in contrast to the suppressive effects of the antibodies. Our current data suggest that the autoantibodies derived from individuals with autoimmune diseases are essentially the same ones expressed in low levels by healthy individuals. Our combined molecular and genetic approach offers new possibilities for diagnosis and potential therapy via novel immunomodulatory mechanisms.

Publications:

Sepulveda RT, Marchalonis JJ, Watson RR. T-cell Receptor vbeta8.1 Peptide Reduces Coxsackievirus-Induced Cardiopathology in Aged Mice. *Cardiovasc Toxicol*, 5(1):21-8.2005.

Yu Q, Watson RR, Marchalonis JJ, Larson DF. A role for T lymphocytes in Mediating Cardiac Diastolic Function. *Am. J. Physiol. Heart Circ. Physiol.*; 289(2):H643-651, Aug. 2005.

Marchalonis JJ, Schluter SF, Sepulveda RT, Watson RR, and Larson DF. Immunomodulation by Immunopeptides and Autoantibodies in Aging, Autoimmunity, and Infection. *Annals New York Acad. Sci.*, (In press).

Elizabeth L. Glisky, Ph.D.

University of Arizona
Award Amount FY05: \$47,771

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

Neuropsychological and neuroimaging data have been collected from approximately 75 older adults. The group currently includes older individuals who score within normal limits on neuropsychological tests of memory and executive function, as well as individuals whose memory performance falls more than 1 standard deviation below the mean and who may, therefore, be at cognitive risk for Alzheimer's disease (AD). In addition, 32% carry a genetic risk and 25% have a family history of AD. Diffusion-weighted magnetic resonance imaging (DWMRI), which has previously been found to be sensitive to inflammation in the brain, indicates a selective age-related decline in the white matter of the temporal stem and in the hippocampus among those individuals with a cognitive risk, genetic risk, or a family history of AD. These results suggest that DWMRI may provide an early indicator of incipient AD pathology, which may allow for more efficacious use of drug treatments.

Water Quality and Cholesterol-induced Pathology

Mounting evidence suggests copper may influence the progression of Alzheimer's disease by reducing clearance of the amyloid beta protein (A β) from the brain. Previous experiments show that addition of only 0.12 PPM copper (one-tenth the Environment Protection Agency Human consumption limits) to distilled water was sufficient to precipitate the accumulation of A β in the brains of cholesterol-fed rabbits. Here we report that addition of copper to the drinking water of spontaneously hypercholesterolemic Watanabe rabbits, cholesterol-fed beagles, rabbits, and PS1/APP transgenic mice produced significantly enhanced brain levels of A β . In contrast to the effects of copper, we found that aluminum or zinc-ion supplemented distilled water did not have a significant effect on brain A β accumulation in cholesterol-fed rabbits. We also report that administration of distilled water produced a reduction in the expected cholesterol-induced systemic pathology. Reduced systemic pathology was identified in cholesterol-fed rabbits administered distilled water compared to animals drinking local tap water; this included pathology of the liver and spleen. Studies directed at determining the effect of the trace metals aluminum, copper and zinc on cholesterol-induced systemic pathology were undertaken. As noted, copper increased Alzheimer-like pathology in the brain but did not augment pathology of the spleen or liver. Aluminum added to distilled water (0.36 PPM) administered to drink exacerbated cholesterol-induced hepatic pathology but not splenic pathology, and addition of 0.36 PPM zinc to the distilled drinking water failed to affect pathology of either the liver or spleen. The overall increase in both central and systemic pathology observed among cholesterol-fed rabbits administered tap water seems to be due to different trace metal contaminants occurring in tap water.

SECTION C

CONTINUING CONTRACTS

Medical Research

Year One

FY 2005

Dianne Lorton, Ph.D.

Sun Health Research Institute
Award Amount FY05: \$50,000

Sodium Narcistatin in Treatment of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is characterized by inflammation and destruction of the articular joints and affects 5% of the population of the United States including Arizona residents. Historically, treatments for RA have been developed from anti-cancer therapies targeting cell invasion and proliferation. These compounds are usually very toxic to immune cells, leaving the patient immuno-compromised. Four novel nontoxic anti-cancer drugs (sodium narcistatin [SNS], Combrestatin-4-phosphate [CA4], Dolastatin 10 and Auristatin PYE) were evaluated for their ability to prevent joint destruction and inflammation in an animal model of RA. Additionally, blood metabolic parameters, liver/kidney enzymes, and white and red blood cell counts were determined to assess toxicity. SNS and CA4P were the most efficacious. Additionally, metabolic parameters, blood cell counts and liver/kidney toxicity measures were unaffected. In conclusion, SNS and CA4P are highly effective in reducing disease parameters and hold great promise for development as safe alternatives to current immuno-compromising treatments for RA.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY05: \$164,500

**Discovery of Novel Anticancer and Anti-infective
Drugs from Endophytic Fungi of Desert Plants**

The overall goal of this inter-institutional and multi-investigator collaborative project is to discover novel anticancer and anti-infective drugs from endophytic fungi (fungi that live in the intercellular spaces) of desert plants. During the course of the first year of this project over 100 endophytic fungi have been cultured, their extracts prepared, and screened for their potential anti-cancer and anti-HIV activity. Anticancer activity was evaluated in NCI-H460 (non-small cell lung) and PC-3M (prostate) cancer cell lines and target oriented *in vitro* bioassays for activation of heat shock response and for the inhibition of the migration of metastatic cancer cell line, PC-3M. Prior to testing of fungal extracts for anti-HIV activity in virus infected T-cells, they were evaluated in A30.1 lymphocyte cell line for their toxicity towards this cell line. Fourteen extracts were selected and these are currently being evaluated in anti-HIV assay. Bioactivity-guided fractionation of an extract active in cell migration inhibition assay yielded a small cyclic peptide identified as lateritin. If extracts active in above assays contain compounds that can inhibit the growth of solid tumors such as lung, breast, colon and prostate cancers, and/or are capable of inhibiting the human immune deficiency virus (HIV), our results will have an impact on the health of Arizona's population.

Publications:

Kithsiri Wijeratne EM, Carbonezi CA, Takahashi JA, Seliga CJ, Turbyville TJ, Pierson, ED, Pierson LS, VanEtten, HD, Whitesell L, Da S Bolzani V, and Gunatilake LAA, Isolation, Optimization of Production and Structure-Activity Relationship Studies of Monocilin I, the Cytotoxic Constituent of *Paraphaeosphaeria quadrisepata*. **The Journal of Antibiotics**, 57: 541-546, 2004.

Zahn J, Kithsiri Wijeratne EM, Seliga CJ, Zhang J, Pierson EE, Pierson LS, Vanetten, HD, and Gunatilaka Leslie AA, A New Anthraquinone and Cytotoxic Curvularins of a *Penicillium* sp. from the Rhizosphere of *Fallugia paradoxa* of the Sonoran Desert. **The Journal of Antibiotics**, 57:341-344, 2004.

Kithsiri Wijertne EM, Turbyville TJ, Zhang Z, Bigelow D, Pierson LS, VanEtten HD, Whitesell L, Canfield LM, and Gunatilaka Leslie AA, Cytotoxic Constituents of *Aspergillus terreus* from the Rhizosphere of *Opuntia versicolor* of the Sonoran Desert, **J. of Nat. Prod.** 66:1567-1573, 2003.

Zhou GX, Kithsiri Wijeratne EM, Bigelow D, Pierson LS, VanEtten, HD, and Gunatilaka LAA, Aspochalasin I, J, and K: Three New Cytotoxic Cytochalasans of *Aspergillus flavipes* from the Rhizosphere of *Ericameria laricifolia* of the Sonoran Desert. *J. of Nat. Prod.*, 67:328-332, 2004.

He J, Kithsiri Wijeratne K, Bashyal BP, Zhan J, Seliga CJ, Liu MX, Pierson EE, Pierson LS, VanEtten HD, and Gunatilaka LAA, Cytotoxic and Other Metabolites of *Aspergillus* Inhabiting the Rhizosphere of Sonoran Desert Plants. *J. of Nat. Prod.*, 67:1985-1991, 2004.

Cherry L. Herald, Ph.D.

Arizona State University
Award Amount FY05: \$164,500

Preclinical Development of Three Anticancer Drugs

Of the 200 or more diseases diagnosed as cancer, head and neck cancer is the focus of this research project. There is an urgent need for the discovery and development of new and effective anticancer drugs for human treatment of these diseases. Based on the ASU-CCR anticancer and vascular targeting agent combretastatin A-4 phosphate now advancing in human clinical trials for anaplastic thyroid and head and neck cancers, we have synthesized new structural modifications with promising anticancer activity. Tyrostatin prodrug and stilstatins 2 and 3 are being further developed and synthesized in sufficient quantity for continued early preclinical development leading towards human clinical trials.

Publications:

Pinney K G, Jelinek C, Edvardsen K, Chaplin D J, and Pettit, G R. The Discovery and Development of the Combretastatins: Emphasis on Combretastatin A-4 (CA4) and Combretastatin A-1 (CA1). *Anticancer Agents from Natural Products*, pp. 23-46, 2005.

Laurence Hurley, Ph.D.

University of Arizona
Award Amount FY05: \$49,746

Targeting the Silencer Element in the PDGF-A Promoter to Suppress Gene Expression

We have identified a signaling pathway that is important in the survival of pancreatic cancer cells. In this pathway the key molecular switch involves an unusual DNA structure and an enzyme that remodels the DNA to activate this signaling pathway. Through this proposal we will gain molecular details of the switching mechanism and how we can externally control this process to inactivate it and selectively kill cancer cells. In the first year of funding we have gained insight into the structure of the switch and shown that a number of small molecules bind selectively to this switch. This is the first milestone in ultimately achieving the development of a drug to treat pancreatic cancer through this mechanism.

Tamara King, Ph.D.

University of Arizona
Award Amount FY05: \$164,500

Prostate Cancer: Model of Bone Metastasis, Pain, and Phenotype

Metastasis of prostate cancer to the bone results in cancer pain that is intense, often unremitting and that requires treatment with strong analgesics such as opiates. This research proposal aims to gain a better understanding of the mechanisms underlying cancer-induced pain and bone damage in order to develop and examine non-sedative analgesic drugs to better treat cancer-induced pain. This year, we successfully characterized the progression of cancer induced pain and bone damage induced by prostate cancer cells injected and sealed into the mouse femur. Cancer-induced pain behaviors and bone loss emerged by 21 days following injection of the cells into the femur. In addition, messages for nicotinic receptors, potential targets for the non-sedative analgesic, nicotine, were observed in the same prostate cancer cells. We also demonstrated that a mutation that changes the adhesion and mobility properties of PC3N cells reduces invasion of cancer into the bone and reduces cancer-induced pain.

Emmanuelle Meuliet, Ph.D.

University of Arizona
Award Amount FY05: \$50,000

Novel Inhibitors of Akt as Anticancer Drugs

Inhibiting the signaling pathways that promote cancer cell survival offers a rational and attractive way of selectively inhibiting cancer growth. We have chosen to target one of the key players in the process of tumor growth, the protein Akt. This protein is an attractive target for the development of drugs to promote death specifically in cancer cells and to increase their sensitivity to cancer drugs. We have adopted a novel approach to interfering with Akt signaling and will design, synthesize and test inhibitors of Akt for their antitumor activity. We have identified a novel lead compound and made analogues of it in order to make them more bio-available and more soluble, in other words, to make it a better drug. The goal of the work is to identify a lead compound for development as a cancer drug.

Joyce A. Schroeder, Ph.D

University of Arizona
Award Amount FY05: \$49,718

Molecular Therapeutic Targeting of MUC1/ β -catenin Interactions in Invasive Breast Carcinoma

Breast cancer is the third leading cause of cancer-related deaths in Arizona. The majority of these patients die when breast cancer spreads from the breast to distant sites in the body (metastasis). While current chemotherapeutic treatments are making small gains against this devastating disease, the need for a treatment that targets a large number of patients is needed. We have identified a molecular event that occurs in a high percentage of transformed cells, but not in normal, non-cancerous cells. Specifically, the tumor antigen MUC1 becomes highly expressed (greater than 90% of patients analyzed have increased MUC1 levels) and interacts in a novel way with the adhesion protein β -catenin during metastasis. Experimental evidence indicates that by preventing this interaction, cells lose the ability to metastasize. We have proposed to develop a targeted anti-cancer therapy that inhibits the growth of metastatic cancer cells, while having no side-effects on normal tissue. We have progressed to the point of developing one form of this therapy and are currently determining how well it works in a mouse model of breast cancer. We are very hopeful that this new specific therapy will prevent breast cancer metastasis in our model.

Protein Interactions Mediated by Cys2-His2 Zinc Finger Domains

One of the most common proteins in human cells are the Cys2-His2 zinc finger proteins, which function by interacting with DNA, RNA and other proteins. Disruption of these protein interactions has been linked to developmental defects and cancer. The goal of this project was to develop assays that could identify protein-interacting zinc finger domains and their binding partners. We studied the protein hOAZ, which contains 30 zinc fingers, each differing slightly in sequence composition. Bioinformatics studies suggested certain sequences in the protein-binding fingers were different than those in the DNA-binding fingers. Biochemical studies suggested a yeast two-hybrid assay might be useful to determine if zinc fingers are the protein-binding type. Both sets of preliminary data will need further verification. A better understanding of how protein interactions occur might provide deeper insights into diseases such as cancer and give us clues on how better to fight them.

Phase II Trial of Topical Perillyl Alcohol in Sun Damaged Skin

Skin cancer is by far the most common cancer (with more than 1.3 million new cases expected in 2003 in the U.S.) and is a tremendous public health problem, especially in Arizona and the southwestern United States where sun exposure is high. As reported by the Southeastern Arizona Skin Cancer Registry, rates of nonmelanoma skin cancer in Arizona are among the highest in the world and are 4-6 fold higher than in the general U.S. population. Incidence rates for melanoma, the most deadly form of skin cancer, are rising faster than almost any other cancer. Topically administered chemopreventive drugs that actually stop or reverse the growth of precancerous lesions in the skin may reduce this burden. Perillyl alcohol is a molecule found in the essential oils of lavender, peppermint, spearmint, cherries, celery seeds, and other edible plants. We have shown that pure perillyl alcohol effectively reduces the incidence of skin tumors when applied topically to the skin in preclinical models of both melanoma and nonmelanoma skin cancers. We have recently performed a Phase I clinical trial of a cream formulation of topical perillyl alcohol developed by our group. Results of this study indicate that this formulation is safe when applied twice daily for 30 days. Further clinical testing is now warranted to determine if perillyl alcohol applied directly to sun-damaged skin can reverse such damage.

The objective of this research is to perform a randomized, placebo-controlled, double-blind, Phase 2a dose-finding clinical trial of topical perillyl alcohol in subjects with moderately to severely sun-damaged skin. The hypothesis being tested is that topical perillyl alcohol, when applied twice daily for three months, can successfully reverse sun damage in skin in a dose-dependent manner as evidenced by histopathologic normalization. As secondary endpoints, we will also determine if topical perillyl alcohol can significantly alter previously-studied surrogate endpoint biomarkers of neoplastic changes including optical coherence tomography (OCT) of skin, as well as p 53 expression, c-Fos expression, and apoptosis (as measured by expression of activated caspase-3) in skin biopsy tissue. In addition, karyometric analysis of nuclear chromatin patterns in skin biopsy tissue will be measured. Establishment of valid biomarkers is vital for demonstrating the activity of this and other drugs in future studies. Safety, tolerance, absorption, and formulation stability will also be monitored. Ancillary studies dependent on performance of this trial (but funded from other sources) will include comparative genomic hybridization analysis of skin biopsy samples for gene copy number changes (in collaboration with the Translational Genomics Research Institute (TGen) in Phoenix, Arizona) and nutritional correlates and dietary assessment. Due to unexpected delays regarding FDA approval of experimental use of topical perillyl alcohol in the proposed clinical trial, this project has been extended while we complete additional preclinical dermal toxicology studies. These studies have been completed (December 2005), and we expect to enroll patients beginning in January 2006. The protocol has been approved by the University of Arizona Institutional Review Board (IRB), and enrollment will commence once final FDA approval is granted. Based on the inclusion criteria, we expect that subject accrual will be completed quickly during the first quarter of 2006.

Robert P. Erickson, M.D.

University of Arizona
Award Amount FY05: \$131,535

**Identification of Genes Involved in Lymphedema by
Single Nucleotide Polymorphism Mapping**

Genetically caused lymphedema (swelling of the limb) is a rare disorder compared to post-breast cancer (secondary to surgery and radiation) lymphedema of the arms. However, by studying these rare disorders, one hopes to find information that will lead to prevention and cure of the more common varieties of lymphedema. One first year goal was to find more families with genetically caused lymphedema and to look for the genes causing lymphedema in those families. During this work period we have both identified new families in Arizona with genetic lymphedema and brought studies of those families to various degrees of completion. We have also continued to study DNA from families reported in the grant application, halving the size of the region on chromosome 11 that was to be studied this year. Finally, we have identified a novel mutation in one known lymphedema causing gene and have identified a new candidate gene for lymphedema.

Douglas Lake, Ph.D.

University of Arizona
Award Amount FY05: \$39,600

Dendritic Cells and Immunity of Valley Fever

In the first year of funding we have evaluated and characterized the phenotype and function of human dendritic cells (DC) when exposed to *Coccidioides* spherule lysate (T27K) and to heat-inactivated spherules. While T27K does not mature DC, MPL is a sub-optimal maturation agent as determined by phenotype and functional analysis. We have chose to move forward with the spherules, as they stimulate lymphocytes from healthy donors who have previously had Valley Fever and are taken up by immature DC prior to maturation of DC. We demonstrated that DC engulf fluorescently labeled spherules and also become mature during or immediately after this process. Endocytosis of spherules by DC suggest they are functionally endocytic prior to maturation by spherules. It also suggests that these DC are capable of processing and presenting antigen to T cells. The nature of this presentation demands investigation, as it sets the stage for how an individual will immunologically respond against the fungus - protective or non-protective. We will embark upon the nature of T cell activation in the next 2 years of funding.

Eric J. Guilbeau, Ph.D.

Arizona State University
Award Amount FY05: \$50,000

Biosensor for Measurement of Breath Acetone

This research is aimed at developing a novel, inexpensive and easy to use sensor that can be employed by individuals with diabetes to monitor their breath acetone concentration. The sensor works by measuring the heat that is generated when acetone reacts with another chemical on the surface of a very sensitive temperature measuring device called a thermopile. The research is significant because a large number of individuals in the U.S. suffer from type 2 diabetes. Individuals with type 2 diabetes are susceptible to a condition called diabetic ketoacidosis (DKA). Under this condition high amounts of acetone are released into the blood stream and unless corrected, the individual may die. During the past year, we succeeded in fabricating a prototype of the sensor and in using it to measure acetone concentrations comparable to those in the breath of individuals with diabetes. Mathematical models were also developed that confirm the experimental sensor response and the theoretical basis for the sensor's operation.

Marek Jan Romanowski, Ph.D.

University of Arizona
Award Amount FY05: \$50,000

Contrast Agents for Optical Coherence Tomography

This report covers Year 1 of a three-year project to develop a new class of contrast agent for skin cancer research and noninvasive diagnosis of skin lesions. This contrast agent will be used in conjunction with optical coherence tomography (OCT), a biomedical imaging technique for visualization of living tissues. We seek to develop this contrast agent by forming dense arrays of gold nanoparticles on the surface of liposome, a biocompatible sphere of diameter ca 100nm. In the period covered here we demonstrated methods of fabrication of liposomes and methods of fabrication of gold nanoparticles. We demonstrated optical resonances of these particles, a property underlying their use in OCT. Furthermore, we developed technological expertise necessary to vary these optical properties so that this contrast agent can be matched (tuned) to optical specifications of OCT. Initial evaluations of contrast enhancement were performed using so-called phantom, a model simulating tissue properties.

NEUROLOGICAL, MENTAL, AND BEHAVIORAL DISEASES AND DISORDERS

Burris Duncan, M.D.

University of Arizona
Award Amount FY05: \$172,841

Acupuncture as Complementary Therapy for Cerebral Palsy

Cerebral palsy (CP) is the most frequent cause of childhood disability in the US. In Arizona, there are 1,601 children and 1,818 adults with a diagnosis of CP enrolled with the Division of Developmental Disabilities at a cost of \$79.3 million in 2002.

Whereas the brain injuries that cause CP are non-progressive, the motor problems often worsen over time resulting in serious disabilities. Current accepted therapies in this country produce less than desired results. This investigation evaluates the standard of care given in China where results are reported to be far superior.

ABRC has seen this as an important and innovative study. If our hypotheses are confirmed, they offer the prospect of improving the lives of children with CP, of preventing many of the complications, of giving these children a greater opportunity to realize their individual potential, and to have a significant impact on healthcare policy and practice at a national level.

Michael R. Sierks

Arizona State University
Award Amount FY05: \$121,369

Morphology Specific Antibodies for Treating Parkinson's

We have shown that scFv fragments directed toward α -synuclein can alter α -synuclein folding and toxicity *in vivo*. Since α -synuclein is a naturally occurring protein abundantly produced in nerve cells, it would be beneficial to obtain antibodies that can prevent folding of the toxic forms of α -synuclein without interfering with the normal function. Therefore, generating a series of epitope and morphology specific antibodies to α -synuclein, all of which can be expressed intracellularly in models of PD, would be very helpful in defining a potential therapeutic route. Here we have demonstrated the feasibility of the approach and have developed the protocols needed to generate and image α -synuclein morphologies, to isolate morphology specific scFv's, to characterize binding and specificity of the scFv's, and to improve affinity of the isolated scFv's. We have also demonstrated that scFv's expressed intracellularly as intrabodies can provide protection against α -synuclein induced toxicity. These results hold great promise for developing a potential therapeutic for Parkinson's Disease.

SECTION D

NEW CONTRACT AWARDS

BEGINNING IN FY 2006

Danny L. Bower, Ph.D.

University of Arizona
Award Amount FY06: \$49,995

A Rapid and Inexpensive Screen for Mutations that Sensitize Cells to Cancer Drugs

Many cancer therapeutic drugs are successful in only a small percentage of patients with a particular type of tumor. In many, if not most, cases this means that a patient is often beyond help by the time the “correct” drug regimen can be tried. Less obviously, the low response rate means that many promising drugs never make it to the clinic because the scale (and cost) of the clinical trials required to find the small percentage of responders is so great that pharmaceutical companies are unwilling to invest the hundreds of millions of dollars to search for a significant number of positive responding patients.

Cancer is caused by genetic mutations in cells, and drug testing in patients can become much more efficient if one can first match sensitivity to a drug with a specific genetic change in cells. One approach to the problem is to define specific molecular targets, typically overactive proteins found in cancers, and design compounds that will inhibit the activity of these proteins. This approach has met with some limited success. Alternatively, one can begin with compounds that have shown some effects in inhibiting the growth of cancerous cells and try to determine which genetic changes make cells especially sensitive to these compounds. Identification of such changes will not only make patient therapy more efficient but will allow molecular prescreening and selection of patients for clinical trials. This means that much smaller numbers of patients will be required and has the potential to reduce the cost of trials dramatically (by more than ten-fold). This means that many more potential drugs can be tested.

We propose to develop a screening procedure that will test potential or existing cancer therapeutic drugs for effectiveness in cells in which the activities of specific genes have been inhibited, using newly derived methods for knocking out the expression of individual genes. In the first rounds we will inhibit the expression of about 100 genes, one at a time, that are involved in the repair of genetic defects. We will then ask if any of these “mutations” makes the cells especially sensitive to the effects of a drug that disrupts the normal copying mechanism of the genes. Some of the drugs we will start with are currently used in the clinic, but fall into the category that the system is designed to assess; that is, they are effective in a small percentage of patients for unknown reasons. Unlike others, we will be doing our initial screening using cells from an invertebrate genetic model system (the fruit fly, *Drosophila melanogaster*). We do this because the underlying biology is similar to that of mammals such as humans, but the technology for inhibition of individual genes is much, much simpler. In part because of this, we can do initial screens for a small fraction of the cost of the same procedure using mammalian cells. This should make the approach affordable for any small enterprise and even a desirable first step for a large pharmaceutical company. This project is designed primarily as a proof of principle; if successful, we will eventually go on to test novel compounds on a large scale.

Barrett's Esophagus, Esophageal Adenocarcinoma and Apoptosis

Barrett's esophagus (BE) is an esophageal lesion that arises as a consequence of chronic heartburn. Heartburn is a very common medical condition. Approximately 40% of adults in Arizona experience heartburn symptoms at least monthly. About 10% of patients suffering from chronic heartburn also have BE, a condition that is associated with a nearly 30-fold increased risk for the development of esophageal carcinoma. This cancer has a poor prognosis with a median survival of less than one year. The incidence of esophageal cancer is rapidly rising for unknown reasons in North America, including Arizona. It is estimated that approximately one hundred patients will die of this cancer in Arizona in 2005 based on deaths rates in prior years. BE is a condition where normal cells are replaced by pre-cancerous tissue. However, the mechanism of development of BE is poorly understood. BE appears to result from chronic heartburn, occurring when the contents of the stomach are refluxed into the esophagus. Gastric acids secreted from the stomach and bile acids secreted in response to a high fat diet from the upper part of small intestine are implicated in the development of BE. Many studies have shown that there is an increased reflux of gastric acids and bile acids into the lower esophagus of patients with BE. In addition, exposure to gastric acids leads to an increase in cell growth. The exposure to bile acids induces the normally occurring process of programmed cell death that is called "apoptosis". Apoptosis is a regulated process of cell suicide that removes stressed and damaged cells. If the damaged cells are not removed by apoptosis, they can establish a cell population with the potential to develop cancer. Repeated exposure of cells to bile acids leads to the development of apoptosis resistance. In our preliminary experiments, we found that interleukin-6 (IL-6), one of the signaling molecules associated with apoptosis resistance, is secreted from BE tissue but not from normal tissue. IL-6 binds to receptors on the surface of cells and activates a cascade of events that result in the increased levels of the actual proteins that can inhibit apoptosis. Importantly, we found increased levels of these anti-apoptotic proteins in BE. Thus, we propose to follow the signaling pathway that is activated by IL-6 in BE and investigate the role of bile acids and gastric acids in the activation of this pathway. An understanding of the signaling pathways that contribute to the development and progression of esophageal carcinoma is crucial for the design of targeted strategies to prevent and eradicate this rapidly increasing cancer.

Hypothesis: Since bile acids and gastric acids appear to be two critical risk factors responsible for the development of BE, we propose that bile acids and gastric acids induce abnormal cellular signaling which results in the increased expression of proteins that inhibit normal cell death (apoptosis). In particular, we plan to evaluate the IL-6 signaling pathway that appears to be activated in BE and investigate the role of bile acids and gastric acids in the activation of this pathway.

The long-term objective of this proposal is to identify molecular pathways that are responsible for the development of BE and esophageal adenocarcinoma, so that targeted therapies can be directed to prevent

the development of Barrett's esophagus and progression to cancer.

To test our hypothesis we are proposing the following three specific aims: 1) to determine if the IL-6 signaling pathway and its components are elevated in BE compared to normal tissue; 2) to evaluate the effects of bile acids and gastric acids on the activation of the IL-6 pathway and the levels of proteins inhibiting apoptosis; and 3) to develop human esophageal cell lines resistant to bile acids and/or gastric acids and to evaluate these cells for the activation of IL-6 signaling and apoptosis resistance.

**Structure and Functional Role of GGA Repeats in c-myb
Promoter Activity in Leukemia**

Leukemias account for more than 20,000 cancer deaths every year in the US, and in spite of some modest improvements in treatment with the development of targeted therapy, there remains an unmet need for novel treatment approaches to this set of diseases. Leukemias arise when an early progenitor of a blood cell becomes cancerous, and acute leukemias are characterized by extraordinary rapid proliferation and a failure of the leukemia cell to “differentiate” or mature into a normal blood cell. The controlled expression of the c-myb oncogene is important for the normal growth and continued repopulation of our blood cells, but uncontrolled, constant and high level of expression of this oncogene can contribute to causing acute leukemia or to allowing leukemia cells to proliferate rapidly (the name “myb” derives from the myeloblastosis virus that causes leukemia in animals and contains a similar gene). Knocking out c-myb appears to be beneficial in test tube and animal experiments with leukemia, but no drugs have been successfully developed that can knock out c-myb expression. Understanding what controls c-myb expression in leukemia cells is important to understanding how these cells grow but do not differentiate. Gene expression is controlled by an on/off switch called a promoter, and we have discovered that part of the c-myb promoter contains a very unusual sequence of DNA where the code “GGA” repeats itself many times. If we clip this segment of the promoter out, c-myb expression becomes almost undetectable in leukemia cells. We have also discovered that the GGA repeat DNA from the c-myb promoter can fold into an unusual four-stranded structure that looks much different than the normal double helix. Because this type of DNA looks so different from double stranded DNA, it may be possible to make drugs that can recognize GGA repeat DNA compared to double stranded DNA. If so, we may be able to force the on/off switch for c-myb into the “off” position and shut down the expression of c-myb with a drug that could then be used in the treatment of leukemia.

Our major goals are to understand how the GGA repeat region of the c-myb promoter controls c-myb expression, to determine whether this region can really fold up into a non-double stranded DNA structure, and to find a drug that can selectively recognize this kind of DNA to knock out the expression of the c-myb gene. It is very likely that one or more proteins that bind to DNA, transcription factors, make use of the GGA repeat region as a docking site to control c-myb expression. Our first objective is to identify the transcription factor binding site in the c-myb GGA repeat region by artificially changing the sequence of DNA to see how that affects c-myb expression. Our second objective is to identify the transcription factor that binds to the GGA repeat by isolating proteins from leukemia cells and finding out which ones bind to a synthetic GGA repeat region in a test tube. We will also use a powerful new technique called “chip” to find out what transcription factors bind to the c-myb promoter in living leukemia cells. Our third objective is to study whether synthetic GGA repeat DNA simulating the c-myb promoter can fold into a four stranded structure. Our fourth objective is to use a series of DNA binding drugs that can recognize four stranded DNA to find the ones that bind best to the c-myb promoter in a DNA binding assay and then use those compounds to show that we can knock down c-myb expression in leukemia cells.

**Discovery and Development of Novel Inhibitors of
Cell Motility from Desert Organisms**

Cancer accounts for a vast number of deaths and suffering. Each year 6.5 million people are diagnosed with cancer worldwide, and in the U.S. alone more than 10 million people are living with a history of cancer. According to the American Cancer Society about 1,372,910 new cases are expected to be diagnosed this year and 570,288 Americans are expected to die of this disease. Arizona's desirable climate attracts many elderly retirees, and thus, the population of cancer patients in the state has been steadily increasing. In 2004, cancer was responsible for 9,710 deaths and 23,560 newly diagnosed cases in Arizona.

Cancer therapeutics, including anticancer drug discovery, is recognized as a near term opportunity by Arizona's Bioscience Roadmap. The majority of anticancer drugs in use today are from natural sources. Our preliminary studies have shown that Sonoran desert organisms are a rich source of biologically active compounds. The overall goal of this inter-institutional multidisciplinary project is, therefore, to investigate Sonoran desert organisms (plants, and plant-associated rhizosphere and endophytic fungi) for cell motility (migration) inhibitors and to conduct structure-activity relationship (SAR) studies of lateritin, a fungal metabolite encountered in an ADCRC funded project, with the broad long-term objective of discovering and developing novel non-cytotoxic agents to treat solid tumors.

The proposed research will lead to testing of our hypothesis that Sonoran desert organisms growing under semi-extreme desert conditions produce secondary metabolites with interesting bioactivity profiles and novel chemical structure similar to other extremophiles.

We are hopeful that the investigation of extract libraries from three different sources and generation of analogs of lateritin, combined with the use of appropriate bioassays and gene profiling techniques, will lead to the discovery of anticancer agents with novel mechanisms of action which can be developed into clinically effective anticancer drugs to treat solid tumors.

Drug Targeting the i-motif in the c-MYC Promoter

A number of human cancers, including pancreatic, colorectal, breast, and prostate, rely upon the overproduction of cancer “oncogenes” for survival. Previous studies have demonstrated that if the production of these oncogenes can be inhibited, then the cancer cells will die. Gleevec, a common cancer drug used to treat gastric and blood cancers, works in this way. We have identified an entirely new switch mechanism for turning off oncogenes that we will target with drug molecules to kill cancer cells.

The long-term goal of this project is to bring into human clinical trials (at the University of Arizona and Mayo Clinic in Scottsdale) a new cancer drug to be used in the treatment of a variety of solid tumors that are presently not amenable to treatment. In order to do this, we will establish the three-dimensional shape of the drug target and its complex with the drug. Using computer modeling of these complexes, we will then design improved drugs for further clinical development. The hypothesis to be tested is that drug targeting of the new cancer oncogene switch we have identified can cure patients of what are now deadly forms of cancer.

**Deconvoluting the Structural Heterogeneity of the Bcl-2 Promoter
Quadruplex to Enhance Drug Targeting**

A variety of human cancers including pancreatic, colorectal, breast, and prostate cancer result, at least in part, from the expression or overexpression of cancer genes or “oncogenes”. It is well known that if these oncogenes can be switched off, the cancer cells will die. A commonly used anti-cancer drug, Glevec, works this way in the treatment of gastric and blood cancers. Oncogenes are attractive targets for anti-cancer drugs, since switching off the cancer causing genes would be expected to have little impact on normal cells. We have discovered an entirely new approach to switching off specific oncogenes like c-MYC and bcl-2. The bcl-2 oncogene is the target of this research, and we expect to develop new drugs that will kill cancer cells by inhibiting the expression of the bcl-2 gene.

The ultimate goal of this project is to discover and/or develop new anti-cancer drugs for the treatment of solid tumors for which there is currently no reliable treatment. In order to exploit our new understanding of oncogene regulation, we will need to develop a three-dimensional picture or model of the gene switch drug target and its complex with drug. Understanding the specific interactions involved in binding the drug to the target will lead to the design of better drugs for clinical development. The long-term objective of this effort is to bring one or more new anti-cancer drugs into clinical trials (at the University of Arizona and Mayo clinic in Scottsdale). The hypothesis to be tested is that drugs designed to target the promoter regions of specific oncogenes may be able to selectively switch off these genes and, thereby, cure patients that currently have untreatable and deadly forms of cancer.

CD8 T cell Priming with Engineered MHC cClass I Molecules

CD8 T cells (also known as cytotoxic T lymphocytes, CTLs) represent a critical component of the body's immune system. CTLs are especially important for protection from intracellular pathogens (such as viruses) and tumors. Regarding the latter, it is clear that under the right circumstances CTLs are capable of specifically recognizing and eliminating cancer cells. Indeed, there is considerable interest in the potential for manipulating CTL responses to target cancer cell elimination in patients. Cancer is one of the most common and serious threats to human health in this country with an estimated 24,000 new cases in 2004 in Arizona, alone. Despite its prevalence, cancer as a whole remains difficult to treat. Thus, new therapies are in great demand, and one promising area of investigation is the aforementioned approach with CTLs. CTLs function to eliminate cells that express at their surface specific "makers" known as antigens which consist of a small protein (peptide) that is bound to MHC class I molecules. Experimentally, it is possible to activate peptide-specific CTLs that can be used therapeutically if the identity of these unique tumor peptides is known. One important peptide, called Her-2/neu, was identified that comes from a normal protein that is over-expressed by 10 to 80% of adenocarcinomas (including breast, pancreas, colon, and ovarian cancers). In this proposal, we will attempt to prime immune responses against this peptide by using a promising new technology.

Recently, we developed a new method for engineering MHC class I molecules to present single peptides, instead of the mixture of different peptides they normally bring to the cell surface. We call the constructs single-chain trimers (SCTs). These pose several remarkable properties that strongly suggest they can be used for many applications, especially priming of CTL responses. Though they have shown promise, it is not yet known whether SCTs can efficiently prime CTLs and what the optimal conditions for priming may be. Here, we will test the hypothesis that DNA vaccination with SCTs will result in better CTL priming and immune protection than more conventional peptide-specific vaccination protocols. A comparative approach will be taken to determine the relative ability of SCTs to activate functional CTLs versus established methods using the important human tumor antigen Her-2/neu as a model. A series of new reagents will be generated and validated for use in a mouse Her-2/neu-expressing tumor model system. Animals will be vaccinated with the SCT DNA constructs and control constructs and, then, challenged with the tumors to determine the effectiveness of the vaccines. This project represents the first systematic test of the ability of SCTs for CTL priming, and it is fully anticipated that its completion will define a new and effective approach for vaccination against Her-2/neu, which could be applied to many other important antigens.

**Discovery, Optimization of Production, and Initial Characterization of Novel
Anticancer Drug Lead Compounds from Bacteria Collected
from the Rhizosphere of Desert Plants**

According to the American Cancer Society, cancer is the second leading cause of death among Americans with one of every four deaths being cancer-related. In 2004, it is estimated that more than 560,000 Americans died of cancer, and about 1.4 million new cases might be diagnosed, even excluding pre-invasive and non-melanoma skin cancers.

Smoking has long been recognized as a major contributor to the incidence of cancers. The 2004 report of the Surgeon General added to the list of diseases caused by smoking the cancers of the kidney, pancreas, cervix, and stomach, in addition to previously established causative links between smoking and esophageal, laryngeal, lung, oral, and throat cancers. Lung cancer accounts for more deaths each year than breast, prostate, and colon cancer combined, making lung cancer one of the deadliest cancers. Almost 90% of lung cancers in the United States are due to smoking. In 2001, out of every 100,000 Arizonians, 69.3 males and 49.1 females were diagnosed and 55.6 males and 37.6 females died of lung cancer alone. A recent study estimated that the cost of treating lung cancer in the United States in 1996 was about \$5 billion dollars per year.

Diagnosed cancer patients are treated by surgery, radiation, and chemotherapy. While chemotherapy is the only alternative for patients with disseminated malignancies, these medicines suffer from problems of general toxicity and cause many undesirable side effects. There are no effective and generally well-tolerated medicines available for solid tumors, and many tumors develop resistance to the drugs currently in clinical use. Thus, continued efforts are necessary to discover relatively safe, cancer cell-specific anticancer compounds that are effective against multidrug-resistant and/or solid tumors.

Natural products from microbes and plants provided us with the majority of anticancer drugs in current clinical use including blockbuster drugs like Taxol. Continued screening of novel sources of microbes and plants thus holds the promise of supplying innovative compounds for anticancer therapy that are not accessible from any other sources, including combinatorial chemical libraries.

Researchers of the University of Arizona have compiled a collection of microbes that grow on or nearby the root of desert plants in the so-called rhizosphere. We and others hypothesized that the extremely harsh environment of the desert calls for fierce competition in the microbial communities, and this competition enriches for microbes that are able to produce chemicals that inhibit the growth of other microbes or help the growth of the plants that host them. Thus, rhizosphere microbes could provide a rich source of biologically active naturally occurring compounds. With initial funding from ADCRC that was

later extended into a larger collaboration, Dr. Gunatilaka and his collaborators successfully screened fungi from his collection for the production of potential anticancer agents using cell lines derived from cancers of the lung, the breast and the central nervous system. Several new compounds were found that were toxic to the cancer cells, supporting the initial hypothesis, and are currently evaluated as drug leads.

The goal of the current proposal is to build on this work and extend the screening effort to evaluate the bacterial part of the microbial collection. Bacteria are known to be at least as prolific producers of biologically active natural products as fungi, and screening programs with bacteria in the past provided many antibiotics, anticancer compounds, immunosuppressants, or otherwise useful chemicals. We propose that this unique collection of desert rhizosphere bacteria represents a novel and unexploited source of anticancer compounds and hope to test this hypothesis by screening around 4% of our collection against a lung cancer-derived cell line. We propose to screen the same bacteria with a novel bioassay that measures changes in the activity of the so-called heat shock proteins that have a supporting role in the uncontrolled cell division that is characteristic of tumor cells. This assay might help us identify compounds with a defined mechanism of action for cancer therapy. We plan to utilize modern microbiological techniques that optimize the production of many different compounds from the same bacterial strains and increase the number of the strains that can be tested in a given time interval. We hope that this screening program will not only prove the unique productivity of our library, but will also provide lead compounds that can be further studied and developed into useful anticancer drugs using chemistry and molecular biology techniques. Such anticancer drugs might eventually alleviate the suffering of many people in Arizona and worldwide.

Lung Cancer Chemoprevention via Inhalation Route

Cancer of the lung is the most prevalent cause of cancer related deaths in the United States. According to the American Cancer Society and International Agency for Research on Cancer, there were 154,900 deaths due to lung cancer in 2002, with an estimated 169,400 new cases diagnosed during the same period. While the five year survival rate for all cancers is 62%, the survival rate for lung cancer is a mere 15%, making it one of the most aggressive and lethal cancers. Unfortunately, current therapeutic strategies such as radiotherapy, cytotoxic chemotherapy and surgical intervention have had minimal effect on the mortality rate for lung cancer. Clearly, the need for a new strategy for the treatment and prevention of lung cancer exists.

The use of inhalation drug delivery for the treatment of lung cancer has several advantages. While there may be identifiable molecular targets for lung cancer, a therapeutic agent can not be effective if it does not reach the target site or is not presented at a high enough concentration. Through the use of inhalation delivery, it is anticipated that relatively high levels of an agent can be achieved in the lung. This should directly correspond with an increase in efficacy, while at the same time decreasing systemic exposure.

A total of 94 million current and former smokers are at risk of developing lung cancer in the coming decades. Indeed, smoking accounts for 87% of lung cancers, and the latency period from initiated tumor cell to clinical diagnosis can be ten years or more. According to CDC statistics for the year 2002, both cigarette smoking and tobacco use in general among the youth population (grades 6 through 8) is higher in Arizona than the national average. Annual smoking-attributable hospitalization expenditures in Arizona topped two hundred million dollars, and total smoking-attributable medical expenditures cost the state of Arizona in excess of one billion dollars annually.

This proposal will help to clearly identify molecular targets for the chemoprevention of lung cancer, specifically 5-LO inhibitors. Moreover, this research will help to define the merits of targeted drug delivery to the lung, which then can be used as a technology platform for other agents that modulate other molecular targets in the lung. This research is designed to examine the following hypothesis: Targeted drug delivery to the lung will increase the efficacy of a 5-LO inhibitor, Zileuton, when compared to oral administration, for the chemoprevention of lung cancer. This specific aims of the research program are as follows: 1) to develop and evaluate a sustained-release aerosol formulation with Zileuton; 2) to determine optimal dose levels for the ethanol-based solution formulation, and determine an appropriate dosing regimen for the sustained-release formulation by monitoring drug levels and biomarker levels from *in vivo* experiments; and 3) to evaluate the effectiveness of the Zileuton formulations when administered via nose-only inhalation in a long-term chemoprevention study.

**Molecular Analysis for the Diagnostic Identification of
Clinically Aggressive Meningiomas**

Meningiomas are typically considered a benign tumor that can be cured by complete surgical removal; however, a percentage of patients have recurrent disease, even after apparently complete removal of a low grade tumor. These patients require additional surgeries, radiation therapy, chemotherapy, or a combination of all three. The ability to recognize these patients prior to recurrence would promote earlier use of additional therapy, thus improving overall patient outcome. Unfortunately, identification of meningiomas with this more aggressive behavior is difficult, and standard pathology techniques rarely suffice. The advent of molecular technologies that allow the analysis of thousands of genes at once (microarray analysis) is opening new possibilities for the subclassification of tumors that pathologically appear to be the same. The correlation of specific expression profiles with known clinical outcomes provides data that can be used to devise molecular biological tests that will provide more accurate diagnostic and prognostic information as well as identifying novel therapeutic targets. Thus, our overall hypothesis is that there are molecular and biochemical changes that can be used to identify meningiomas that will have a more aggressive clinical course. This information can then be translated into a clinically useful diagnostic test. The goals of the proposed research are to define a molecular profile to identify meningiomas that are invasive and/or those that are likely to recur rapidly despite complete surgical resection. We will use microarray-based gene expression profiling and molecular cytogenetics to create this molecular profile. We will then use this information to define a list of proteins that can be analyzed by immunohistochemistry, a technique commonly used in pathology laboratories. We will use a panel of 1200 meningiomas samples to determine which of these proteins and/or molecular cytogenetics can be used to identify meningiomas with a more aggressive clinical behavior. The overall objective of these studies is to identify those patients who would benefit from early additional therapy, thus improving overall survival and quality of life.

Meningiomas are the most commonly reported brain tumor in the United States, accounting for approximately 27% of all primary brain tumors. These tumors are graded I-III based on criteria established by the World Health Organization (WHO). This grade influences the postoperative clinical management of the patient. Grade I tumors are generally benign while grade III tumors behave in a malignant fashion and grade II tumors fall between these two extremes. The majority of meningiomas are grade I; however, 5-11% are grade II and 1-3% are grade III. Patients with grade III meningiomas have a median survival from diagnosis of approximately 1.5 years and a 5-year mortality rate of 68%. Grade II meningiomas are associated with a 40% 5-year recurrence rate even when total removal is achieved. Grade I tumors are thought to be surgically curable; however, a significant percentage of these tumors behave aggressively, and 10-20% of these tumors grow back after surgery. Thus, like other brain tumors, meningiomas display individual variability within a given grade. Current diagnostic tests cannot adequately predict their clinical behavior.

The development of diagnostic tools that identify molecularly-defined subsets of meningiomas that will behave aggressively would allow the use of additional therapy prior to tumor recurrence resulting in an overall improvement in patient survival and quality of life. This “personalized” approach to patient management based on the profile of an individual tumor is a goal of the Medical Biotechnology facet of the Arizona Biosciences Roadmap. We are uniquely poised to contribute to Arizona’s success in this arena due to the clinical expertise at the BNI which results in a large patient population, the molecular expertise in our Neuro-Oncology Research Laboratory, and our use of the molecular profiling capabilities at the Translational Genomics Institute (TGen).

Deregulation of Translation Initiation by eIF3f in Melanoma

The incidence of malignant melanoma has been rising faster than any other human cancer in Arizona and nationally over the past three decades. The death rate from melanoma has tripled in the past four decades. The molecular mechanisms involved in melanoma pathogenesis are only beginning to be defined. Although several tumor suppressors and oncogenes have been shown to be involved in melanoma pathogenesis, relatively little is known about the molecular mechanism of melanoma tumorigenesis and tumor progression.

Regulation of protein synthesis at the level of translation initiation is fundamentally important for the control of cell growth and proliferation under normal physiological conditions. The eukaryotic factor 3 (eIF3) is a multi-protein complex that plays an important role in translation initiation. eIF3f is the p47 subunit of eIF3 complex. We identified eIF3f as a protein associated with the caspase-processed isoform of the cyclin dependent kinase 11 (CDK11p46), which appears to be down-stream effector in apoptotic signaling. Initial studies demonstrate the eIF3f can inhibit melanoma cell growth. eIF3f gene is located at human chromosome band 11p15.4. Aberrations of 11p15.4 occur in many solid tumors including melanoma. Our long term goal is to elucidate the regulatory mechanisms of eIF3f on translation initiation and apoptosis as a prerequisite to the development of therapeutic protocols that can be used to treat cancer. Our hypothesis is that eIF3f is a negative regulator of translation initiation during apoptosis; disruption of eIF3f function contributes to melanoma tumorigenesis by increasing eIF3 activity and deregulating apoptosis. The experimental focus of this proposal is on the abnormalities of eIF3f in melanoma and the mechanism of its regulation of translation initiation. The specific aims are designed to provide a comprehensive assessment of the eIF3f alterations in melanoma cell lines and human melanoma specimens and the regulatory functions of eIF3f in translation initiation, apoptosis and tumorigenesis.

The number of melanoma cases in Arizona has risen 55 percent since 1997, according to the Arizona Cancer Center. Melanoma is the most frequent cancer among women aged 25 to 29 and the second most frequent (after breast cancer) among women aged 30 to 34. The goal of this study is to identify genes involved in the transformation of melanocytes and melanoma tumor progression. Initial studies suggest that the eIF3f gene locus might be involved in melanoma tumorigenesis. We expect that eIF3f gene may be a tumor suppressor gene and, thus, may be an appropriate target to treat melanoma as well as anticipate tumor progression, disease prognosis and survival rate. Accomplishing the specific aims outlined in this proposal will provide the foundation required to assess that possibility by understanding the relationship between eIF3f gene and tumorigenesis (Specific Aim #1), and elucidating the mechanism by which eIF3f regulates apoptosis via regulation of translation initiation (Specific Aim #2).

**Targeting Angiogenesis in Human Kidney Cancer with
G-quadruplex Interactive Compounds**

Cancer related deaths account for about 20% of all deaths in both men and women in Arizona. Thus, there is an urgent clinical need for the development of new treatment for this disease. The main problem with the treatment of cancer patients lies in the spreading of tumors from the primary site to distant sites. The spreading of tumors, called tumor metastasis, is highly dependent on the recruitment of host blood vessels by the growing tumor. Vascular endothelial growth factor (VEGF) is one of the most important factors that stimulate the growth of new blood vessels surrounding tumor tissue, providing nutrients and oxygen for growing tumors. Thus, therapeutic strategies interfering with the normal function of this VEGF protein have proven to be effective in preventing tumor angiogenesis in several preclinical and clinical studies. In this proposal, we will test the hypothesis that certain drugs will slow down or stop the formation of the human VEGF factors. Eventually, the studies proposed here will directly lead to the discovery and evaluation of new types of anticancer drugs based on G-quadruplex interactive agents in a preclinical model of human kidney cancer, a tumor type characterized by the abnormal overexpression of VEGF due to the frequent loss of the von Hippel Lindau (VHL) tumor suppressor gene.

The overall objective of this proposal is to explore a new therapeutic strategy aimed at preventing the growth of new blood vessels during tumorigenesis by targeting G-quadruplex structures formed in the promoter region of VEGF gene with small molecules to repress the transcription of these genes. The specific aims of this proposal are to: 1) to determine the role of secondary structures in VEGF promoter region on the regulation of this gene; 2) to explore the potential application of G-quadruplex interactive agents as a new class of anti-angiogenic agents, which can repress the transcriptional activity of the VEGF gene; and 3) to discover new classes of G-quadruplex compounds or new lead compounds based on known classes of G-quadruplex interactive agents that specifically inhibit the transcriptional activation mediated by the promoter activity of VEGF gene.

Cancer Immunotherapy by TCR-Modified HSC Transfer

Cancer is a major public health problem in Arizona and nationwide. In 2004, it was estimated that 1,368,030 people in the United States would be diagnosed with cancer including 23,560 individuals in Arizona. One in four Arizona residents is a “baby boomer”. As the baby boomers become age 55 and older, the number of cancer cases in Arizona could significantly increase. In fact, 80% of Arizona residents who are diagnosed with cancer are age 55 and older. Although traditional therapy, including surgery, chemotherapy and radiotherapy, has increased the survival rate of cancer patients, approximately 563,700 Americans were expected to die of cancer in 2004. In Arizona alone, it was estimated that 9,710 people could die from cancer in the same year. Therefore, new strategies are urgently needed to fight against cancer. In fact, no traditional treatment can remove all the malignant cells from the patient. Immunotherapy, which harnesses the cells of the patient’s immune system to eliminate tumors, is becoming a promising approach and attracting more attention. Combinations of traditional therapy with immunotherapy could not only improve the survival of cancer patients but also decrease the recurrence of cancer.

During the process of cancer development, cancer cells express certain proteins that can be recognized by the host immune system. However, most cancer patients do not mount efficient immune responses against their tumors. Among the immune cells, T cells are a major player in the control of cancer. T cells recognize tumor antigens through specific T cell receptors (TCR) and destroy tumor cells. Transfer of tumor specific T cells into patients could result in protection against tumors to some extent. A limiting factor in this approach is the insufficient doses of transferred T cells and inability of these cells to persist long enough in high numbers within the body of recipient. Hematopoietic stem cells (HSC) are the cells that normally give rise to every type of blood cell including T cells. Immunization results in T cell proliferation. Therefore, it is possible to generate more tumor specific T cells using genetically modified HSC within the body. We hypothesize that transplantation of tumor specific TCR - expressing HSC followed by genetic immunization will be an effective approach for cancer immunotherapy. The transfer of TCR gene-modified HSC will increase the frequency of tumor-specific precursor T cells. Genetic immunization, which means immunizing animals with DNA encoding the tumor antigen, will stimulate immune responses directed against tumors and result in expansion and activation of tumor specific T cells. The objectives are to 1) generate tumor-specific TCR gene-modified HSC, 2) determine the T cell production capacity of transferred tumor-specific TCR-modified HSC, and 3) treat cancer using TCR gene modified HSC transfer and genetic immunization. Our long-term goal is to improve the efficacy of cancer immunotherapy. We believe the combination of adoptive cellular therapy with active immunization should maximize the immune response against cancer and induce prolonged protection against recurrence.

Mohamed A. Gaballa, Ph.D.

University of Arizona
Award Amount FY06: \$50,000

Human Umbilical Progenitor Cell-based Therapy for Myocardial Infarction

Coronary artery disease is the leading cause of death and congestive heart failure in the western world. Although medical therapy prolongs survival and improves symptoms, it does not cure the disease. A curative therapy has to restore healthy vessels in ischemic areas to prevent progressive loss of viable tissue, infarct extension, and fibrous replacement. The heart is unable to regenerate and repair the damaged tissue since cardiac cells are end-stage differentiated cells. The ability of one's tissue stem cells to repair tissue damage is usually sufficient up to and for some time after reproductive maturity. However, the regenerative capacity or the numbers of such cells are often insufficient to meet the body's needs as one grows older. Thus, cell replacement therapy has spawned the development of regenerative medicine. Although fetal/embryonic stem (ES) cells have the ability to become virtually any cell type and tissue, no studies have shown a controlled differentiation into a specific cell type and only that cell type. Furthermore, unless the ES cells are derived by nuclear transfer techniques, they will be viewed as allogeneic by the immune system and subject to rejection by the body's defenses. Umbilical cord blood (also referred to as placental/cord blood or simply cord blood), the blood left over in the placenta after the birth of a child, has long been utilized as a source of blood cells in patients. Preliminary data from our laboratory show that one may be able to repair the damaged heart after heart attack using subsets of the cord blood cells. This is the main goal of our research.

Preliminary data for our research indicate that one can improve heart function after heart attack by injection of precursor cells obtained from human umbilical cord blood. Since cord blood, similar to bone marrow, contains a number of different cell types, it is unclear which subset(s) of these cells are responsible for the improvement of heart function after the transplant. Therefore, one objective of this work is to identify the contribution of some precursors commonly found in the cord blood. The second objective of this work is to determine how these umbilical cord blood cells improve heart function. Possible mechanisms, which will be studied in this application, are: 1) the transplanted cells can incorporate into the newly-formed vessel wall, 2) they can result in increase in the number of vessel within the heart, or 3) these cells may release chemicals (known as growth factors) that simulate the resident cells to proliferate and create new vessels. These newly-formed vessels typically improve blood perfusion in the heart muscle and lead to improvement in function and relief of symptoms.

David Duggan, Ph.D.

Translational Genomics Research Institute

Award Amount FY 06: \$50,000

Genetic Basis of Auriculo-Condylar Syndrome in 2 Arizona Families

Auriculo-condylar syndrome (ACS) is a genetic disorder passed from affected parents to roughly half of their children. This syndrome affects development of the head and might also cause problems with hearing. It is characterized by ear malformations, deformities of the chin, temporomandibular joint (TMJ) abnormalities, small jaw, small mouth, and a round facial appearance with chubby cheeks. Affected individuals have varying degrees of respiratory difficulties, trouble chewing, and various dental problems. The exact genetic mutation responsible for ACS remains unknown. Over the past year our group of University of Arizona doctors and medical students along with TGen researchers have identified two unrelated families from Arizona through clinics at the University of Arizona with many affected individuals. Never before has a large enough family been identified to allow for successful genetic research into the causes of the disease. Our goal is to further define the features of people affected with this disorder and to determine the specific genetic mutation(s) of ACS in these two Arizona families. In addition, this work may identify the first genetic etiology of temporomandibular joint abnormalities, a common disorder affecting up to 15% of the elderly in the state of Arizona.

We have developed the following Specific Aims to accomplish this goal:

- 1) continued DNA sample collection from relatives of those affected with ACS and further study into the facial features and hearing of people affected with ACS in these two Arizona families;
- 2) perform a genetic search through over 3 billion bases and 25,000 genes that make up the human genome in every individual in these two Arizona families to determine the region(s) of the human genome which contain the disease causing mutation;
- 3) perform finer searches through identified region(s) of interest to determine the region(s) of most likely involved in causing ACS; and
- 4) scan the prioritized regions to identify the mutation responsible for ACS in each of these families.

Sanjay Ramakumar, M.D.

University of Arizona
Award Amount FY06: \$141,969

**Gene Delivery Using Photosensitive Nanogels for Renal Regeneration
and Prevention of Ischemia Induced Injury**

Kidney failure is a widespread disorder with no known cure facing most clinical specialties. Despite major advances in intensive care, dialysis and kidney transplantation, the death rate among these patients has not decreased appreciably over the past 50 years. It ranges from approximately 7% of admitted patients to more than 15-20% of patients after major surgery. In ICU patients with kidney failure, the death rate can be as high as 88% with longer hospital stays and substantially increased healthcare costs of about \$15,000 per patient. There are multiple clinical causes of short term kidney failure including decreased blood flow, medications that injure the kidney and obstructed urine flow. It is known that ischemia injury, a lack of blood flow carrying oxygen, is a major cause of short and long term kidney failure. This process is mediated by a factor known as intercellular adhesion molecular-1 (ICAM-1) which causes a massive inflammatory response, resulting in congestion of the blood vessels and damage. Another factor involved in kidney regeneration after damage, Activin A, has also been identified. Numerous studies using gene therapy have targeted these factors; however, delivery of genes to a specific organ has been the most difficult aspect of this treatment and has been the reason that the use of gene therapy is very limited today.

In the early nineties, over 400,000 Arizonans were suffering from impaired kidney function. In 2000, about 9000 people were suffering specifically from end stage renal failure as a result of ischemia caused by primary diseases such as diabetes, hypertension and glomerulonephritis⁴. Despite public and private spending of over \$387 million on the disease in Arizona in that year, 1446 treated patients died from it. Progression to chronic renal failure in the remaining survivors imposed greater pressure on the state-wide transplant network which has exceeded 2,850 cases since 1988 in Arizona.

The goal of this project is to utilize a novel, nanoscale, bioengineered synthetic polymer to deliver gene therapy against the two major genes involved ischemia-related kidney failure. The nanogel polymer, poly-N-isopropylacrylamide (PNIPAAm), has chemical and electrical properties which allow it to strongly bind DNA genes, protect it from breakdown, and facilitate its passage into the cell. Moreover, the addition of a spiropyran molecule will enable it to contract or expand with changes in light exposure. Therefore, it can be used to selectively release therapeutic DNA genes within cells that are exposed to light. The overall hypothesis is that this novel nanogel vector will overcome the limitations of conventional gene therapy, offering a treatment which will stop the inflammatory injury caused by ischemia and regenerate kidney cells. Nanoscale materials with multiple functions are expected to play a significant role in the future of molecular medicine, especially with its outstanding control of delivery. *If the proposed research results in an effective nanogel delivery vehicle for DNA, this would revolutionize the field of gene therapy and would drastically reduce the morbidity, mortality, and treatment costs associated with kidney failure in Arizona.* Our

objectives are: 1) refine the synthesis of novel PNIPAAm nanogel polymers and determine their properties. Specific Hypothesis: Modified PNIPAAm nanogels can be customized to be delivery vehicles of DNA genes; 2) characterize and model the interactions between DNA and the tailored PNIPAAm nanogel for optimal DNA delivery. Specific Hypothesis: The interaction of the nanogel molecule with DNA can be optimized by characterizing its relationships, making it an ideal gene carrier; 3) evaluate the ability of genes in a nanogel carrier to have an effect on the kidney cells by using a cell culture model. Specific Hypothesis: ICAM-1 and Activin-A using the nanogel carrier will be effective in blocking the synthesis of these proteins; and 4) evaluate the effectiveness of the developed treatment using a live rabbit model. Specific Hypothesis: Rabbit kidneys injured by lack of blood flow will be partially protected from damage and will regenerate if treated by the gene therapy delivered in the novel nanogel polymer.

Diabetic Kidney Disease in American Indians

Diabetes is the leading cause of kidney failure in developed countries, and people with diabetic kidney disease are often disabled or die prematurely. Inherited factors appear to strongly influence the risk of developing diabetic kidney disease. The risk of developing diabetic kidney disease is higher in American Indians than in most other populations. Studies conducted in one group of American Indians, the Pima Indians of Arizona, suggest that genes on chromosome 3 increase susceptibility to diabetic kidney disease; this region was also implicated subsequently in other populations of Caucasians. The present proposal will systematically investigate all genes in this region that, because of their biological function and expression in the kidney, might affect susceptibility to diabetic kidney disease. In addition, in Pima Indians and other populations, inhibition of the renin-angiotensin system (RAS), which is an important regulator of fluid balance in the kidney, has been shown to delay the progression of diabetic kidney disease. Levels of at least one of the components of this system, angiotensin II converting enzyme (ACE), are under genetic control. Thus, this proposal will also investigate those genes of the RAS to delineate the role of this pathway in mediating susceptibility to diabetic kidney disease in Pima Indians. The identification of susceptibility genes for diabetic kidney disease will enhance our understanding of the inheritance and pathogenesis of the disease, provide markers for patients at high risk of developing the disease, and potentially lead to improved treatment and prevention strategies.

The goal of this study is to identify variants in genes that increase the risk of diabetic kidney disease in American Indians and determine the specific functional consequences of these variants. By doing so, we will be better prepared to develop improved treatments to slow the progress or even prevent the disease. This study proposes three major goals. The first goal is to systematically investigate all genes on chromosome 3 where linkage to diabetic kidney disease has been previously identified. Genes will be selected based on their known biological function and screened to identify all genetic variants. The second goal is to thoroughly screen the five genes comprising the RAS to identify genetic variants. In both goals, all variants will be investigated in diabetic individuals with or without kidney disease to identify those which are specifically associated with susceptibility to diabetic kidney disease. The final goal will begin to delineate the functional consequences of those variants which are found to underlie diabetic kidney disease in the first two objectives.

Jie Wu, M.D., Ph.D.

Barrow Neurological Institute
Award Amount FY06: \$49,694

Nicotine Acetylcholine Receptors in the VTA and Nicotine Dependence

Tobacco use in Western society, including in Arizona, is estimated to be the largest single cause of premature and preventable death. Dependence on nicotine, a major component of tobacco, is thought to drive tobacco use. Nicotine is likely to express its addictive nature by acting on nicotinic acetylcholine receptors (nAChRs), which are widely distributed throughout the brain and body and are important mediators of chemical and electrical signaling. The brain's pleasure-reward centers have been implicated in drug seeking and dependence. In brain regions within these centers, various subtypes of nAChRs are found on nerve cells that signal using the chemical messenger dopamine (DA) on other kinds of nerve cells and on nerve endings that help regulate electrical activity of DA and non-DA nerve cells. It is generally accepted that alterations of DA nerve cell function in the brain's pleasure centers contribute to nicotine addiction. However, mechanisms of nicotine action and the specific roles of nAChR subtypes in direct or indirect modulation of DA nerve cell function remain poorly understood.

The long-term goal of a line of research in the applicant's laboratory is to improve the understanding of cellular and molecular bases of nicotine dependence. Toward this objective, the project's specific aims are to define distributions, functions, and sensitivity to nicotine exposure of diverse nAChR subtypes expressed in a pleasure-reward region called the ventral tegmental area (VTA). This will be accomplished principally by using state-of-the-art electrical recording of DA nerve cells in the VTA in response to nicotine and related drugs. The cells to be studied will be isolated using a set of manipulations that very effectively preserve their chemical sensitivity to nicotine to an extent not previously achieved by using brain-slice preparations. These studies also will define the kinds of nAChRs found on specific nerve cells and profiles of their chemical sensitivity to nicotine and related drugs. One aspect of the project involving measures of effects of long-term exposure to nicotine at levels like those found in the tissues and blood of human smokers is of particular relevance to an understanding of cellular and chemical bases of nicotine action. By doing so, the project seeks to elucidate roles for nAChR activation and, perhaps, inactivation upon short and long-term exposure to nicotine and VTA nerve cell activity that may be relevant to drug reinforcement and dependence. Valuable insights can be anticipated into improved treatment for nicotine dependence which afflicts 25% of Arizonans and other addictions as well as roles for nAChRs in the pleasures and rewards of everyday life.

Roles of Estrogen in BACE Regulation *in vitro* and *in vivo* Systems

Alzheimer's disease (AD) is the most common cause of dementia in the elderly, affecting up to 15% of people over the age of 65 and nearly half of all individuals by the age of 85. Given the fact that Arizona has the second highest population of elderly people in US, AD is very prevalent in our state. Increasing evidence indicates that postmenopausal women have a higher risk of developing AD than age-matched men. This increased risk may be due to the loss of estrogen in postmenopausal women. Research also demonstrates that estrogen appears to enhance performance in elderly women and might delay the onset of AD. Such findings have suggested that the reduction of estrogen in postmenopausal women might be a risk factor for developing AD and that estrogen treatment may have the potential to prevent AD.

We recently discovered β -secretase (BACE) enzymatic activity is significantly increased and this elevation is correlated with amyloid load in AD brains (Li, *et al.*, PNAS, 2004; Yang *et al.*, Nature Medicine, 2003). However, it is not known why BACE is elevated in AD and whether estrogen plays a role in BACE activity.

In vitro studies suggest that estrogen exerts its effects by altering A β generation. To study roles of estrogen *in vivo*, we have combined genetic deletion of estrogen synthase aromatase (ArKO) with the over-expression of a mutant APP. To further investigate mechanisms of BACE changes in APP/ArKO brains, our first goal in this proposal is to examine whether BACE is altered at the transcription and translation levels in brains of APP/ArKO mice. As we have identified an estrogen-binding site in BACE promoter, we will secondly, examine whether BACE transcriptional activity is mediated by estrogen in an Alzheimer transgenic model with deletion of estrogen. Through accomplishment of the two goals, we will test the hypothesis that *estrogen can regulate upstream events of BACE translation which, in turn, control APP processing.*

**Long-Term Activation of Pain-Enhancing Systems Following
Short-Term Opioid Use**

Morphine and similar drugs, classified as the analgesic opioids, are the most effective and commonly used drugs for the treatment of moderate to severe pain. Despite their tremendous effectiveness, they also have properties that may worsen pain, potentially causing unintended harm to patients. These properties may be a significant problem, given the widespread use of opioids for the treatment of pain resulting from causes such as surgery, trauma, illness, and cancer, and given the lack of effective alternatives. Understanding the mechanism of these unwanted effects of opioids may allow the design of therapies to counteract them. Recent animal research has shown that after opioid administration, pain sensitivity returns to normal, but a state of increased pain sensitivity can be evoked at later times (weeks or months after the opioid administration) by conditions such as stress or repeat opioid exposure. The state that predisposes to evoked pain hypersensitivity is called latent pain sensitivity. This discovery is particularly noteworthy since many physicians who treat pain have the clinical impression that pain sensitivity and responses to opioids are altered in patients who have previously received opioids. In addition, the potential clinical importance of these findings is clear since many patients who receive opioids later experience stress or are again treated with opioids. A better understanding of the mechanisms that produce long-lasting changes in pain pathways following opioid use might allow us to design ways to prevent the undesirable effects of opioids without inhibiting their pain-relieving effects.

This research is of importance to the citizens of Arizona because pain treatment remains imperfect, with many pain patients (30-50% in most studies) experiencing residual pain despite attempts at treatment. This research may allow us to improve pain treatment, allowing many Arizonans to experience more complete relief.

The proposed research is a pilot study in a new area of research for us, designed to gather data that can be used to prepare a grant application for the National Institutes of Health. This work will achieve three goals: 1) Further test the hypothesis that short-term opioid administration induces long-term activation of pronociceptive processes leading to latent pain sensitization. This aim tests the duration of opioid exposure that is necessary to cause latent pain sensitization. This is important because it gives us an idea of which patients may be at risk for evoked pain hypersensitivity. It also measures the duration of latent pain sensitization after opioid exposure. This is important because it gives us an idea of how long after opioid exposure patients remain at risk for evoked pain hypersensitivity; 2) Test the hypothesis that this activation of pronociceptive process results from an opioid-mediated increase in the expression of excitatory peptide neurotransmitters and TRPV1 receptors in primary afferent neurons. This aim tests whether three pain-sensitizing molecules that are increased in amount during opioid administration

remain increased after opioid administration is stopped, predisposing to evoked pain hypersensitivity; 3) Test the hypothesis that long-term activation of pronociceptive process results from prolonged activation of descending facilitatory pathways, acting through spinal dynorphin to enhance spinal release of excitatory neurotransmitters. During opioid administration, nervous system pathways that travel from the brain to the spinal cord are activated, increasing pain sensitivity. This aim tests whether these pathways remain increased after opioid administration is stopped, predisposing to evoked pain hypersensitivity.

Microactuated Microelectrodes to Assess Cortical Role in Memory Deficits

Learning and memory disorders resulting from normal and pathological aging represent a major public health concern. The development of an understanding of brain mechanisms of memory is critical to the ultimate development of effective therapeutic interventions for these disorders. The encoding, storage, retrieval and consolidation of memory involve temporally extended interactions of large neural populations distributed widely over the brain. However, current technologies for simultaneously studying the function of large populations of neurons in intact brains are very limited at best. The technologies are either extremely unreliable in long-term situations or lack the spatial and temporal resolution to monitor single neuronal function with precision. We propose the neurophysiological analysis of the effects of normal aging and experimentally induced hippocampal dysfunction on memory-related neural ensemble interactions within a linked system of brain regions that are important in mnemonic processes.

The key goals of this proposal are 1) to develop a novel high-density independently movable microelectrode array technology that will enable us to monitor simultaneously the function of large populations of neurons in cortical brain regions involved in memory processes of behaving rodents in long-term experiments, and 2) test the hypothesis that with aging or hippocampal dysfunction, the neurons in different cortical layers progressively lose the ability to differentiate spatial and behavioral contexts.

The state of Arizona has a large proportion of population who are elderly in whom the occurrence of learning and memory disorders due to normal and pathological aging leads to significant deterioration in the quality of life and productivity. The proposed research addresses fundamental questions concerning memory deficits that will lead to novel discoveries and potentially novel therapeutic strategies.

In addition, the proposed novel technology to monitor large populations of neurons in real-time will immediately impact a variety of other Neuroscience and Neuropathology studies such as stroke and neural repair, auditory studies, etc. that critically depend on understanding precise cellular mechanisms of function. Successful development of this technology will also address a critical need in the development of several emerging neural prosthetic devices for Parkinson's disease, epilepsy, schizophrenia, etc. The proposed development of an exciting Neural Engineering technology that addresses a critical Neuroscience problem builds on the strengths of Arizona identified in the Arizona Bioscience Roadmap.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY 06: \$50,000

Ultra - Miniature Endoscopes for Biomedical Imaging

Endoscopes are used in a variety of clinical imaging applications. Common applications include imaging the colon (colonoscopy), esophagus and stomach (gastroscopy), and internal body cavity (laparoscopy). Commercial instruments are available in sizes ranging from centimeters down to about one millimeter in diameter depending on the application. Ultra small diameter endoscopes with diameters much less than 1mm are currently not available due to the need to provide an imaging channel, illumination channel, irrigation, and positioning control all in a very small diameter package. Nevertheless, there are a number of important scientific and clinical applications for such ultra-miniature endoscopes. Development of such instruments will require novel technical approaches such as those described in this proposal.

This research is aimed at developing and building ultra-miniature endoscopes. A primary goal is to build a system with a single illumination and imaging channel in order to make it small. Another goal is to build a system that allows both standard white-light imaging and a newer kind of imaging based on fluorescence, which has the potential to improve identification of disease. Although there are many scientific and clinical applications, this research will collaborate with an ongoing scientific project employing a mouse model of Barrett's esophagus, a precursor of esophageal cancer. This is an important scientific project in its own right that can benefit from this new imaging technology as well as serve as a useful model to test the instrument prior to evaluating clinical applications in humans.

Alyssa Panitch, Ph.D.

Arizona State University
Award Amount FY06: \$250,000

Biomimetic Scaffolds for Spinal Cord Regeneration

Considered one of the most challenging areas for regenerative medicine, reparation of the central nervous system continues to face substantial obstacles. However, well-designed biomimetic materials will create an environment that will allow the nerve regeneration necessary to restore ambulation to paralyzed individuals. Annually, new cases of spinal cord injury affect 28-50 per one million people in the United States with the highest rate of injury occurring in younger populations. The impact upon the workforce is thus disproportionately substantial because of the younger age of most persons at injury. Spinal cord injuries lead to a lack of not only physical mobility and sensation, but also affect vital functions including bladder and bowel control, and often lead to serious morbidity from decubitus ulcerations of the skin. A therapeutic approach that combines 1) the mechanical support of scaffolding with 2) biological signals that are present during neural embryonic development and 3) molecular cues to the regenerating axons will improve the potential for functional recovery after spinal cord injury. Consistent with the ABRC roadmap, the overall goal of this research is to utilize information from developmental biology, neurophysiology, neuroscience, bioengineering and medicine to develop a scaffold that provides the biological signals required for nerve regeneration in the injured spinal cord.

The hypothesis of this investigation is that complementing the biologically relevant scaffold hyaluronic acid (HyA) with cell signals will improve neurite outgrowth from primary cortical neurons and improve rate and number of regenerating neurons after spinal cord injury.

The specific aims are to:

1. optimize hyaluronan (HyA) concentration and poly(ethylene glycol) (PEG) diacrylate crosslink density with respect to neurite outgrowth and correlate that to mechanical properties of the scaffold;
2. develop and characterize HyA-based scaffolding that is crosslinked with PEG diacrylate and complemented with cell signaling peptides;
3. develop and optimize methods to control clustering of ligands for presentation to the extending neurites; and
4. compare cellular behavior among scaffolds containing different combinations of optimized peptide presentations.

To accomplish the specific aims, we will combine peptide chemistry, organic chemistry, primary cortical neuron isolation and culture, engineering design of experiments and *in vivo* evaluation in a rat spinal cord injury model. *In vivo* evaluation will occur at each step along the way to ensure that optimization of a material *in vitro* correlates to optimization *in vivo*. The results from this study are expected to greatly improve regeneration in the rat spinal column. Future work will translate the therapeutic materials to larger animal models.

SECTION E

COLLABORATIVE TRANSLATIONAL RESEARCH

ARIZONA PARKINSON DISEASE CENTER
SCORPION TREATMENT AND IMAGING OF NEUROTOXICITY GROUP
COLLABORATIVE DATA MANAGEMENT SYSTEM
ARIZONA BIO-SPECIMEN ALLIANCE
ALZHEIMER'S DISEASE COLLABORATION

**Arizona Parkinson Disease Center Prevention of Progression to Parkinson's Disease
and Parkinson's Disease with Dementia: Development of Biomarkers
and Novel Treatment Strategies**

Parkinson's Disease (PD) is a progressive neurodegenerative disorder that is currently diagnosed clinically by finding slowness of movement along with either rest tremor or rigidity. The only definitive diagnosis for PD is by autopsy. While usually thought of as a motor disorder, 30-75% of patients with PD go on to develop dementia. As the disease progresses, dementia is often more disabling than the motor symptoms leading to higher rates of nursing home placement and death. While there are treatments (medications and surgical procedures) that improve the motor symptoms, there are no treatments that slow or halt disease progression nor prevent dementia in PD.

We have designed our proposal to include cores and projects whose theme is the development of biomarkers and putative treatments for preventing the development of PD and progression of PD to PD with dementia (PD-D). The work will be performed through the Arizona Parkinson Disease Center, a consortium of investigators at multiple institutions throughout the state: Sun Health Research Institute, Mayo Clinic Scottsdale, Barrow Neurologic Institute, Banner Good Samaritan Medical Center, Arizona State University, and the Translational Genomics Research Institute.

This grant application is geared towards expanding the Arizona Parkinson Disease Center Brain Donor Program to develop biomarkers that have high predictive values for the development of both PD and PD with dementia. The clinical data will be validated by autopsy confirmation, the "gold standard" for diagnosing PD. Multiple projects have been included that will investigate potential biomarkers in cerebrospinal fluid (CSF) and brain tissue for PD and PD-D. Additionally, studies will be undertaken investigating possible mechanisms for the development of PD and dementia in PD with the goal of finding new targets for treatment. This program will then utilize the antemortem data to predict the likelihood of individuals to progress to PD or PD-D.

The Clinical Core will enroll and longitudinally evaluate subjects in the Brain Donor Program categorizing them as being controls, PD's, and PD-D's. One main goal of the Clinical Core will be to determine clinical biomarkers that will be used to predict who will develop PD and PD-D. In addition, the Clinical Core will provide the Neuropathology Core and projects with extensive clinical description obtained during life of the autopsy material. The Neuropathology Core will perform all autopsies, provide data on Lewy bodies and other markers to correlate with the clinical data, and provide brain tissue and CSF for the projects. One project will investigate whether a loss of brain-derived neurotrophic factor (BDNF) in the cortex of PD-D may lead to neuronal susceptibility to cell death. A second project will determine whether mitochondria from cases of PD-D have different levels of the TNF type 1 death receptor (TNFR1) and

wether this influences changes in α -synuclein and DJ-1. A third project will investigate compounds that may alter the role of inflammation in cell death (utilizing assays for RAGE receptor for advanced glycation end products and CD200 and the CD200 receptor). The fourth project will utilize microarray fingerprinting of neurons susceptible to cell death in PD-D to identify the genetic pathways leading to α -synuclein/DJ-1 aggregation and cell death. The fifth project will utilize proteomics in CSF to investigate for biomarkers that distinguish PD without dementia from PD-D. As a whole, these projects will provide new biomarkers and new therapeutic targets for eventual testing with subjects at the Clinical Core sites.

At the completion of the three-year funding period, we propose the submission of a NIH funded program project or center grant that will build on these themes. Additionally, we will propose randomized controlled trials of treatments that will be designed to stop or slow the progression to PD or PD with dementia. The design of these clinical treatment trials will be a result of information gathered from the Clinical Core allowing us to choose enriched populations of subjects with high probability of developing PD or progressing to PD-D. Given the size of the Arizona Parkinson Disease Center clinical population, the controlled trials would be able to be performed here in Arizona by the consortium.

Scorpion Treatment and Imaging of Neurotoxicity Group

This study combines treatment of scorpion sting with a new antivenom and the use of long-distance video technology to create a standard for diagnosing scorpion sting toxicity and then a teaching tool for healthcare providers.

In nearly all Arizona emergency rooms, doctors have treated scorpion stings with antivenom manufactured at Arizona State University for over 50 years. The supply of this product was depleted by early 2005, threatening the life and health of approximately 100 children annually. In Mexico, safer modern scorpion antivenom has become the standard of care for serious scorpion stings, but the process for the drug to meet the U.S. FDA standards and to become licensed for use in the U.S. will likely take until 2008 to complete. This project will provide the new antivenom treatment to patients who otherwise would not be able to receive antivenom despite a severe sting. The patients' neurological symptoms will be videotaped in a standardized way and these video tapes will be edited and reviewed by a panel of experts to ensure that scorpion sting symptoms can be diagnosed over long distances. The tapes, in turn, can then be used to develop a teaching tool for healthcare providers. In addition, a work station at the Arizona Poison and Drug Information Center will provide trained-observer support to distant emergency services providers involved in the care of patients with scorpion stings.

The short-term goal is to avoid a public health crisis by providing a new antivenom to patients with serious scorpion sting and to prove that videotaping scorpion sting symptoms at outlying sites can be used to accurately diagnose scorpion envenomation. The long-term goals are to provide supporting information for requesting license by the FDA for U.S. distribution of the new antivenom, and to establish a "gold standard" for the diagnosis of serious scorpion sting and train health care professionals to recognize the symptoms of serious scorpion sting using the "gold standard" that we establish in the study. Two study phases include:

1) Video Imaging -

- Choose the symptoms that expert doctors agree will indicate serious scorpion sting
- Test and confirm the ability to videotape scorpion symptoms and have experts make an accurate diagnosis
- Develop a teaching program for doctors, nurses, pharmacists and other health professionals to recognize serious scorpion sting
- Test the educational program
- Use the educational program to prepare the staff of the Arizona Poison and Drug Information Center (APDIC) to provide real-time consultation to emergency facilities statewide over a broadcast clinical network called telemedicine

2) Clinical Treatment -

- Evaluate safety of the antivenom immediately following treatment and 14 days later
- Signs of serious scorpion sting will resolve after treatment.

Brent Gendleman

5AM Solutions
Award Amount FY05: \$100,000

5AM Illumine

5AM Illumine™ is a web-based data management system that elevates collaborative clinical research. Easy access to real-time study status; organized data views for collaborators, regulators, sponsors and grantors; and clinical and genomic data integration are all benefits to multi-disciplinary investigators. We have deployed the supporting infrastructure at the state of the art facility in downtown Phoenix and have launched the first global study. Led by Dr. Michael Berens, this study involves 15 research partners from Asia, Europe and North America submitting brain cancer tumors in paraffin block and the accompanying clinical annotation from each subject. TGen will assemble tissue micro-arrays from these samples and redistribute slides back from staining. All the data, including the tracking and staining is managed by the system. The software will adopt several new diverse studies in FY 2006, add features and provide the first open-source enterprise clinical research system to Arizona investigators.

The Arizona Bio-Specimen Alliance

Arizona is well positioned to become a leader in the Biosciences. In response to Arizona's Bioscience Roadmap prepared by the Battelle Memorial Institute, the Molecular Profiling Institute (MPI) is uniquely positioned to respond to the need for a centralized bio-specimen repository, thereby accelerating the process of bringing new discoveries in medicine to patient care. Two major hurdles that were identified are 1) access to tissue, both normal and diseased, to validate research discoveries and 2) the ability to coordinate and distribute medically relevant information. MPI recognizes that the rate of development of useful genomic information on human disease is inhibited by the relatively small sample size that can be generated at one institution. Also recognized is the heterogeneity in sample processing and analytic techniques for genomic profiling between different institutes, thus precluding a useful or instructive combination of multi-institution data. MPI, with its experience in quality bio-specimen procurement and its access to state-of-the-art computer systems, is spearheading the development of an Arizona Bio-specimen Alliance which will standardize protocols for bio-specimen procurement storage, annotation and tracking. This will greatly improve the quality of bio-specimens and data, thus broadening Arizona researchers' access to tissue and increasing the usability of data across sites and studies, and enabling Arizona researchers to lead the way in genomic medicine.

The goal of this proposal is to establish the initial phase from which to build a centralized Arizona Bio-specimen Alliance (ABA), owned by all participating institutions, as a unique bio-specimen resource and database for the Arizona research community. In the initial phase the alliance will consist of Translational Genomics Research Institute (TGen), the International Genomics Consortium (IGC), the Molecular Profiling Institute (MPI), Scottsdale Healthcare (SHC), and Sun Health Research Institute (SHRI). The Alliance aims to standardize patient consent and bio-specimen procurement, processing, and tracking protocols at multiple specimen collection sites. Bio-specimens will arrive at a centralized laboratory for confirmatory pathology diagnosis and long-term storage of frozen and paraffin embedded bio-specimens which will be available for re-distribution to Alliance partners and others for further approved studies, ensuring linkage of their research findings to the database. In compliance with HIPAA regulations, specimens entering MPI will be de-identified (personal patient information is removed) using bar-coding schemes for subsequent bio-specimen tracking.

These efforts will provide a framework for providing well-characterized clinical bio-specimens for researchers in Arizona.

Translational Research on A β Metabolism, from Synthesis to Clearance

Alzheimer's disease (AD) is a chronic, progressive neurologic disorder leading to dementia. It is estimated to currently afflict well over 4 million Americans and, because its most pervasive risk factor is old age, that number will increase dramatically in the coming years as population demographics shift to more and more elderly citizens. With some of the world's most concentrated senior populations (e.g., the Sun Cities, Sun Lakes, Green Valley, and many developments in Yuma, Sedona, Prescott, and elsewhere), Arizona also includes, by definition, some of the world's most concentrated AD populations. If we do not come up with something to diagnose, treat, or prevent AD in the near future, Arizona's highly concentrated AD populations will engender human, community, and economic crises of major proportions.

Current treatments for AD provide only temporary respite and do little to halt its inexorable progression to dementia. This is easily understood when one views first-hand the ravages to the higher centers of the brain that occur in AD. It will be many decades before we can realistically hope to reverse such damage. Our best treatment option, then, is to find new ways to halt AD and to apply them early. Translating the basic research discoveries made by the present program project investigators into new therapies that halt AD is the first overall objective of this application. However, knowing when AD starts and when a new treatment should be initiated is also a major problem as we do not now have any certain way to diagnose the disorder except by examination of the brain at autopsy. A second major goal of the Program Project is, therefore, to develop and test a new diagnostic approach that has shown promise in preliminary studies by the program project investigators.

Aggregated deposits of a molecule called amyloid β peptide (A β) have been a defining hallmark of AD for the last 99 years, and A β is widely viewed as a major cause of damage to the AD brain. The present program project represents a highly collaborative, multi-institutional effort to translate new discoveries by its investigators about the synthesis and clearance of A β into new treatments, diagnostics, and biomarkers for AD disease progression.

Project 1 tests the hypothesis that a particular molecular pathway – many elements of which are known to be increased in the AD brain – helps regulate A β synthesis and may drive A β increases in AD. The Project is highly collaborative with Project 3, which attempts to translate the findings into a new treatment approach using molecules called antibodies that have been specifically engineered to inhibit the A β synthetic pathway studied in Project 1. Project 2 seeks to develop and validate a simple, inexpensive blood test that is diagnostic for AD and may serve as a biomarker for AD disease progression. Preliminary data show that A β gets into the blood and may be cleared by a classic mechanism called immune adherence. The mechanism uses receptors on red blood cells to ferry pathogens to the liver and spleen for removal. Previous studies of A β in blood have typically thrown the red blood cell fraction away,

but our experiments suggest that it is the A β in this very fraction that may be diagnostic for AD. Like Project 1, Project 2 is also tightly linked to Project 3, which explores how the mechanism implicit in the Project 2 diagnostic could be translated into a new treatment approach. Here, novel antibodies will be engineered to enhance or to inhibit the immune adherence, A β removal mechanism. In animals models, enhancing the mechanism should reduce A β in the blood and brain suggesting a new treatment approach that would not require introducing chemicals or antibodies into the brain, only the blood. This would be advantageous as previous attempts to put anti-A β antibodies into the brain have resulted in major adverse events including the deaths of three patients in the study.

SECTION F

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