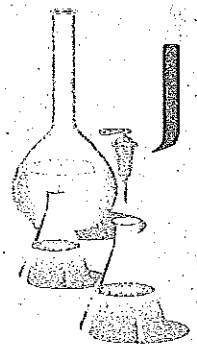
Arizona Disease Control Research Commission



2003 - 2004 Annual Report

January 2005

ARIZONA DISEASE CONTROL RESEARCH COMMISSION

ANNUAL REPORT
2003– 2004

Janet Napolitano, Governor

Henry Reeves, Ph.D., Chairman

COMMISSION MEMBERS

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

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

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

Staff
Executive Director: Dawn C. Schroeder, D.D.S., M.A.
Executive Staff Assistant: Damika D. Brock
Accountant II: Ismene Quintanilla
Program Project Specialist II: Daniel J. Powell
Administrative Assistant II: Cecelia Tosie

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January 2005

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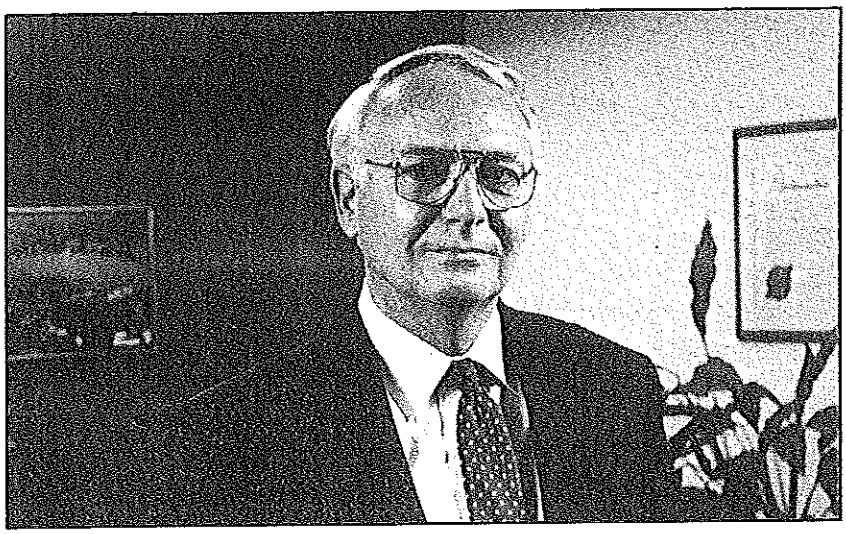


Message from the Chairman

Fiscal year 2004 has been a year of leadership and change for the Arizona Disease Control Research Commission. The Commission has continued to play an important role in the furtherance of Arizona as a center of biomedical research excellence. We are pleased that Arizona serves as a national model for the funding and management of biomedical research.

Eighteen 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 have enabled 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. New patent applications have been submitted. We anticipate the issuance of two to five new patents in FY 2005. We have continued our marketing efforts on current patents and are nearing completion of our second licensing agreement.

The Commission has extended its intensive involvement in the development of translational research. The Commission provided $5,500,000 to the support of the Translational Genomics Institute. One million dollars was provided to the Alzheimer's Disease Research Institute. Additional funding was provided for Parkinson's Disease research and other brain related diseases and disorders. Funding was provided for brain tissue and prostate tumor tissue banks that support scientific research nationwide. All of these areas are key to the future health and welfare of Arizona residents.

Commission activities have broken new ground in the promotion of the goals found in the Arizona Biosciences Roadmap. (A link to the Roadmap may be found at www.adcrc.com.) Arizona researchers are being asked to align their research objectives with those of the Biosciences Roadmap. The Commission and the Flinn Foundation have entered into a joint contract with the Battelle Memorial Institute to help make the Roadmap objectives for translational research a reality.

In the coming year the Commission and the Flinn Foundation with the assistance of Battelle will encourage the development of multi-institutional collaborations in the pursuit of discoveries in neurosciences including Parkinson's and Alzheimer's disease, cancer, bioengineering, and imaging. Part of this effort will be the formation of a Translational Research Project Advisory Committee and three subcommittees to investigate streamlining Institutional Review Board and HIPAA processes, harmonize approaches to key business practices, and address how to most effectively collaborate with special populations. In an effort to bring together key Arizona researchers the Commission with the Flinn Foundation will sponsor a statewide biomedical research symposium in 2005.

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 Disease Control Research Commission that encouraging 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 2003 – 2004 are presented below.

Henry Reeves, Ph.D., Chairman

Professor Emeritus
Arizona State University

Commissioner Reeves is a member of the Scientific Community. He was a Professor of Microbiology at Arizona State University from 1969 to 1993. During that time he also served as chair of the Department of Microbiology from 1970 to 1973 and as Vice President for Research from 1985 to 1991. On leave from Arizona State University, he served as director of the Division of Physiology, Cellular and Molecular Biology at the National Science Foundation from 1976 to 1979. Commissioner Reeves received his B.S. from Franklin and Marshall College and his M.A. and Ph.D. from Vanderbilt University. He was first appointed to the Commission by Governor Symington to complete the term of Commissioner James Bloedel in 1995. He was reappointed in 1996. Governor Hull appointed him to his second full term in 1999 and a third term in 2002. His term will expire in May 2005.

General Public

C. Eileen Bond, J.D. — Prescott

Private Practice
Specializing in Child Welfare Law

Commissioner Bond received her B.A. in History (Far Eastern Studies) and Master of Library Science from 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.

Lyra McCoy, M.P.H. — Mesa

Program Administrator
Governor's Division of Drug Policy

Commissioner McCoy received her Masters Degree in Public Health with a specialization in Health Education and Promotion from the University of Arizona. Commissioner McCoy became the CEO for ICAN, Improving Chandler Area Neighborhoods (ICAN) in 2004. Commissioner McCoy was the Program Administrator for the Governor's portion of the Safe and Drug Free Schools and Communities program from the U.S. Department of Education prior to assuming her present post. In August 2003, she became grants administrator for the Arizona State School Readiness Board programs including the Head Start State Collaboration Project and the Childcare Development Fund for school age childcare. She has been involved in statewide collaborative projects including the Arizona Youth Survey and the Arizona Program Design and Evaluation Logic Model. Prior to joining the Governor's Office, Commissioner McCoy worked for the American Cancer Society and the Arizona Program for Nicotine and Tobacco Research in tobacco prevention with youth and tobacco policy change initiatives. She was appointed to the Commission in 2001 by Governor Hull, and her term expired in May 2004.

General Public

Steven Weinberg, J.D. Phoenix

Greenberg Traurig, LLP

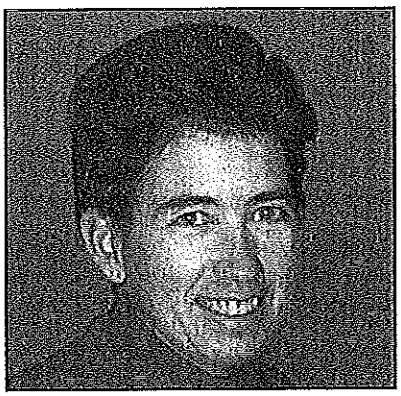
Commissioner Weinberg received his B.A. from State University of New York at Buffalo and his J.D. *cum laude* from St. John's University. He has been representing major corporations in trademark, copyright, software, and advertising litigation, principally in federal courts for over 22 years. He also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

Medical Community

Colleen M. Brophy, M.D. Scottsdale

Chief of Vascular Surgery

Carl T. Hayden VAMC



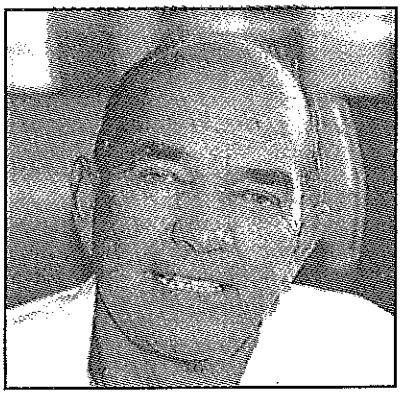
Commissioner Brophy received her undergraduate and medical degrees at the University of Utah. She completed her surgical residency at Yale University followed by a fellowship in vascular surgery at Harvard University. She is a Research Professor of Bioengineering at Arizona State University, a Clinical Professor of Surgery at the University of Arizona and the director of the Proteins and Peptides as Pharmaceuticals Center in the 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 2005.

Medical Community

William Crisp, M.D. Paradise Valley

Resident Education Gynecology/Oncology

Samaritan Regional Medical Center

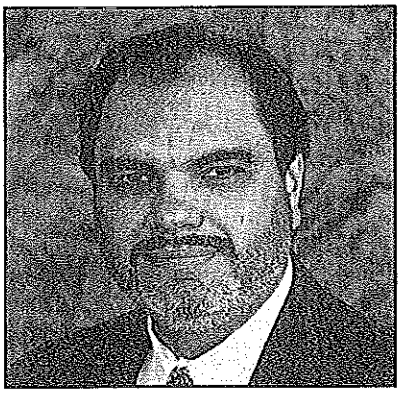


Commissioner Crisp received his M.D. degree from George Washington University College of Medicine. He is Board Certified in Obstetrics and Gynecology and holds an Advanced Certificate in Gynecology Oncology. He also serves as an Adjunct Professor in the Cancer Research Institute and the Bioengineering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

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

Chief, Internal Medicine

Mariposa Community Health Center



Commissioner Pereira received a B.S. in Chemistry from Georgia Tech in 1979. He graduated *magna cum laude* from Emory University School of Medicine in 1983. After completing his internal medicine residency, he joined the staff of Mariposa Community Health Center as a Scholar of the National Health Service. He returned to Emory University in 1990 as Assistant Professor of Medicine and Director of the Intensive Care Unit, Grady Memorial Hospital, Atlanta. He has been a Fellow of the American College of Physicians since 1993. In 1998 he was named Chief of the Medical Staff and Clinical Services at Mariposa, overseeing an 11-physician group that provides 60 percent of the medical care in Santa Cruz County. He was appointed to the Commission by Governor Symington in 1995 to complete the term of Commissioner Carlos Gonzales whose term expired in May 1996. He was reappointed in 1996. Governor Hull appointed him to the Commission in 1999 and reappointed him in 2002. His term will expire in May 2005.

Scientific Research Community

T. Lon Owen, Ph.D. — Flagstaff

Professor of Medical Anatomy and Physiology

Northern Arizona University

Commissioner Owen received his B.A. in Zoology from the University of California, Davis; a master's degree in Biology from California State University, Sacramento; and his Ph.D. in Physiology from U. C. Davis. He was a National Institutes of Health Postdoctoral Fellow at Michigan State University and Visiting Associate Professor in the Pharmacology Department of the University of Arizona College of Medicine. He has chaired the Research Committees of the American Heart Association at both the Arizona Affiliate and Southwestern Regional levels. He is a member of a research Platform Committee and the Steering Committee for the Arizona Biosciences Roadmap. He has published in the areas of cardiovascular, aging and environmental physiology. Commissioner Owen was appointed to the Commission in June 1998 by Governor Hull, reappointed in 2001. His term expired in May 2004.

Walter Williams, Ph.D., M.D. — Tucson

Professor, Department of Nuclear Medicine

and Radiology, University of Arizona

Commissioner Williams received his B.S. with majors in Chemistry and Physics from the University of Missouri in 1963, his Ph.D. in Physical Chemistry from Purdue University in 1968 and his M.D. from Yale University in 1980. 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 prior to returning to school to study medicine. From 1985 to 1987, he was a clinical instructor in the Joint Program for Radiology and Nuclear Medicine at Harvard. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and reappointed in 1997. Commissioner Williams was reappointed by Governor Hull in May 2000 and by Governor Napolitano in May 2003. His term expires in May 2006.

Summary of 2003-2004 Commission Activities

The Commission administers 55 contracts in two programs—unrestricted medical research, brain research and Parkinson's disease research—with medical researchers in Arizona as of July 2003. In addition to the regular Commission programs, ADCRC will be supporting the Translational Genomics Research Institute in the amount of $5,000,000 per year for a period of five years and a $500,000 annual award for a period of ten years. The section headings list each program and whether the project is in its first, second or third year of funding. Research abstracts outlining the progress made during the year are contained in Sections A-D. Citations for scientific publications and abstracts arising out of the research are also listed.

Lay summaries for new medical research and brain research projects awarded in 2004 can be found in Section E. The medical research projects began in July, FY 2004, while year one of the Parkinson's disease research projects began on April 1, FY 2003 and ended March 31, 2004.

Approximately 945 Requests for Proposals (RFPs) for 2004-2005 awards were mailed to potential applicants in September 2003. The amount available for new unrestricted medical research was approximately $1,800,000. In response to the RFPs, the Commission received 117 unrestricted medical research proposals. There were 4 requests for bridge funding that were evaluated separately. This left 113 proposals that were evaluated for new awards.

ADCRC Projects Accepted/Projects Submitted FY 2004

Institution	Accepted	Submitted	Percent	Amount
Arizona State University	4	16	25.00%	$385,864
Northern Arizona University	1	3	33.33%	$49,992
Sun Health	1	10	10.00%	$50,000
University of Arizona	11	71	15.49%	$1,196,171
Others	1	13	7.69%	$100,000
Total	**18**	**113**	**15.93%**	**$1,782,027**

In November and December the medical research proposals were sent to a panel of national and international scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more than 174 out-of-state peer reviewers. Three reviews were sought for each proposal. The proposals and evaluations were distributed to the Commissioners who were formed into three, three-person subcommittees to facilitate the final review process. In the spring and summer of 2004 the Commission selected 18 proposals for funding. During 2004-2005 the ADCRC will be managing 55 contracts.

ADCRC Total Project Awards for FY2004

Institution	Award	Amount	Percent
Arizona State University	7	$1,013,750	16.26%
5AM Solutions	1	$100,000	1.60%
Intrinsic Bioprobes	1	$138,927	2.23%
La Frontera	1	$108,131	1.73%
Northern Arizona University	1	$49,940	0.80%
St. Joseph's	1	$155,321	2.49%
Sun Health	3	$818,750	13.14%
University of Arizona	41	$3,848,228	61.74%
Total	**55**	**$6,233,047**	**100.00%**

SECTION A

CONTINUING CONTRACTS

MEDICAL RESEARCH

YEAR THREE

FY 2004

Alyssa Panitch, Ph.D.

Arizona State University
Award Amount FY04: $50,000

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

Suspension of the polysaccharide dextran in physiological saline solutions are sometimes used to replace vital fluids depleted during blood loss in trauma victims. Limitations of this treatment include a) the control of bleeding is not treated; these substitutes do not include factors that will help clot blood and stem bleeding, b) tissue-damaging inflammatory responses are not suppressed. The bodies natural wound response can be extensive in these situations causing further damage to the victim. We have shown that peptide-dextran conjugates consisting of peptide for the CD 11b/CD18 binding pocket (A-domain) inhibits inflammatory cell adhesion to activated endothelial cell in static cell culture and in an *in vivo* ear model. We have also shown that dextran conjugated to assembly peptides of fibrin will associate with fibrin upon clotting *in vitro* or to prevent clotting as desired. Future funding is being sought from the NIH for continuation of the successful anti-inflammatory therapeutic.

Harris Bernstein, Ph.D.

University of Arizona
Award Amount FY04: $300,000

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

We showed that reduced apoptosis capability in Barrett's esophagus tissue likely leads to progression to esophageal adenocarcinoma, and that elevated levels of interleukin-6 likely contribute to the development of this apoptosis resistance. We completed a proteomic study of resistance to deoxycholate-induced apoptosis that clarifies the mechanisms by which apoptosis resistance arises during progression to colon cancer and which suggests potential targets for cancer treatment and biomarkers for assessing colon cancer risk. We showed that inhibitors of mitochondrial complexes I-IV protect against endoplasmic reticulum and mitochondrial stresses and apoptosis induction. We published an extensive review of the evidence bearing on whether bile acids are carcinogens in gastrointestinal cancers. We found that reduced expression of a particular mismatch DNA repair gene has promise as an early biomarker of risk. We also showed that, among several available immunohistochemical markers of apoptosis, cleaved cytokeratin-18 was the most useful in colon tissue.

Publications:

Dvorakova K, *et al.* Increased expression and secretion of interleukin-6 in patients with Barrett's esophagus. Clinical Cancer Research, 10: 2020-2028, 2004.

Bernstein H, *et al.* A proteomic study of resistance to deoxycholateinduced apoptosis. Carcinogenesis, 25(5): 681-692, 2004.

Bernstein H, *et al.* Bile acids as carcinogens in human gastrointestinal cancers. Reviews in Mutation Research, (In press).

Bernstein H, *et al.* Recent molecular evidence illuminates the decades-long debate on the role of dietary fat in colon carcinogenesis. Journal of Cancer Integrative Medicine, (In press).

M. Donner Denton, Ph.D.

University of Arizona
Award Amount FY04: $64,766

A Unique Mass Spectrometer for Biomedical Studies

The past year has established many significant applications for H2 laser mass spectrometry (MS) of biomedical samples, yielding very promising results. H2 laser MS has been employed in the detection of anti-neoplastic drugs and anti-microbials of interest to the community such as Altretamine, Levamisole, Methotrexate, and Rifampicin.

An extensive survey of various classes of molecules has been completed to illustrate the wide range of therapeutic drugs that are amenable to this technique. Studies have been conducted showing the potential for analyzing drugs in complex biological matrices such as blood, urine and plant extracts. This technique has also been adapted for analysis of steroids, barbiturates, and flavonoids through covalent attachment of a photo ionizable tag. Derivatization was further utilized to exhibit the unique potential of the H2 laser mass spectrometer in detecting reaction products between DNA nucleic acids and mutagens such as poly-aromatic hydrocarbons.

The ability of H2 laser MS to rapidly detect these adducts lends it to screening patient samples for certain types of cancer-causing agents. This photo ionization technique has also been employed in the screening of alkaloid drugs in crude natural product extracts which can further the discovery of new medicines and can improve the control of active ingredients in commercially available nutraceuticals.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY04: $50,000

Development and Clinical Evaluation of a Confocal Microendoscope

The project proposed to develop and evaluate a new type of instrument called a fluorescence confocal microendoscope. The goal is to demonstrate that the instrument can improve the accuracy of diagnosis of lung disease and other organ systems.

The first and second years of the project built and evaluated catheter systems. The third year of the project developed and evaluated clinical applications of the systems. Lungs, esophagus, colon, cervix, uterus, and ovaries were imaged. Image contrast of tissue autofluorescence of pig lungs was poor. However, collaborations with clinical investigators produced successful images of human gastrointestinal tract and female reproductive systems. Images were collected using both a 3 mm catheter applicable to many clinical endoscopes and with a bare fiber bundle 1 mm in diameter allowing deeper lung imaging.

A database of confocal microendoscope images from both normal and pathologic tissue specimens has been started. Four peer reviewed publications were produced during the project. Significant progress has been made and further research is being proposed to refine clinical applications to the lung as well as other areas.

Publications:

Rouse AR, *et al.* Design and demonstration of a miniature catheter for a confocal micro-endoscope. Appl Opt, 2004, (In press).

Brewer MA, *et al.* Imaging of the Ovary. Technology in Cancer Research and Treatment, 2004, (Accepted for publication).

Abstracts

Udovich JA, *et al.* Confocal micro-endoscope for use in OB/GYN applications. Proc. 2004 IEEE International Symposium on Biomedical Imaging, 2004.

Gmitro AF, *et al.* Confocal micro-endoscopy for GI and OBGYN applications. Optical Society of America Annual Meeting, Frontiers in Optics, 2003.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY04: $187,419

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

The overall goals of this project were to discover, optimize production and evaluate novel anticancer drugs from microorganisms associated with rhizosphere of desert plants growing under semi-extreme conditions. During the course of the final year of this project, 79 fungi have been cultured, their extracts prepared, and screened for their potential anti-cancer activity utilizing cell-based and a target-oriented *in vitro* bioassay for activation of heat shock response. Bioactivity-guided fractionation of extracts derived from six fungi yielded several novel and known compounds with significant *in vitro* anti-cancer activity. If these compounds are active against solid tumors such as lung, breast, colon and prostate cancers, our results will have an impact on the more elderly and/or tobacco-dependent portions of Arizona's population since these cancers are prevalent in our State.

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

University of Arizona
Award Amount FY04: $131,520

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

Significant changes and improvements were made to the Arizona Prostate Cancer Tissue and Serum (APCTSB) database. The Arizona Cancer Center's Bioinformatics Shared Service is leading an initiative to consolidate tissue data resource across the center and to develop technology infrastructure, based on the database developmental work from ADCRC funding. Security and reliability of the system has been strengthened and new data entry and query forms were developed. The database system has grown into a sophisticated system consisting of 74 web pages, 29 database tables, and hundreds of Java programming objects to coordinate the application. We anticipate the APTSB database system will continue to be a vital resource for the long term, with additions and improvements being made over time. Without ADCRC funding, this resource would not exist for prostate cancer research.

Karl B. Kern, M.D.

University of Arizona
Award Amount FY04: $50,000

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

After cardiac arrest and successful CPR the heart struggles to pump effectively. The mechanism of this failure of the heart pumping function is not known. We investigated the role of an enzyme system within the heart, the nitric oxide synthase (NOS) enzymes, that if overly stimulated could contribute to such heart failure. Measurement of two forms of NOS enzymes following cardiac arrest shows the inducible form (iNOS) remains high at 5-6 hours after resuscitation, when the pumping function is especially poor, while the other form (iNOS) returns to its pre-arrest baseline level at 6 hours. This suggests that iNOS is more likely contributing to the poor heart function seen after resuscitation. We then studied the effect of blocking this enzyme system, both non-selectively and selectively for iNOS, in an attempt to improve heart pumping function after resuscitation. Non-selective blockade of the NOS system was detrimental and resulted in worse pump function and poorer outcome than either other group. No significant difference in outcome or pump function was seen between the groups receiving the selective iNOS inhibitor and the placebo. Unresolved issues include the optimal timing for administration as well as the best dosing regime for the selective iNOS blocker.

Publication:

Gaballa MA, *et al.* Differential expression of myocardial constitutive and inducible nitric oxide synthase in cardiac arrest and after resuscitation. Circulation 108:N-380, 2003.

Marlys H. Witte, M.D.

University of Arizona
Award Amount FY04: $49,985

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

Lymphatics parallel blood vessels and return leaked plasma in tissues back to the bloodstream. Lymphatic failure from obstruction or growth defects [angiodysplasia (AD)] promotes lymphedema (LE), a brawny disabling tissue swelling afflicting thousands in Arizona and millions worldwide. We and others have demonstrated that various growth factors/receptors and master genes [Vascular Endothelial Growth Factors/Receptor (VEGF-C/D/R3); Angiopoietins (Ang)1,2; FOXC2] modulate lymphatic growth; corresponding gene alterations underlie distinctive human and genetically-engineered mouse LE-AD syndromes. By enhanced lymphatic imaging, this project has delineated specific patterns of lymphatic/ lymph node UNDER-development in LE mice lacking the Ang2gene (preventable by Ang1 replacement) and contrasting dysfunctional lymphatic OVER-development in FOXc2-deficient (and also over-expressing) mice with distichiasis (double row of eyelashes), mimicking human LE-distichiasis with FOXC2 mutations. Greater understanding of how gene defects produce lymphatic maldevelopment in these mice and man should promote understanding early detection, and new treatments of LE-AD syndromes.

Publications:

Witte MH, Erickson RP, Witte CL. Cardio(blood-lymph)vascular genomics: Need for a terminology adjustment. **Lymphology**, 36:159-161, 2003.

Northup KA, Witte MH, Witte CL. Syndromic classification of hereditary lymphedema. **Lymphology**, 36:162-189, 2003.

Bernas M, Witte M, Erickson R. Genetic advances in pediatric/adolescent lymphedema. **LymphLink**, 16:9-10, 2004.

Bernas M, Witte M. Alternative/complementary treatment in lymphology: Trying the untried and testing the untested. **Lymphology**, 37:43-44, 2004.

Bernas M, *et al.* Massage therapy in the treatment of lymphedema: Rationale, results, applications. **Proc. World Congress on Medical Physics and Biomedical Engineering**, (In press).

Dagenais SL, *et al.* Foxc2 is expressed in developing lymphatic vessels and other tissues associated with lymphedemadistichiasis syndrome. **Gene Expression Patterns**, (In press).

Book Chapters

Witte MH, MJ Bernas, KA Northup, CL Witte. Molecular lymphology and genetics of lymphedema-angiodysplasia syndromes. In: *Textbook ofLymphology for Physicians and Lymphedema Therapists.* Foldi M, E Foldi, S Kubik (Eds), Urban & Fischer Verlag, Miinchen, Germany (English text revised by Biotext, LLC, San Francisco), 2003, Chapter 16, pp.471-493.

Witte CL, MH Witte. Lymph circulatory dynamics, lymphangiogenesis, and pathophysiology of the lymphvascular system. In: *Vascular Surgery. Sixth Edition.* Rutherford RB (Ed.), W.B. Saunders Company, Philadelphia, Pennsylvania, Chapter 31, (In press).

Abstracts:

Bernas M, *et al.* Massage therapy for lymphedema treatment. Abstract booklet, 19th ICL, Freiburg, Germany, p. 16,9/16/03.

Witte MH, Witte CL. Advances in molecular lymphology, lymphangiogenesis and the genetics of lymphedema-angiodysplasia syndromes. Ceremonial lecture presented at 19th International Congress of Lymphology, Freiburg, Germany, 9/1-6/03, Abstract booklet, p. 193.

Lynch M, *et al.* Comparative efficacy of massage (manual lymph drainage) therapy components in reducing limb swelling in a rodent model of post-cancer treatment lymphedema. 19th ICL, Freiburg, Germany, 9/1-6/03, [Presidential Prize] Abstract booklet, p. 92.

Noon AB. *et al.* FoxC2 gene insufficiency and imbalance in the genesis of the distinctive lymphatic and ocular phenotype of lymphedema-distichiasis syndrome. Poster Presentation, AFMR, Chicago, IL, 4/16-17/04, J Inv Med, 52:S386, 2004.

Witte M, Witte C. Found in Translation: Advances from Molecular to Clinical Lymphology. Keynote lecture at Society of Vascular Biology & Medicine, Anaheim, CA, 6/4-8/04, Abstract booklet, p. 91-92.

Bernas M, Witte CL, Witte MH. A fantastic voyage through the lymph circulation: A patient-oriented research perspective. AHSC Frontiers in Biomedical Research Poster Session, Tucson, Arizona, 9/15/04.

Noon AB, *et al.* Foxc2 gene insufficiency and imbalance in the genesis of the distinctive lymphatic and ocular phenotype of lymphedema-distichiasis syndrome. AHSC Frontiers in Biomedical Research Poster Session, Tucson, Arizona, 9/15/04.

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

University of Arizona
Award Amount FY04 : $48,862

Mechanism of Cigarette Smoking on Human Infertility

In Arizona, approximately one-third of the women of reproductive age smoke cigarettes. It has been demonstrated that cotinine, a metabolite of nicotine is significantly increased in ovarian follicular fluids in smokers. It is unknown how cotinine damages the ovaries at the molecular level. We, for the first time, demonstrated that cotinine stimulates the nuclear transcription factor (Nfk-B) and messenger RNA levels of inducible Nitric Oxide Synthase (iNOS), and also induces the cell death in the ovarian granulosa cells. The results were confirmed at gene transcriptional level (mRNA) and protein level using methods of polymerase chain reaction (PCR) and Western Blot analysis for protein expression. We also demonstrated that cotinine acts through Nfk-B and iNOS independently, which further revealed molecular mechanisms of toxic effect of cotinine on ovarian granulosa cells. The damage for the ovarian cells would affect the oocyte development and luteal function, which would result in early pregnancy loss. The outcome of this research will lead to better understanding the mechanisms of the toxicity of cigarette smoking, which should provide scientific information for the people in Arizona, especially the people at the reproductive ages.

Linda Garland, M.D.

University of Arizona
Award Amount FY04: $103,724

A Pilot Study of Lung Cancer Prevention with Selenium Supplementation

There are currently more than 40 million former smokers in the U.S. Former smokers remain at elevated risk of lung cancer even 20 years after quitting smoking. Lung cancer is difficult to diagnose at an early stage, when it may be curable. Lung cancer continues to be the leading cause of cancer-related death in both men and women in the New Millennium.

Selenium is a micronutrient that has shown promise as an oral supplement for preventing cancer. The largest early study of selenium supplement was conducted at the University of Arizona by Dr. Clark and colleagues, who showed a lowered risk of lung cancer in persons with low selenium blood levels who took selenium as compared to the group who took a placebo yeast tablet. It has been proposed that selenium may act to un-silence important genes whose function has been silenced by a process called DNA methylation. In addition, selenium may act as an antioxidant, whereby it decreases the body's level of destructive oxidation (termed oxidative stress) that has been linked to inflammation and, from there, to cancer development. The overall goal of this study is to help define whether selenium may alter underlying biologic processes that have been linked to lung cancer development.

We have randomized 37 former smokers who have smoked the equivalent of $\geq$1 pack of cigarettes per day for 20 years to either selenium yeast tablet or placebo yeast tablet for 9 months. Dr. Bernard Futscher's laboratory analyzed sputum and buccal (cheek) cells for evidence of abnormal gene methylation in two genes important in the development of lung cancer. In our samples from relatively "healthy" former smokers who did not have highly compromised lung function, we did not see abnormal DNA methylation changes in sputum or buccal cells.

We are analyzing the levels of oxidative stress in the blood before and after selenium supplementation to see if selenium has influenced oxidative stress. Many factors can influence oxidative stress and include some vitamins and dietary intake of substances such as tea. We will analyze participant use of these antioxidants from our dietary questionnaires.

Karl H. Schram, Ph.D.

University of Arizona
Award Amount FY04: $93,150

Biomarkers of Systemic Fungal Infections

The objectives, and results, for the year were to 1) complete the analysis of fungal extracts; the analysis of these samples is currently being completed; 2) determine the structures of two metabolites unique to the extract of a rabbit branchial lavage sample infected with A. fumigatus; samples were analyzed in the laboratory of Dr. Vladmir Havlicek at the institute of Biochemistry in Prague, Czech Republic using a variety of methods; no cyclic peptides were identified, 3) continue the analysis of blood samples; analysis of these samples is ongoing, and 4) determine the limits for detection of cyclic peptides in blood samples; electrospray ionization provides femptomole detection limits for these compounds. Because none of the methods used have shown the presence of cyclic peptides, our focus has been broadened to include fungal lipids.

Publications:

Miketova P, *et al.*GC/MS profiling of fatty acids in medically important fungi. Scientific Papers of the University of Pardubice, Series A, Faculty of Chemical Technology 9:49-67, 2003.

Abstracts:

Miketova P, *et al.*Analysis of fungal products in growth medium, fungi and human blood. 52nd Annual Conference on Mass Spectrometry and Allied Topics, Montreal, Canada, 2004.

Miketova P, *et al.*Fungal biomarkers of opportunistic infections in bone marrow transplant patients by pyrolysis metastable atom bombardment time of flight mass spectrometry. 52nd Annual Conference on Mass Spectrometry and Allied Topics, Montreal, Canada, 2004.

Kemmons A. Tubbs, Ph.D.

Intrinsic Bioprobes, Inc.
Award Amount FY04: $138,977

Proteomic Analysis of Nicotine Receptor Structure and Composition

The knowledge base from which to understand potential dependence and addiction from tobacco and tobacco related products requires continued study of targeted nicotine based biological receptors, the nicotinic acetylcholine receptors (nAChR). This receptor forms a barrel shaped structure residing in cellular membranes whose individual subunits resemble the staves of a barrel. The intricacies of these subunit based macromolecules are key to understanding structure-function associations within the receptor as well as with other cellular proteins. Of further interest is the ability to track changes during and after subunit formation. Our understanding about how nAChR function is altered by prolonged and repeated exposure to nicotine require overcoming these deficiencies in our knowledge base. Habitual use of tobacco and tobacco related products contribute to these effects and continue to advance adverse health and economic consequences to a significant number of adult Arizonans. To understand the structural and compositional inadequacies of diverse forms of human nAChR, we have proteomic based techniques to generate and characterize complex proteins like nAChR that reside in cell membranes. In addition, we are using molecular biology techniques to engineer other nAChR subtypes in full-length, truncated and epitope-tagged forms. The native and expressed protein characterization uses mass spectrometry based techniques in concert with traditional protein techniques. Proteomic techniques have been used to initiate characterization of diverse forms of nAChR containing different subunit building blocks. These techniques have been applied to identify and characterize subunits that assemble to create different forms of human nAChR generated by genetically engineered cells or found naturally in human tissues and to identify and characterize other cellular components that interact with specific forms of nAChR or receptor assembly partners.

Publication:

Bieber AL, Tubbs KA, Nelson RW. Metal ligand affinity pipettes and bioreactive alkaline phosphatase probes: Tools for characterization of phosphorylated proteins and peptides. Mol and Cell Proteomics 3:266-272, 2004.

Garth Powis, D. Phil.

University of Arizona
Award Amount FY04: $50,000

Thioredoxin Peroxidase, A Novel Mechanism for Protection Against Lung Toxicity

The mitochondrial thioredoxin-reductase 2/thioredoxin-2 (Trx-2)/peroxiredoxin-3 (Prdx-3) is an important regulator of mitochondrial redox status and protects mitochondria from oxidant damage. We have shown that Prdx-3 is an important cellular antioxidant that regulates physiological levels of H^2O^2 leading to decreased cell growth while protecting cells from the apoptosis inducing effects of high levels of H^2O^2. We have also shown that mice with both thioredoxin-2 Trx-2 genes inactivated die early during embryo development at a time when mitochondrial respiration is just starting. The mouse model we have developed points to the importance of Trx-2 in protecting against reactive oxygen species damage. Dynamic contrast enhanced (DCE) magnetic resonance imaging was used to show acute hemodynamics changes in human lung cancers growing in mice caused by oxidant anticancer drugs. There are 4 manuscripts (3 published one in press) resulting from work on the contract and a federally funded RO1 grant.

Publications:

Nonn L, Berggren M, Powis G. Increased expression of mitochondrial peroxiredoxin-3 (thioredoxin peroxidase-2) protects cancer cells against hypoxia and drug-induced hydrogen peroxide-dependent apoptosis. Mol Cancer Res, 1(9): 682-699, 2003.

Nonn L. *et al.* The Absence of mitochondrial thioredoxin 2 causes massive apoptosis, exencephaly, and early embryonic lethality in homozygous mice. Molecular Cell Bio, 23(3): 916-922, 2004.

Welsh SJ, *et al.* Antitumor activity and pharmacodynamic properties of PX-478; an inhibitor of hypoxia inducible factor-1 ex. Mol Cancer Therap, 3:233-244, 2004.

SECTION B

CONTINUING CONTRACTS

MEDICAL RESEARCH

YEAR TWO

FY 2004

Lokesh Joshi, Ph.D.

Arizona State University
Award Amount FY04: $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, these two molecules and other such drugs have been obtained from mammalian sources (including humans). However, because of the inefficiency of the production method and the inherent threat of cross-contamination, such as prions, viruses and other adventitious agents, there is a strong need for alternative methods of production. We are developing plants as the source of these human proteins that are able to prevent and/or treat diseases. Plants are safe and can be scaled up to produce large quantities of medically-important proteins for patient care. In the past year we were able to introduce human genes encoding the proteins of interest in plants. We have successfully generated plants that contain a protein which modulated our immune system to combat cancer growth. These plant derived proteins will be characterized and tested for their efficacy in cell-based assay. This presents a novel approach that has significant therapeutic and biotechnology potential.

Molly A. Brewer, DVM, MD

University of Arizona
Award Amount FY04: $143,548

Fluorescence Spectropscopy as a Biomaker for Prevention-Early Diagnosis of Ovarian Cancer

We will evaluate 10 cancers comparing measurements to the data in the first portion of the study and calculate a power analysis to determine numbers needed to reach significance. Focusing on only epithelial cells has been a shortcoming of prior studies and this technique not only allows the correlation between fluorescence and molecular markers, but also the study of the interactions between stromal and epithelial cells. We hypothesize that much will be learned in ovarian oncogenesis by perfecting the technique of organ culture in the ovary. We will use this information to develop fluorescence spectroscopy as a screening tool for the early diagnosis of ovarian neoplastic changes, and potentially be able to identify ovarian tissue that is undergoing early neoplastic changes in situ. We will also have the information to use fluorescence as a biomakers of drug activity in the ovary for women using chemopreventive agents to prevent ovarian cancer.

Publications:

Brewer MA, *et al.In vitro* model of normal, immortalized ovarian surface epithelial and ovarian cancer cells for chemoprevention of ovarian cancer. Gynecologic Oncology, 2004 (In press).

Kirkpatrick N, *et al.* Endogenous fluorescence spectroscopy of cell suspensions for chemopreventive drug monitoring. Photochemistry and Photobiology, 2004 (In press).

Megan M. McEvoy, Ph.D.

University of Arizona
Award Amount FY04: $50,000

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

The goal of this proposed research is to understand the mechanism of asymmetric cell division by determining the structures and interactions of proteins that are involved in this process. A subset of the proteins which form the apical complex were selected for characterization, Par-6 and Bazooka. We were pursuing the NMR structure determination and the interactions of domains from these proteins. However, after much effort with these systems, we were still finding the constructs we made to the intractable for full NMR structural studies. A new cryogenically cooled probe will be installed in November 2004, and with the improved sensitivity of this system, we expect to again make progress with the system. As an alternative approach, we have been working with Cdc42 and Staufen, both of which work in apical complex formation. The NMR spectrum of the Staufen domain show that it is largely unfolded. We are preparing a construct of Inscuteable that interacts with Staufen, to see if folding can be induced and to study the structure of the complex. This work will lead to better understanding of the fundamental process of asymmetric cell division, which may in the long term have implications in the treatment of cancerous tissue where cell proliferation is unrestricted.

Claire M. Payne, Ph.D.

University of Arizona
Award Amount FY04: $175,000

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

Our laboratory has determined that stable populations of colon cells that fail to undergo programmed cell death, can develop when persistently exposed to agents that induced DNA damage (e.g. bile salts associated with a high fact diet). These stable populations are at risk for becoming cancerous since they have a higher propensity to develop mutations. We have analyzed these death-resistant cells at the molecular and cellular levels and determined that there is an increase in the signaling pathway that is activated by nitric oxide (NO) when compared to death-sensitive cells. We also showed that mitochondria, the main producers of energy in the cell, contribute to apoptosis resistance. Therefore, we have identified several targets for intervention that may prevent colon cancer on an individual basis. In addition, we have opened up novel potential biomarkers that may help identify those individuals at risk for cancer.

Publications:

Payne CM, *et al.* Caspase-6 mediated cleavage of guanylate cyclase alpha 1 during deoxycholate-induced apoptosis: Protective role of the nitric oxide signaling module. **Cell Biology and Toxicology**, 19:373-392, 2003.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY04: $250,000

Development of New Anticancer Drugs for Improving Human Cancer Treatment

The principle goal of this project is the discovery, development, and synthesis of anticancer drugs. Progress has been made in both discovery of new anticancer drugs and in synthesizing existing organic based drugs.

When combined with other known effective anticancer drugs dolastatin 10 is proving successful in anaerobic bacterial treatment of cancer tumors in nude mice. Dolastatin 10 is also in a human cancer clinical trial under the auspices of the National Cancer Institute. Cell line evaluation of dolastatin 18 shows significant activity in treating human tumors. However synthetic dolastatin 18 needs further refinement.

During the past year total synthesis of dolastatin 16 and 17 has almost been completed. Auristatin 15-DMO and 15-F synthesis are also close to completion. These compounds were originally discovered in living organisms. The clinical trials and future applications of these anticancer drugs are dependent upon a continuing supply of the drugs. Synthesis will assure that supply.

Several research papers have been published. ADCRC funded research has been combined with other sponsored research resulting in an expanded ability to achieve the goals of the project.

Publications:

Dang LH, *et al.* Targeting Vascular and Avascular Compartments of Tumors with C. *novyi-NT* and Anti-Microtubule Agents. Cancer Biology & Therapy, 3:326-337, 2004.

Bai R, *et al.* Direct Photo affinity Labeling by Dolastatin 10 of the Amino Terminal Peptide of -Tubulin Containing Cysteine-12. J Biol Chem, 279:30731-30740, 2004.

Pettit GR, Hogan F, Herald DL. Synthesis and X-Ray Crystal Structure of the *Dolabella auricularia* Peptide Dolastatin 18. J Org Chem, 69:4019-4022, 2004.

Pettit GR. Antineoplastic Agents 510. Isolation and Structure of Dolastatin 19 from the Gulf of California Sea Hare *Dolabella auricularia.* J Nat Prod, (In press).

Danzhou Yang, Ph.D.

University of Arizona
Award Amount FY04: $49,940

DNA and Topoisomerase I Interactions of Novel Homocamptothecin Anticancer Drugs

The campotothecin (CPT) derivatives are among the most promising anticancer drugs recently introduced in the clinic. Topotecan (TPT; Hycamtin) has been approved for the treatment of advanced ovarian cancer and for second-line therapy in small cell lung cancer. Irinotecan (CPT-11; Camptosar) has been approved for the treatment of colorectal carcinoma. Current clinical trials indicate that camptothecin derivatives will be useful in a variety of other human malignancies. Homocamptothecins (hCPT) are a group of novel CPT analogues with a modified seven-member lactone ring by the insertion of a methylene group in the E-ring hCPts fully conserve the topoisomerase I inhibiting activity and stimulate high levels of DNA cleavage. This E-ring modification enhances the stability of the lactone ring, which, in the case of classical CPTs, opens to an inactive carboxylate form at physiological pH. This enhanced stability may account for the superior antitumor activity of hCPT *in vitro* and *in vivo*.

Human topoisomerase I (top I) is an essential cellular enzyme that manipulates the topological states of DNA and is pivotal for DNA replication, transcription, recombination and chromosome condensation. By transiently breaking one strand of duplex DNA to relax DNA supercoiling, Topo I is the sole molecular target for homocamptothecins. Understanding the structural details of human topo I is critical for elucidating the interactions of homocamptothecins with DNA/Topo I. The C-terminal domain of topo I contains the active site tyrosine (Tyr723) that forms the covalent linkage with DNA and a number of residues that are involved in interactions with DNA and camptothecin. It has been suggested that the C-terminal domain of topo I is independently folded and is readily associated with the core domain to form the catalytically active enzyme. We carried out structural analysis of human topo I C-terminal domain using different biophysical techniques.

Stephen Massia, Ph.D.

Arizona State University
Award Amount FY04: $175,000

Local Gene Therapy Targeting Vascular Graft Hyperplasia

Cardiovascular disease remains a major health problem in the nation and in Arizona. Bypass graft placement is widely used as an interventional therapy for treating obstructions in arteries due to atherosclerotic disease. This research focuses on developing gene therapy that can be placed in synthetic graft materials and delivered locally after surgery to inhibit the process leading to graft occlusion. During the first year of this project, we developed materials designed for sustained release of therapeutic genes from synthetic vascular grafts. Gene therapies have recently failed clinically and await new technologies to overcome these problems. The second year of this project has developed alternatives to the original gene therapy approach and show that these alternatives outperformed the originally proposed gene therapy *in vitro*. The third year studies will evaluate how these alternative therapies perform in preventing vascular graft failure in a pre-clinical animal model.

Rodney D. Adam, M.D.

University of Arizona
Award Amount FY04: $49,999

Gene Expression in *Giardia Lamblia*

The overall goal of the current project is to understand the role of epigenetic inheritance in controlling gene expression in *Giardia lamblia*. Epigenetic expression refers to inherited gene expression independent of the DNA sequence and is controlled by methods such as DNA methylation or modification of histones, a set of proteins that are wrapped around the DNA. Since *Giardia* does not have DNA methylation, we focused on the role of histone acetylation/deacetylation. We found that *Giardia* had enzymes for DNA acetylation, deacetylation, and methylation. We have used inhibitors of DNA acetylation to determine their affect on *Giardia*. We found no effect on antigenic variation or on encystation/ excystation. We found that these agents prevent the completion of cell division, so that two cells are attached together after the division of their nuclei. It appears that *Giardia* histone acetylases/deacetylases have a role in control of cell cycle (cell division).

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY04 : $175,000

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

AIDS in children is increasing at a significant pace worldwide because more women of childbearing age are becoming infected with HIV-1. Infants born to these infected women are at risk of acquiring HIV-1 infection and subsequently developing AIDS. In Arizona, the number of AIDS cases is increasing and Tucson has been included in the top 50 metropolitan areas in the country with the highest annual rates of AIDS cases. One striking feature of HIV disease is that it follows a more rapid course in infected infants compared with infected adults. The molecular mechanisms of HIV-1 infection in infants are not clearly understood, making it difficult to develop strategies for prevention and treatment of HIV-1 infection in children. Our hypothesis is that HIV-1 replicates more efficiently and destroys more rapidly infants' target mononuclear cells compared with mature adults' target cells. This may result in an accelerated course of disease progression in infants. We are using cord blood in place of infants' blood because of its closeness to neonatal cells in terms of immaturity (high in CDA45RA+ and low in CD45RO+) and adult blood mononuclear cells to investigate the mechanisms of HIV-1 infection. We found that HIV-1 replicates better in cord blood lymphocytes and monocytes/macrophages compared with adult blood lymphocytes and monocytes/macrophages, without significant difference in the expression of HIV-1 receptor (CD4) and co-receptors CCR5 and CXCR4). We found that the enhanced HIV-1 replication in cord blood cells compared with adult blood cells could be attributed to up-regulation of HIV-1 gene expression. The higher levels of HIV-1 in cord blood cells may cause more rapid destruction of T cell precursors. The findings may be helpful in explaining why HIV-1 replicates better in cord blood mononuclear cells compared with adult blood cells. This may provide some insights into the development of strategies for the treatment and prevention of HIV-1 infection in children.

Jorge A. Giron, Ph..D.

University of Arizona
Award Amount FY04: $50,000

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

Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is recognized as a major cause of severe bloody (hemorrhagic colitis) and non-bloody diarrhea in humans. Bacterial pathogens produce adhesins, called pili, that mediate their close interaction with the host cells. We have recently identified and purified a novel pilus structure (HCP) produced by EHEC strain EDL933 and other O157:H7 strains. Antibodies in human sera from individuals infected with EHEC recognize purified HcpA, the major pilim subunit, suggesting that these pili are expressed within the host.

We have constructed an HcpA'-lacZ transcriptional fusion to study the environmental signals that trigger expression of HcpA. We have also created a strain with an in-frame deletion of HcpA which will be used in future animal studies to determine the importance of HCP in colonization of a natural host. Understanding the role of HCP in EHEC pathogenesis may provide knowledge that will help control outbreaks in humans.

Marlene P. Freeman, M.D.

University of Arizona
Award Amount FY04: $49,988

Omega-3 Fatty Acids for Postpartum Depression

Postpartum depression (PDD) is common disorder and has broad public health implications. Omega-3 fatty acids are polyunsaturated fatty acids with many associated health benefits. Some women are unwilling to accept antidepressant medications while breast feeding.

Twenty-one women with PDD entered a dose-finding trial of omega-3 fatty acids. Subjects were randomly assigned to daily doses of 0.5 g, 1.4 g, or 2.8 g. Subjects were assessed at weeks 0, 1, 2, 4, 6, and 8. Assessments include the Edinburgh Postnatal Depression Scale (EPDS) and the Hamilton Rating Scale for Depression (HRSD). For subjects who returned for $\geq$1 visit, average decrease in EPDS and HRSD was 51.5% and 48.8%, respectively.

This study provides data about a novel treatment for PPD. The limitations thus far include small sample size and lack of placebo group. We have utilized this data for the development of a placebo-controlled trial of omega-3 fatty acids for perinatal depression.

Michael H. Ossipov, Ph.D.

University of Arizona
Award Amount FY04: $173,026

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

Chronic pain states are often treated with opiate-type analgesic like morphine or fentanyl, but these are associated with tolerance and increased pain. We found that tolerance develops substantially more slowly with fentanyl when compared to morphine. Long-term opiate use causes increases in the spinal levels several neurochemicals, dynorphpin, substanc P and bradykinin, that contribute to pain signaling pathways. We found that either prevention of the production, or blocking the receptors where these transmitters act, prevents the development of abnormal pain and tolerance to opiates. The development of drugs that block the activities of these transmitters should permit the long-term treatment of chronic pain patients without the development of tolerance and the need for increasing doses and eventual loss of analgesic activity. We also believe that fentanyl is a strong analgesic with considerable promise for the long-term management of chronic pain related to nerve injury and abnormal pains related to cancer.

Patricia E. Penn, Ph.D.

La Frontera Center, Inc.
Award Amount FY04: $108,131

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

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

- PSMI smoke nearly half of the cigarettes in this country.
- Their smoking rate is 2-3 times higher than in the general population.
- Public funding provides most of PSMI support; cessation could mean cost savings.
- Cessation interventions for this population are understudied, and most studies exclude PSMI.

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

Participants are from three La Frontera Center, Inc. clinics. To date we recruited 210, enrolled 163 (77.6% of those referred/recruited), and intervened with 109 PSMI in the treatment groups. Preliminary data suggest that by week 20 (end of active intervention) nearly half (48.5%) of our participants have quit or are smoking much less. By week 36 (final follow-up), 39% still had no or low use.

Barry M. Pryor, Ph.D.

University of Arizona
Award Amount FY04: $49,982

Characterization of Alternaria Isolates Associated with Allergenic Asthma

Soil and plant debris samples were collected from 22 locations in native desert and urban landscape environments around Tucson, Arizona, and *Alternaria* isolates recovered. Methods for qualification of conidiation pattern were developed and used to characterize morphology of 20 representative (type) *Alternaria* species and all Tucson isolates. Morphological examination revealed that variation among Tucson isolates encompassed the range of variation exhibited by 10 different *Alternaria* species.

AFLP fingerprinting of fungal DNA from Tucson isolates also revealed a range of genetic variation that encompassed 8 different *Alternaria* species. Most of the Tucson isolates were included in the alternata species-group and divided in to four subgroups. Less than 10% showed close relationships with the type isolate of *Alternaria alternata*. Methods for recovery of fungal proteins (allergens) have been optimized and are currently being used in immunoblot analysis of fungal allergen diversity based upon hybridization with human sera obtained from asthmatic children in Arizona.

Publications:

Hong SG, Pryor BM. Development of selective media for the isolation and enumeration of *Alternaria* species from soil and plant debris. Canadian Journal of Microbiology, 50:461-468, 2004.

Hong SG, Liu D, Pryor BM. Restriction mapping of lGS regions from *Alternaria* spp. and their taxonomic implication. Mycological Research, 108: (In press).

Hong SG, *et al.* Alt a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. Fungal Genetics and Biology, 43, (In press).

Bradley S. Moore, Ph.D.

University of Arizona
Award Amount FY04: $50,000

Engineered Biosynthesis of "Unnatural" Natural Products for Drug Discovery

The biosynthesis of prenylated natural products from different actinomycete strains was evaluated at the chemical, biochemical and genetic levels with the goal of isolating new prenyltransferase enzymes for the chemoenzymatic synthesis of new chemical entities. Towards this goal, we have cloned several putative biosynthetic enzymes involved in the synthesis of these bioactive agents and have developed conjugative protocols for gene inactivation and complementation experiments in the natural product producing strains. In the case of the neuronal cell protecting agent aestivophoenin from *Streptomyces purpeofuscus*, we have cloned a dNDP-glucose dehydratase gene that may be involved in the biosynthesis of this glycosylated and prenylated compound. Concerted effort is underway to clone and sequence this biosynthetic gene cluster along with those for (neo)marinone, lavanducyanin and CNQ-525518.

Publications:

Kalaitzis JA, *et al.* Biosynthesis and structure revision of neomarinone. Org Lett, 5:4449-4452, 2003.

Moore BS, Kalaitzis JA, Xiang L, Exploiting marine actinomycete biosynthetic pathways for drug discovery. Anton Leeuw J Microbiol, (Accepted).

SECTION C

CONTINUING CONTRACTS

MEDICAL RESEARCH

YEAR ONE

FY 2004

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

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

Vagal Anti-inflammatory System

Systemic inflammation/sepsis is a leading cause of morbidity and mortality. In search for novel treatments, it has been proposed that the vagus nerve (the largest nerve in the body servicing internal organs, including the liver) plays a key role in natural defense against systemic inflammation and that the protective action of the vagus is mediated by nicotinic receptors, possibly on the cells called macrophages in the liver. Data obtained during Year 1 of this project suggest that only a certain type of fibers in the vagus nerve (efferent) is involved in this protective action. We also have performed initial pharmacological characterization of macrophages on which the vagus nerve may act to inhibit the overproduction of harmful inflammatory mediators (such as tumor necrosis factor). These results have been reported to the prestigious Nobel Conference in Stockholm, Sweden. One article has been published in a leading pharmacological journal; two manuscripts are in preparation.

Publication:

Dogan MD, *et al.* Lipopolysaccharide fever is initiated via a capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. Br J Pharmacol, 2004.

Elizabeth L. Glisky, Ph.D.

University of Arizona
Award Amount FY04: $49,977

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

In the first year of the project, we focused on the recruitment and testing of our normally aging older adults and those with a family history of Alzheimer's disease (AD). We are now testing people in our other two groups, those with mild cognitive impairment (MCI), defined as 1.5 standard deviations below the mean on tests of memory, and those diagnosed with AD. Preliminary analysis of data reflecting the integrity of white matter, which were obtained using diffusion-weighted magnetic resonance imaging (DWMRI), indicate an age-related decline in white matter integrity in normally aging older adults. However, the same measures obtained from one AD patient and one person with MCI indicated much greater loss of white matter integrity. Those early findings suggests that DWMRI, which is particularly sensitive to inflammation of white matter in the brain, may provide an early indicator of AD pathology.

Indraneel Ghosh, Ph.D.

University of Arizona
Award Amount FY04: $49,500

Inhibiting Protein-Protein Interactions Involved in Cancer

In the year 2004, there will be an estimated 1,368,030 new cancer cases diagnosed in the United States, of which 23,560 will be in Arizona. We are in process of developing a technological platform for drug design that is both simple and powerful and will not only aid in designing cancer therapeutics but also impact other diseases such as diabetes and Alzheimer's. We have created a library of over 1 billion drug-candidates from which we can isolate therapeutics that specifically target two cancer-linked proteins, the vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6). VEGF is implicated in solid tumor development and IL-6 in multiple myeloma. We have made excellent progress towards our goals, having designed and created over 1 billion drug candidates and isolated an initial set of 12 drug-candidates that directly target VEGF. We are currently in the process of testing the activities of our drug candidates.

Haiyong Han, Ph.D.

University of Arizona
Award Amount FY04: $49,881

Development of New Anti-Renal Cell Carcinoma Agents Using Pharmacological Syntheric 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 establishment of isogenic cell lines for the VHL gene. We cloned the VHL gene and re-expressed it in a renal cancer cell line 786-0 which has a loss-of-function mutation in the VHL gene. This new cell line and the parental line 786-0 form a pair of isogenic cell lines for VHL. We have also used the siRNA technology to knock down the expression of VHL in an embryonic kidney cell line from a second pair of isogenic cell lines for the VHL gene. These two sets of isogenic cell lines will be used to screen compound libraries to identify small molecules that specifically kill renal cancer cells harboring VHL deficiency.

Nancy C. Horton, Ph.D.

University of Arizona
Award Amount FY04: $44,825

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

While Arizonans enjoy many beautiful sunny days year-round, an unfortunate side-effect of the exposure to sunlight is damage caused to skin cells by ultraviolet radiation (UV). UV light damages cellular DNA in a manner that can give rise to mutations and hence increase the risk of skin cancer. Fortunately, our cells have several systems that can repair such damage. The primary mechanism in our cells in repairing UV damage has been termed nucleotide excision repair (NER). Currently, as a result of funding from ADCRC, we isolated the DNA damage recognition region of the NER protein XPC and its binding partner hHR23B. These two proteins are thought to be the initial DNA damage recognition proteins that initiate NER. It is our goal to further characterize the structures of these proteins while bound to UV damaged DNA in order to understand how they function. This information will be invaluable for the design of potent chemotherapeutics to treat and prevent skin as well as other types of cancer.

Natalia A. Ignatenko, Ph.D.

University of Arizona
Award Amount FY04 $55,000

Effects 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 supressor gene acts as a gatekeeper for colorectal adenoma formation. All patients with Familial Adenomatous Polyposis (FAP) and the majority with sporadic colon cancer have APC mutations. Polyamine levels are significantly higher in cancers with APC mutations. Polyamine levels are significantly higher in cancers with APC mutations than in normal tissue.

Valuable systems for studying tumor initiation and progression, target tissues of carcinogenic agents and preclinical evaluation of potential chemopreventive and therapeutic drugs are provided by animal models. In this study we used the multiple intestinal neoplasia (MIN) ice which have a genetic mutation similar to the FAP patients. We created a new mouse model of colon carcinogenesis to study the role of the transcriptional regulator of the ODC gene, the c-myc protooncogene, in colon carcinogenesis. The preliminary data show that deletion of the c-myc gene in the intestinal tract of MIN mice suppresses colon tumorigenesis.

We evaluated the effects of two chemopreventive agents, non-steroidal anti-inflammatory drugs (NSAIDs) α-difluoromethylornithine (DFMO), a specific inhibitor of polyamine pathway on colon tumorigenesis. We found these agents to be at least additive in suppressing intestinal tumor numbers. These data support the rationalle for combination therapy employing NSAIDs and polyamine inhibitors in FAP patients. Putrescine (1%) increased intestinal putrescine concentrations and partially reversed the anti-tumor effects of sulindac.

Emmanuel Katsanis, M.D.

University of Arizona
Award Amount FY04: $96,492

Chaperone Rich Cell Lysate (CRCL) Vaccine for Ovarian Cancer

Special types of proteins called heat shock proteins (HSPs) or chaperone proteins can be extracted from cancer cells. These HSPs have been found to carry cancer proteins and, therefore, can function as unique anti-cancer vaccines. We have developed a relatively simple, rapid, and efficient procedure that generates multiple HSPs called Chaperone Rich Cell Lysates (CRCL). Our efficient CRCL enrichment technique has enabled us to overcome the lack of cancer protein material that may often be encountered by purifying individual HSPs using the other methods. We have found that when CRCL is used to immunize mice with different cancers, they provide significant protection against all cancers. Our hypothesis is that several types of HSPs together (such as found in CRCL) induce the maximally effective anti-cancer responses by providing more cancer proteins that can stimulate T cells by improving the function of dendritic cells that can then present these cancer proteins to T cells more effectively. Our goals are to prove that this is true in the human system as we have shown in mice.

We have successfully generated adequate CRCL from 6 human ovarian cancers. This amount would be enough to vaccinate patients. We tested CRCL to see if it activates dendritic cells and found that like in the mouse experiments, CRCL improved the function of human dendritic cells which will allow them to present these cancer proteins to T cells more effectively. We have also done experiments studying the effectiveness of ovarian cancer CRCL in stimulating T cells and found that CRCL can make T cells attack and kill cancer cells. CRCL therefore appears that it would be an effective vaccine against ovarian cancer.

Publications:

Graner MW, *et al.* Tumor-expressed albumin inhibits T cell activation and responses. Cancer Research, (In press).

Abstracts:

Li G, *et al.* Human ovarian tumor-derived chaperone-rich cell lysates (CRCL) activate dendritic cells and elicit T cell responses *in vitro.* 19th Meeting of the Society of Biological Therapy, San Francisco, California, December 2004.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY04: $166,250

Anticancer Drug Preclinical Development

The development in Arizona of new anticancer drugs leading to clinical trials forms the overall objective of this project. Specifically the focus is on phenpanstatin, pancratistatin3, 4-0 cyclic phosphate prodrug, iodocomstatin phosphate prodrug, and auristatin MO.

Cancer cell line testing of pancratistatin and its derivatives demonstrated promising antitumor activity. Testing is ongoing to discover the precise mode of action of these compounds. Dolastatin 10, auristatin 15-PE, auristatin PYE, and pancratistatin 7-O-phosphate were combined with other anticancer drugs as part of a collaborative clinical trial of human cell line cancer tumors. In combination with other drugs the compounds were shown effective in stopping blood flow to the tumor site. The project provided the synthesized drugs.

The Arizona Cancer Institute is a leading provider of innovative anticancer drugs. After clinical evaluation, the next steps are to develop and evaluate the pharmacology and formulation necessary to proceed into Phase I clinical trials.

Publication:

Rinner U, *et al.* Synthesis and biological activity of some structural modifications of pancratistatin. Bioorg Med Chem Lett, 14:2911-2915, 2004.

Pettit GR, *et al.*Antineoplastic Agents 510. Isolation and structure of dolastatin 19 from the Gulf of California sea hare *Dolabella auricularia.* J Nat Prod, (In press).

Pettit GR, Melody N. Antineoplastic Agents 527. Synthesis of7-Deoxy-narcistatin, 7deoxy-trans-dihydronarcistatin, and Trans-dihydro-narcistatin 1. J Nat Prod, (In press).

Pettit GR, *et al.* Synthesis and Evaluation of 3' Position Structural Modification of (2)- and (E)-Combretastatin A-4. J Med Chem, (In press).

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY04: $50,000

Eluding Drug Resistance in Cancer Chemotherapy

Drug resistance in cancer chemotherapy can result from expulsion of the drug from cancer cells by a biochemical pump in the cell. This negates the effectiveness of anticancer drugs. Our strategy to prevent this is 1) to knock out a key enzyme in cell division by forming a covalent bond between the drug and the enzyme so the biochemical pump cannot reverse the drug's effects; and 2) to allow the target enzyme to carry out its job but to provide it with a faulty substrate that results in nonfunctional cancer cell division proteins. More than two dozen compounds in five different chemical categories have been designed (some by computer simulation methods), prepared, and/or tested. Several were found to effectively inhibit the enzyme and/or inhibit the growth of human cancer cells in culture. This work may lead to new anticancer agents for the benefit of Arizona residents.

Daekyu Sun, Ph.D.

University of Arizona
Award Amount FY04: $89,183

Development of Human Telomerase Inhibitors as New Anticancer Drugs

Telomerase is a potential molecular target of anti-cancer drugs because it is highly specific and essential for survival and growth of cancer cells. To identify the effective ways of targeting human telomerase or telomere maintenance mechanisms, selective telomerase inhibitors and G-quadruplex interactive compounds have been synthesized, and their biological activities have been evaluated using MiaPaCa human pancreatic cancer cell line as a model system. At nontoxic concentrations, TMPyP4, which is both a G-quadruplex interactive agent and a potent telomerase inhibitor, induced MiaPaCa cell growth arrest, senescence, apoptosis and telomere length shortening within 4 weeks, while similar biological effects came out in 8-9 weeks with treatment of other known G-quadruplex interactive agent telomestatin. Our data suggested that the different biological effects of telomestatin and TMPyP4 could be attributed to their selectively for interaction with intramolecular or intermolecular G-quadruplex structures. Recently, a potent telomerase inhibitor BIBR 1532 and a new G-quadruplex interactive agent Sapphyrin have been synthesized and the biological evaluation of these compounds are in progress.

Nafees Ahmad, Ph.D.

University of Arizona
Award Amount FY04: $50,000

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

The molecular mechanisms of HIV-1 pathogenesis and disease progression in infected infants are not known, making it difficult to define better and effective strategies for prevention and treatment of HIV-1 infection. Our hypothesis is that there are specific molecular and biological properties of HIV-1 that are critical determinants of HIV-1 pathogenesis and disease progression in infected infants. 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.

In this study, we characterized HIV-LTR sequences from 6 mother-infant pairs following perinatal transmission and determined its biological activity in mammalian cells. The LTR region was PCR amplified from 6 mother-infant pairs' PBMC DNA and cloned into a Gateway vector system that allowed expression of the CAT gene driven by the cloned HIV-1 LTR. Ten to 15 clones from each patient were sequenced and analyzed by using GCG, PAUP, Coalesce and NEIGHBOR programs. We found that there was a low degree of HIV-1 heterogeneity within mothers, within infants and between epidemiologically linked mother-infant pairs. However HIV-1 heterogeneity between epidemiologically unlinked sequences was greater than epidemiologically linked sequences. Analysis of the promoter (TATAA), enhances (three SP-I, two NFkB, two NFAT and two AP-I sites) and the TAR region revealed that these important motifs were generally conserved. Phylogenetic analysis demonstrated that each mother-infant pair's sequences clustered with each other, suggesting that epidemiologically linked sequences are closer to each other than epidemiologically unlinked sequences. We then determined the promoter activity of the mother-infant pairs HIV-1 LTR by transferring these DNAs into HeLa cells and measuring CAT activity in cell lysates. We found that the HIV-1 LTR derived from 6 mother-infant pairs were functional. These results may be helpful in understanding the molecular mechanisms of HIV-1 vertical transmission and pathogenesis.

Dominick DeLuca, Ph.D.

University of Arizona
Award Amount FY04: $70,000

Effect of Nicotine on T cell Development

Over 20% of pregnant patients enrolled in Arizona's pregnancy programs continue to smoke throughout their pregnancy. During the past year, we have shown that human T cell progenitor development in newborn infants is significantly altered by nicotine exposure of the mothers during pregnancy in that 26 infants born to smoking mothers showed a decrease in T cell development compared with 15 infants born to non-smoking mothers. In addition, the degree of inhibition of fetal T cell development was correlated with the degree of nicotine exposure as determined by the amount of nicotine present in the urine of the mothers at the time of delivery. Thus, mothers exposed to relatively little nicotine had infants which were more greatly affected than infants exposed to moderate levels of the drug. At very high doses of nicotine, however, T cell development decreased again. This pattern was also reflected in the ability of T cell progenitors from the mother's own blood to develop into T cells. Interestingly, it was the mature T cells which were inhibited by nicotine exposure. Immature T cells were relatively resistant to nicotine. In addition, previous exposure to nicotine in the mother's body generated T cells which were resistant to additional nicotine added to the cultures used to generate T cells. This result suggests that the receptors for nicotine on T cells might be decreased by nicotine exposure, making them relatively resistant to the drug.

Our initial results in examining the expression of these receptors confirms that they are, indeed, lower in mothers exposed to nicotine. Genes involved with T cell receptor expression are activated by nicotine which may cause cancer. Understanding how nicotine specifically effects lymphocyte development may help us to better appreciate the impact that smoke exposure imposes on the delicate balance of the developing fetal immune system.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY04: $166,250

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. Furthermore, smoking causes a more rapid progression of RA associated lung disease and significant increases in need for clinical intervention. Under previous support of the ADCRC, we found that RA patients have significantly elevated levels of autoantibodies directed against recognition molecules of their own thymus derived lymphocytes(T cell receptor). We have generated monoclonal autoantibodies from RA patients and determined that these are novel recognition molecules that have the potential to modulate the T cell arm of the immune system. Autoantibodies of RA patients are usually of the IgM (immune macroglobulin class), whereas those of SLE patients tend to be of the IgG or major serum immunoglobulin class. Our ability to generate these monoclonal molecules give us the unique opportunity to determine the gene usage in the generation of these in the two autoimmune disease and to study their biological functions in interaction with T cells and in modulating the immune response. It is essential to determine whether these disease-related molecules represent populations correlated with the rheumatic diseases or whether these are essentially the same ones expressed in low levels by healthy individuals in immunmodulation. Our combined molecular and genetic approach offers new possibilities for diagnosis and potential therapy.

Publications:

Adelman MK, Schluter SF, Marchalonis JJ. The natural antibody repertoire of sharks and humans recognizes the potential universe of antigens. The Protein J, 23:103-118, 2003.

Sepulveda RT, Marchalonis JJ, Watson RR. T-cell receptor Vr38.1 peptide reduces coxsackievirus induced cardiopathology in aged mice. Cardiovascular Toxicology, (In press).

Paul F. Torrence, Ph.D.

Northern Arizona University
Award Amount FY04: $49,940

Nucleic Acid Therapeutics for West Nile Virus Infections

Infection by West Nile virus continues to grow as an emerging health problem. In Arizona alone there have been 360 confirmed cases of West Nile virus infection and five deaths as of mid-September of this year. We have employed a nucleic acid-based drug discovery approach to find a lead compound that could be pursued as a potential therapeutic agent for West Nile virus infections. Using West Nile virus genomic RNA sequence information together with known aspects of viral RNA structure and conserved sequence regions, we designed and prepared a series of modified antisense oligonucleotides which were screen in Vero cell culture for antiviral activity against the West Nile virus. Several promising active compounds have resulted from this effort so far. These now provide the basis for further chemical modification to enhance their pharmacodynamic and pharmacokinetic properties.

David L. Sparks, Ph.D.

Institution: Sun Health Research Institute
Award Amount FY 04: $166,250

Water Quality and Cholesterol-Induced Pathology

The objective of this project is to confirm that copper ion found in drinking water exacerbates accumulation of B-amyloid in the brain and that the accumulation can be reversed. Our previous studies have shown that the addition of copper ion to drinking water augments cholesterol induced Alzheimer's like pathology in the brain.

By following an experimental design in an animal model of controlled diet, the interjection of cholesterol, the addition of copper ion to distilled drinking water, or a combination of the three, the data suggest that low levels of copper ion in drinking water could promote Alzheimer's disease by increasing the accumulation of B-amyloid in the affected patients. Normally the brain will clear out excessive B-amyloid as measured by circulating levels of ceruloplasmin. It appears that the combination of copper ion in drinking water and cholesterol in the diet cause the brain to not be able to clear the B-amyloid. Over time Alzheimer's-like plaques build up in the brain.

Additional testing was conducted to see if ceruloplasmin circulation could be reduced by eliminating the copper ions from the diet. While the data tends to support the concept that cholesterol and copper independently and additively influence circulating ceruloplasmin levels, replacing copper supplemented drinking water with distilled water produced little effect on circulating ceruloplasmin levels.

It is a preliminary finding of this study that levels of copper ion below the EPA drinking water standard combined with cholesterol in the diet may result in Alzheimer's like plaque build up in the brain. Further research is needed to understand the activities and confirm the findings.

SECTION D

CONTRACTS

PARKINSON'S DISEASE RESEARCH

YEAR TWO

FY 2003

Jeffrey N. Joyce, Ph.D.

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

Arizona Parkinson's Disease Center

The Arizona Parkinson's Disease Center (APDC) provided an administrative structure that supports the shared mission, productivity, and growth of our consortium of Parkinson's related Institutes.

The key personnel of the APDC were fully integrated into all aspects of the program through twice-monthly meetings of the Clinical Core personnel and yearly meetings of all scientific personnel. The APDC developed enrollment programs and clinically evaluated all individuals who have registered for the Brain Donation Program at the Sun Health Research Institute (SHRI). There have been 821 movement disorders examinations, 380 olfactory tests (UPSIT), 225 electrophysical tests, 500 general neurological examinations, and 650 neuropsychological tests. The Neuropathology Core has performed 56 autopsies on cases with clinical diagnoses (from medical records and the SHRI Center for Clinical Research) of Parkinson's disease, Parkinsonism, or normal control, allowing for clinico-pathological characterization. It also provided tissue for other projects.

There was progress towards elucidating mechanisms of neuroprotection against death of DA neurons afforded by DA agonists, specifically that D3 receptor preferring agonists act through D2 and/or D3 receptors and via brain derived neurotrophic factor (BDNF) dependent pathways to produce protection against the Parkinsonian toxin MPP+.

Progress was made towards determining if the increased frequency of Marinesco bodies and ubiquitinated nuclei in DA neurons of old as compared to young patients and in Diffuse Lewy Body disease can lead to Parkinson's disease. Increased frequency of Marinesco bodies and ubiquitinated nuclei in DA neurons of old patients produces subclinical damage to the nigrostriatal dopamine system.

There was significant progress towards understanding the causes of inflammatory responses and dopamine (DA) neuron attach in Parkinson's disease, providing the first evidence that the neurotransmiter DA, itself, is stimulant for microglial activation and migration.

Publications:

Joyce JN, *et al.* A low dose of pramipexole is neuroprotective in the MPTP mouse model of Parkinson's disease, and acts to downregulate the dopamine transporter via the D3 receptor. **BMC Biology**, 2: 22, 2004.

Presgraves S, *et al.* Neuroprotective effects of S32504 and pramipexole against 1-methyl-4-phenylpyridinium (MPP+) in terminally differentiated SH-SY5Y cells are mediated by the D3 receptor and BDNF dependent pathways. **Experimental Neurology**, (In press).

Presgraves S, Ahmed T, Joyce JN. Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. **Neurotoxicity Research**, 5(8): 579-598, 2004.

Joyce JN, *et al.* Neuroprotective effects of the novel D3/D2 receptor agonist and antiparkinson agent S32504, *in vitro* against 1-methyl-4-phenylpyridinium (MPP+)and *in vivo* against 1-methyl- 4-phenyl 1,2,2,6-tetrahydropyridine (MPTP): a comparison to ropinirole. Exp Neurol, 184(1):393-407, 2003.

Beach TG, *et al.* Substantia Nigra Marinesco Bodies are associated with decreased striatal expression of dopaminergic markers. J Neuropathol Exp Neural, 63(4):329-337, 2004.

Adler CH, *et al.* Motor impairment in normal aging, clinically possible Parkinson's disease, and clinically probable Parkinson's disease: longitudinal evaluation of a cohort of prospective brain donors. Parkinsonism and Related Disorders, 9:103-110, 2002.

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

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

Gene Targeting TNF Receptors Reveals Dopamine Cell Death

In the past two years, we have made significant progress by analyzing cytokine contents in human brains with Parkinson's disease. We found that TNFα is the most abundant of the oxidative stress-stimulated cytokines (compared to IL1b, IL6 and TGF) in the striatum and cortext of PD. These studies provide rationale for our projects. As PD is an age-related disorder and cyorkines may have different roles in fetal neurons as well as adults neurons, we have thus developed an adult neuron culture in our laboratory. First, we have examined the effect of TNFα on adult versus fetal neurons. Interestingly, our results demonstrated that DA neurons (TH-positive) from 6 months old mice are much more vulnerable to TNFα compared to fetal DA neurons. By using techniques of LDH relase, TUNEL staining and DNA fragmentation analysis, we found that TNFα causes the most cell death compared to IL1 and IL6 *in vitro* (6month old), and TNFα-induced DA neuron death is significantly reduced in neurons from TNFRI knockout mice. Thus, this degenerative process is TNFRI dependent. Moreover, we found that numbers of TH-immunoreactive neurons with depletion of estrogen are significantly reduced compared to that in WT. Interestingly, depletion of estrogen b-receptor cause lower survival rate of TH-immunoreactive neurons than that in WT DA neurons.

To examine other PD-related proteins, in addition to the study directly from our ADCRC grant, we have also extended our queries examining whether TNFRI interacts with amyloid protein, complement protein and other receptors etc.

As we study human neurodegenerative diseases, including Parkinson's disease, we need to understand that exactly is happening in the diseased brains. Thus, we have used rapidly autopsied brains with neurological disorders. In the past two years, we have successfully detected enzymatic activity such as beta-secretase in rapidly autopsied brains with PD and AD. These studies provide us solid foundation for further study mechanisms of PD at the molecular and cellular levels.

Publications:

Li R, *et al.* Aβ load is correlated with elevated BACED activity in sporadic Alzheimer patients. Proc Natl Acad Sci, 101(10):3632-3637, 2004.

Li R, *et al.* TNF death receptor signaling cascade is required for amyloid-β-protein induced neuron death. Journal of Neuroscience, 24(7):1760-1771, 2004.

SECTION E

NEW CONTRACT AWARDS

BEGINNING IN FY 2005

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 U.S. In Arizona, there are currently 1,601 children between birth and 18 years of age and 1,818 adults over the age of 18 with either a primary or secondary diagnosis of CP enrolled with the Division of Developmental Disabilities (DDD). During the 2002 fiscal year, DDD paid over $79.3 million in services for these 3,419 individuals for an average individual annual cost of $23,204.

CP results from an injury or insult to the brain that occurs before, during or shortly after birth - at the time when the brain is in its most rapid development. This damage leads to severe neurological deficits, motoric and otherwise. Whereas this injury or insult is non-progressive, the effects from the motor deficits generally worsen with time. The results may be quite severe. Unfortunately, despite impressive progress in other medical fields, current standards of treatment for children with CP in the U.S. are not producing the results that patients, families and health care providers hope for. Dissatisfaction with outcomes has prompted on-going efforts to identify and evaluate new therapies or combination of therapies that would optimize the long-term course of CP.

One such effort that warrants careful attention is the Chinese comprehensive approach to CP. In China, the standard package of care for CP is administered early and intensely. It consists of an intense 12-week program of the same physical and occupational therapies that are administered in the U.S. plus acupuncture. In the Chinese medical literature, both practitioners of modern medicine and of traditional Chinese medicine have documented that, as a result of using an intense level of this comprehensive approach to care, function is improved and that many serious complications are, to a large extent, averted. These reports, however, have not been subjected to vigorous scientific scrutiny. Hence, our overarching goal for this study is to use rigorous scientific methods to systematically evaluate the effectiveness of this intense and comprehensive approach to the care of children with the most common form of CP, spastic CP.

Toward that end, we have established a strong international alliance with one of the most reputable medical institutes in China. This alliance builds upon the strength of the respective but different expertise of the Chinese and the U.S. partners. Thus, the study will be conducted at two locations: a) the Beijing Children's Hospital, where participants' recruitment, intervention therapies, videotaped evaluation, and data collection will be done; and b) the University of Arizona, where scoring of the videotape evaluations and data analyses will be done and from where logistic support will be provided.

For the study itself, we propose to conduct a prospective clinical trial that will compare the results of two distinct packages of care in children, 1-6 years of age, with spastic CP. Based on chance randomization, half of the children (group 1) will be given acupuncture at the same time as intense conventional therapies (physical, occupational, and hydro-therapies) right from the outset of the study. The other half

(group 2) will be given first the intense conventional therapies, followed, after a 12-week delay, by the acupuncture component. Results will be assessed at six different time points during and after treatment is given. To avoid bias in the assessment, all the evaluations will be blind, meaning that the evaluator will not know to which of the two groups the children belong. Use of state-of-the-art Web-based technology will further ensure objectivity and trustworthiness of data.

Two hypotheses underlie this study. First, that adjunctive acupuncture administered concurrently with intense conventional therapies will improve the gross and fine motor function and the health related quality of life of children with spastic CP more than intense conventional therapies alone. Second, that when compared three months after termination of therapy, the level of gross and fine motor function and health related quality of life issues achieved with acupuncture administered concurrently with intense conventional therapies will be higher than those achieved with intense conventional therapies alone.

We are pleased that the state of Arizona, through this funding mechanism, has taken a leadership role in providing initial funds for this important and innovative study that promises to have a significant impact on healthcare policy and practice as related to CP at the national level. If our hypotheses are confirmed, this study offers the prospect of improving the lives of children with CP, of preventing many of the complications that accompany CP, and of giving these children a greater opportunity to realize their individual potential.

Robert P. Erickson, M.D.

University of Arizona
Award Amount FY05: $131,535

Identification of Genes Involved in Lymphedema by Single Nucleotide Polymorphism Mapping

Lymphedema is the accumulation of tissue liquid (lymph) in the limbs or other parts of the body, e.g. the genitalia, due to a defective lymph drainage system. In Arizona, the most common cause of lymphedema is surgery and radiation therapy afterwards, which is performed for breast cancer that involves lymph node removal and frequently results in lymphedema of the operated arm or arms. Lymphedema can be disfiguring, disabling, and emotionally scarring and is at risk for repeated infection and delayed occurrence of aggressive malignancies. Lymphedema can also occur as a genetically inherited disorder. Our group has a long standing interest and has published numerous articles on lymphedema and the genetics of lymphedema. Several Arizona-based families have been pivotal in the progress made to date. The identification of the genes involved in hereditary forms of lymphedema may help explain the more common kinds of lymphedema. For instance, only some women (~15-30%) develop lymphedema after breast cancer surgery and these may be more susceptible because of their genetic makeup. Thus, if it was possible to identify genes influencing the causation of lymphedema, variation in those genes might predict who is most likely to develop lymphedema after breast cancer surgery. A less destructive operation, or perhaps preventive methods, could then be targeted at those individuals. Since cancer frequently spreads (metastasizes) through lymphatics, genes involved in lymphatic growth are also possible targets for cancer prevention and treatment.

To date, only three (of the many) genes involved in human lymphedema syndromes have been identified. The Arizona lymphedema families offer opportunities to identify more such genes with the new methods of the proposed research. We have previously identified 3 chromosomal regions which appear to contain genes involved in susceptibility to lymphedema. The establishment of the Translational Genomics Research Institute (TGen) in Phoenix provides new collaborators and powerful new techniques to extend our previous research. Thus, in collaboration with TGen, we will use new, more rapid and precise technologies for detecting DNA variation to refine our search and ultimately identify single genes that would allow early detection of the condition as well as new approaches to gene/protein therapy.

Brent D. Gendleman

5am Solutions
Award Amount FY05: $100,000

The System for Collaborative Translational Research

The absence of a standardized platform for geographically disparate, multi-institutional individuals and organizations in Arizona who want to collaborate to perform translational research presents a tremendous impediment to their success. The translational research initiatives underway and those being planned will be significantly hampered in their effectiveness without a platform that can support efficient organization, collection, storage, analysis, monitoring, integration and sharing of clinical and genomic research data and results. The number of participating institutions, private clinicians and patients will be far less than optimal if there is no collaborative initiative and will negatively affect the ability to further clinical advances and acquire grants for Arizona, both in number and value. The net affect is that the promise of translation research to improve the health of Arizonans will be compromised.

Specifically, the lack of such a platform collaborators to:

- use paper-based forms that require significant discipline and lead to redundant key entry, data loss and physical storage issues
- be compromised by the inability to quickly and conveniently acquire, share and explore vital clinical, pathological, molecular, and genomic data
- lose interested collaborators who lack the personnel, logistical and computational resources to separately capture data for patient care and research protocols
- design and build stove-piped and typically incompatible data entry systems in accordance with 21 CFR guidelines governing electronic data capture
- give their "word" to patients and institutions who have confidentiality fears that relate to HIPAA compliance governing the privacy of patient data.

Presently, there are no complete systems commercially available today that can tackle the problem presented, though parallel efforts are underway addressing a subset of the problem. The opportunity exists to not only build a complete working system that solves Arizona's infrastructure issues for efficient execution of translational research, but serve as a model for national and international adoption and the creation of a new product vein for Arizona's biotechnology growth.

The primary goal is to build an internet-based platform to provide the infrastructure and tools for greater collaboration between Arizona institutions, doctors, and researchers to affect the diagnosis and treatment of their patients. This platform will provide the means to securely connect a patient or subject to a larger quantity of relevant data and influence the workflow and ease the procurement of medical and research results. We are bringing the platform to Arizona that is currently serving researchers, clinicians and patients at the Hospital for Special Surgery in New York City, where they are successfully running 10 longitudinal studies, are tracking 1200 patients and are collaborating with 50 distinct users with no defects since the software deployment in 2002. We are targeting a pilot Alzheimer study involving Translational Genomic Research Institute and the Banner Good Samaritan Medical Center who will need to work with roughly 5,000 patient samples. Access to, and active participation by, the pre-eminent expertise in the clinical and genomic fields of translational research in Arizona ensure that the platform and its extensions will meet the needs of the entire community. The talent, collective infrastructure

resources, and complimentary missions of 5AM and TGen and Banner are the building blocks of a statewide system with the following objectives:

- Facilitate participation by securely centralizing data storage and presentation while minimizing the local and individual technology costs and integration.
- Provide oversight for data capture, authentication and accurate reporting.
- Adapt data-entry to the most workflow sensitive format at the location of acquisition.
- Build an extensible system that will allow for both functional improvements and expand the user community over time.
- Protect the safety and identity of research subjects without compromising effective participation.
- Make Sample/Tissue data public for data mining purposes.
- Use the pilot study to create a process for study submission for IRB Approval.

Eric J. Guilbeau, M.S., Ph.D.

Arizona State University
Award Amount FY05: $50,000

Biosensor for Measurement of Breath Acetone

We plan to develop a novel, inexpensive and easy to use sensor that can be used by individuals with diabetes to monitor their breath acetone concentration. Acetone is a common solvent. It is often used to clean the polish off of women's fingernails. 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). DKA occurs in diabetics when there is not enough insulin and blood glucose levels are high. Under these conditions high amounts of acetone are released into the blood stream and unless corrected, the individual may die. There are larger numbers of individuals with type 2 diabetes in Arizona than in other states in the U. S. because of Arizona's large Native American Indian and Hispanic populations. These racial groups are more susceptible to diabetes than the rest of the population. Additionally, the incidence of diabetes is increasing because of the national obesity epidemic and the aging of the general population. If successful in developing and characterizing the proposed breath acetone sensor, it might be feasible to manufacture a medical device that could be used in the home for daily testing of breath acetone levels so that individuals with diabetes would know whether a hospital visit is warranted. The device might also be used to monitor the burning of fat during diets and increase compliance with prescribed diet regimes.

Our goal is to develop the sensor and define its operational characteristics in gas mixtures that contain quantities of acetone known to be present in patients with diabetic ketoacidosis. The gas mixtures will also contain substances in the breath of individuals suffering from diabetic ketoacidosis that might produce a component of the sensor signal that is not attributable to acetone.

Our hypothesis is that antimony (Sb) - bismuth (Bi) thin film thermopiles have the necessary sensitivity to measure the heat of reaction that is generated when acetone reacts with an appropriately selected chemical reactant that has been immobilized on the measuring junctions of the thermopile. The resulting thermopile electrical signal (emf) is proportional to the amount of acetone in the gas stream contacting the thermopile. To answer this question and prove our hypothesis, we plan to perform a series of experiments guided by the following set of specific aims:

To achieve our goal and prove our hypothesis, seven specific aims are described in the proposal. We plan to a) develop a mathematical model of the proposed acetone biosensor embodiment, b) fabricate thin film antimony-bismuth thermopiles that will be used as the primary sensing element of the acetone biosensor, c) after assembly of the sensor, we will measure the thermopiles electrical characteristics, d) identify and e) select chemical reagent candidates for immobilization on the measuring junction of the thermopile, and f) measure the response of the acetone biosensor when the biosensor is exposed to stagnant and flowing gas mixtures that contain acetone concentrations characteristic of breath acetone levels in patients with diabetic ketoacidosis with and without interfering substances.

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

Among the leading world health problems, cancer and infectious diseases remain the two primary causes of human morbidity, mortality and suffering. Cancer, a disease especially of the elderly and a major killer in the U.S., was responsible for 9,700 deaths and 20,300 newly diagnosed cases in Arizona in 2003. Though the number of deaths from infectious diseases in the U.S. is currently on the decline, it has shown a resurgence in troublesome areas particularly associated with the spread of AIDS. Opportunistic infections especially in patients whose immune systems are compromised by AIDS, cancer, diabetes, age and other causes are especially susceptible.. Incidence and deaths due to cancer and infectious diseases are on the rise in Arizona as many elderly continue to move here due to the desirable climate.

Cancer treatment relies on surgery, radiation and chemotherapy. Chemotherapy is the only possible treatment of a disseminated cancer and for infectious diseases including AIDS. Unfortunately, there are no good chemotherapies available to treat major solid tumors, AIDS, and infectious diseases associated with cancer and AIDS. There is thus an urgent need for the discovery of new, effective and non-toxic anticancer and anti-infective drugs to treat these life-threatening diseases.

The majority of anticancer and anti-infective drugs in use today are from natural sources. Our preliminary studies and work by others have shown that endophytic fungi, fungi that live in the intercellular spaces of plants, to be a rich source of bioactive molecules. The overall goal of this inter-institutional and multidisciplinary project is to discover novel anticancer and anti-infective (anti-HIV, antibacterial and anti-fungal) drugs from endophytic fungi of desert plants with the broad long term objective of creating a library of this unexploited source of natural products for this and future drug discovery programs.

The proposed research will lead to the testing of our hypothesis that endophytic fungi of plants growing under semi-extreme desert conditions will produce secondary metabolites with interesting bioactivity profiles and novel chemical structures similar to other extremorphiles.

We are hopeful that having access to this unique source of bioactive natural products, combined with the use of appropriate bioassays, will lead to the discovery of anticancer and anti-infective agents with novel mechanisms of action which can be developed into clinically effective anticancer and anti-infective drugs. We are also hopeful that with the preliminary data generated in this project we will be able to attract funding from National Institutes of Health for continuation and expansion of the project.

Cherry L. Herald, Ph.D.

Arizona State University
Award Amount FY05: $164,500

Preclinical Development of Three Anticancer Drugs

Human cancer continues to be associated with a high incidence of mortality in the United States. Of the nearly 600,000 annual deaths from cancer, over 200,000 are related to tobacco use. It is estimated that about 48% of all cancer patients can now be treated and subsequently be considered disease free. The need for additional curative anticancer drugs continues to be a high priority in this country and worldwide. Among the 200 or more related diseases that are included under the diagnosis, cancer, head and neck cancer will be the focus of this request for ADCRC research funding to advance the treatment of tobacco related disease through drug discovery and development. The incidence of head and neck cancer has been increasing in the U.S. and accounts for 3% of all cancers. Some 85% of all head and neck cancer are related to the use of tobacco.

The Arizona State University Cancer Research Institute (ASU-CRI) is completely committed to the discovery and development of new, effective anticancer drugs for human treatment. Specifically, for head and neck cancer, the currently available anticancer drug treatments have achieved responses in approximately 34% of the cases with only some 8% of patients experiencing a complete response. Based on the ASU-CRI anticancer and vascular targeting agent, combretastatin A-4 phosphate, now in advanced clinical trials for anaplastic thyroid and head and neck cancers, we have synthesized new modifications with promising anticancer activity. Three of them, tyrostatin prodrug and stilstatins 2 and 3, require synthesis of sufficient quantities for continued early preclinical development towards eventual human clinical trials. The preliminary evidence obtained with the three drugs indicates that one or more of these lead compounds could prove to be quite useful in the treatment of head and neck cancer.

Steven A. Hoffman, Ph.D.

Arizona State University
Award Amount FY05: $50,000

Autoantibodies in CNS Lupus

Autoimmune diseases and mental disorders are a major burden on both the individual and the health system. It is also a particular problem for an aging population. The elderly are a significant part of the population of Arizona and the high levels of sunshine are related to the exacerbation of autoimmune diseases like systemic lupus erythematosus (SLE). In general, the proposed studies would test one hypothesis of how the immune system can affect the brain and lead to neurologic and mental disorders such as those seen in autoimmune diseases like SLE, contributing to a better understanding of disorders like Alzheimer, multiple sclerosis (MS) and AIDS. To date the mechanisms by which the immune system can contribute to mental disorders (often incorrectly assumed to be due simply to vascular damage) are largely unknown. There is accumulating evidence in the research literature that mental disorders like schizophrenia and autism have an autoimmune component that contributes to brain dysfunction. It has been too long without this autoantibody hypothesis being adequately tested; the methods and knowledge are available to do this. The current proposal will characterize and identify the specific reactivity of the type of autoantibodies found in CNS lupus.

To date there is no understanding of how mental disorders such as those seen in schizophrenia, autism, and SLE can arise. Patients with SLE often manifest symptoms of central nervous system dysfunction (CNS-SLE) including the mental disorders of emotional dysfunction, cognitive impairment and psychoses (including schizophreniform behavior). The proposed studies are designed to lay the foundations for investigating how the immune system can affect brain functioning, contributing to mental disorders. In this project we will focus on the role of autoantibodies in contributing to mental disorders. These studies will move us a long way in better understanding the pathogenesis of the mental disorders associated with autoimmune disease. This will provide new means of diagnosing and treating autoimmune-related mental disorders such as CNS-SLE.

The primary focus of this project will be to characterize the autoantibodies reactive with brain in an animal model of CNS-SLE. We will use phage-displayed cDNA libraries in combination with the hybridoma technology to identify the specific proteins to which these autoantibodies bind. This is essential for understanding how antibodies can alter normal neuronal functioning leading to neurobehavioral changes. In this project, we will focus on the role of autoantibodies reactive with components of brain. The autoantibody hypothesis to be tested states that a subset of brain reactive autoantibodies contribute to some of the mental disorders seen in SLE and may play a role in other mental disorders. This hypothesis has not been adequately tested although there is a good deal of suggestive evidence. The methods and knowledge, using a combination of immunologic, neurobehavioral and genetic techniques, are available to do this. The current proposal will lay important groundwork for rigorous testing of this hypothesis. Neurobehavioral testing (including neurological measures, motor activity, tests of affect, and memory and learning) of autoimmune mice will study the connection of immune processes to brain function. Correlations will be made between both brain reactive autoantibodies, as determined by ELISA and immunoblot, and the behavior. More importantly, a library of monoclonal autoantibodies, obtained from mice with CNS involvement will be generated and tested using a phage-displayed, cDNA library of mouse brain. The monoclonal antibodies will be characterized

by immunoblotting and immunohistochemistry. Phage-displayed, cDNA libraries of mouse brain will be used to identify the proteins to which the monoclonal antibodies bind. The above information, and knowledge of the areas within brain to which the autoantibodies bind, would provide an understanding of what molecular functions of cells are likely to be affected, as well as what psychological and behavioral manifestations to expect. The monoclonal antibodies can be used in future studies to reproduce the behavioral manifestations seen, allowing a causal connection to be made between autoantibodies and their functional effects. These studies will move us a long way in better understanding the pathogenesis of neuropsychiatric involvement in autoimmune diseases, providing new avenues for investigating specific diagnoses and treatments of mental dysfunction, as well as elucidating some of the links between the brain and immune system.

Laurence Hurley, Pharm.D.

University of Arizona
Award Amount FY05: 49,940

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.

The PDGF signaling pathway has been demonstrated to provide a growth advantage in pancreatic cancer. We have identified a new paradigm for control of oncogene expression involving secondary DNA structures and DNA remodeling proteins. In the case ofPDGF-A, a complex mechanism using a nuclease hyper-sensitivity element (NHE) together with a silencing element (SHS) and NM23-Hl is involved in control of gene expression. In this proposal we identify three specific aims:

1. to determine the structure of the molecular complex between the NHE in the promoter region of PDGF-A and the 5 -SHS element,
2. to determine the mechanism for remodeling of the silencer element complex by NM23-Hl, and
3. to determine the effect of drugs on this process.

With results from these studies, we will prepare an NIH grant with the objective to provide a new class of drugs to treat pancreatic cancer that will elevate the standard of care for chemotherapy in advanced pancreatic cancer by increasing the longevity and quality of life of patients in Arizona.

Tamara King, Ph.D.

University of Arizona
Award Amount FY05: $164,500

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

The spread of cancer to the bone is the most common cause of pain in patients with malignant tumors. Prostate cancer is one of the most common causes of pain from malignant bone disease. Skeletal metastases are present in 60-90% of men who die from prostate carcinomas. In 2003, the American Cancer Society estimated that 4,300 new cases of prostate cancer would be diagnosed among men in Arizona and that 600 men would die from prostate cancer in Arizona. Metastasis of cancer to bone is a catastrophic event in the life of the patient and the major factor which affects quality of life. Cancer pain is intense, often unremitting and requires treatment with strong analgesics such as opiates. However, treatment with opiates is associated with severe side effects, including heavy sedation and mental impairment that interfere with the patient's ability to interact with family and friends. For these reasons, non-sedative alternatives to the use of opiates for strong pain relief in cancer pain is highly desirable. In order to develop effective treatment for pain management, it is necessary to understand the mechanisms underlying cancer-induced pain. This proposal uses a new mouse model of bone cancer in which human prostate cancer cells are injected and sealed into the femur. This model allows localized analysis of changes in the bone and in the pain pathways that underlie cancer-induced pain. In addition, this proposal will examine the ability of two analgesic drugs, a COX-2 inhibitor (Vioxx) and nicotine, to eliminate cancer induced pain without the sedation and other side effects associated with opiates. This proposal will also examine a novel drug designed to prevent tumor growth and mobility for its ability to prevent tumor growth, bone destruction, and cancer-induced pain when injected into the bone with the human prostate cancer cells. These studies will yield significant new information that may lead to novel treatment approaches for human bone cancer and pain associated with these cancers, and anticipated discoveries are likely to be of great benefit to the citizens of Arizona.

We plan to examine the behavioral and neurobiological changes induced by human prostate cancer cells using a novel bone cancer model in mice. The use of these human cancer cells allows comparisons of pain expression and mechanism, bone biology and neurobiological changes induced by osteolytic PC3N or mixed osteolytic/ osteoblastic LNCaP types of prostate cancer cells, thus modeling many features relevant to the human disease. Our goal is to compare the effectiveness of nicotine and a COX-2 inhibitor, two non-opioid analgesic drugs, and a novel peptide which may prevent tumor adhesion and growth on behavioral measures of cancer pain, tumor proliferation, and neurochemical changes within the spinal cord and cell bodies of primary afferent nociceptive fibers in the dorsal root ganglia. Aim I evaluates the effects of the 2 cell lines on spontaneous and evoked pain behaviors, bone density and tumor growth. We hypothesize that the expression of spontaneous or evoked cancer pain and the consequences on bone density and time-course to bone fracture will emerge at different times after injection. Aim 2 characterizes the associated neurobiological changes induced by the PC3N and LNCaP cells in the dorsal horn of the spinal cord and dorsal root ganglia across time. We hypothesize that these cancer cells lines, known to have different effects on bone structure and density, will produce a different molecular signature of pain as reflected by the phenotypic features in the dorsal root ganglia and the spinal dorsal horn. Aim 3 evaluates the effects of COX-2 inhibitors or nicotine on the expression of bone cancer pain, tumor proliferation, and bone density in osteolytic and mixed osteolytic/ostesoblastic prostate cancers. We hypothesize that administration of COX-2 inhibitors and nicotine will inhibit the pain of prostate bone

cancer and tumor proliferation without producing sedative actions. Aim 4 determines whether reducing tumor adhesion and growth diminishes cancer growth and cancer-induced pain using HYD-1, a D-amino acid containing peptide which blocks adhesion of human prostate tumor cells to the extracellular bone matrix. We hypothesize that blocking tumor adhesion reduces proliferation and migration of the cancer cells within the bone, lowers PSA levels, and diminishes changes in bone density, cancer induced pain, and changes in neurobiological phenotype.

Douglas F. Lake, Ph.D.

University of Arizona
Award Amount FY05: $39,600

Dendritic Cells and Immunity to Valley Fever

Coccidioidomycosis, known commonly as Valley Fever, is caused by the dimorphic fungus *Coccidioides immitis*. C. *immitis* is, perhaps, the most virulent fungus known to infect humans. As such, C. *immitis* is the only fungus among over 50 other organisms listed as a potential bioterrorism agent. Coccidioidomycosis is known to be endemic in Arizona and is also a costly healthcare problem for Arizona, as prolonged hospital stays are often required for some cases of disseminated coccidioidomycosis. Even in less severe cases, loss of work and productivity is economically significant. Importantly, the geographic epicenter of Coccidioides spp. coincides with the major population centers in Arizona. Unfortunately, very little is known about the initial immune response to C. *immitis*. Specifically, it is not known why some individuals respond to the fungus with protective immunity while others fail to mount a protective response. Approximately 60% of those infected are completely asymptomatic after infection and their only evidence of infection is expression of a specific cell-mediated immune response to coccidioidal antigen. The remainder develop a self-limited pulmonary illness which usually resolves with the expression of specific cell-mediated immunity. However, approximately 1- 5% of all individuals who are infected develop chronic disease, either persistent pulmonary disease or infection disseminated beyond the lung. Disseminated disease commonly presents as bone and joint, skin and soft tissue, or meningeal involvement. A better understanding of the mechanisms of non-responsiveness in human coccidioidomycosis will lead to improved therapy for patients with severe and disseminated disease, for which prolonged and expensive antifungal therapy is the current rule. Moreover, the immune response to human coccidioidomycosis is likely to be related to other granulomatous diseases such as tuberculosis, leprosy and leishmaniasis. Therefore, our work on immunity to C. *immitis* may have a significant impact on other diseases.

The overall objective of the research proposed in this application is to evaluate recombinant antigens derived from C. *immitis*-specific in the human dendritic cell (DC) system, with the goal of validating these antigens for immunotherapy of patients with disseminated disease. We have previously demonstrated that DC loaded with C. *immitis* antigens are capable of stimulating T cells from healthy individuals and patients with disseminated coccidioidomycosis. We hypothesize that DC pulsed with defined recombinant antigens derived from the Coccidioides spherule (in the presence of adjuvant) will elicit a protective TH1 cellular response and reverse coccidioidal non-responsiveness in lymphocytes from patients with disseminated coccidioidomycosis. We propose to compare cell surface phenotype, function and cytokine profiles of DC pulsed with C. *immitis*-derived lysate and recombinant antigens in the

presence and absence of adjuvant from healthy non-immune donors and immune patients (recovered from infection). To address this we will demonstrate that DC-pulsed with recombinant antigens show similar phenotypes as DC loaded with lysate. We will also evaluate phenotype and cytokine profiles of T cells (isolated from healthy and immune donors) responding to DC pulsed with C. *immitis* lysate or recombinant antigens. Lastly, we propose to evaluate the ability of DC loaded with *Coccidioides* recombinant antigens, that induce a protective THI cellular response in healthy individuals, to activate non-responsive T cells from patients with disseminated coccidioidomycosis.

Dianne Lorton, Ph.D.

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

Sodium Narcistatin and Treatment of Rheumatoid Arthritis

Amaryllidaceae alkaloids, constituents from the bulb of the tropical spider lily plant (Hymenocallis littoralis; Amaryllidaceae), have anti-cancer and anti-viral activities. Potent anti-cancer properties have been reported for the Amaryllidaceae alkaloid, narciclasine. An efficient procedure was found for the synthetic synthesis of biologically active narciclasine and a water-soluble derivative that retains its anticancer properties called sodium narcistatin. In addition to Amaryllidaceae alkaloids having anti-cancer properties, Mikarni and co-workers have reported that narciclasine significantly reduced disease severity in an adjuvant-induced arthritis (AA) rat model for rheumatoid arthritis (RA). Similarly, in preliminary studies, we have found that sodium narcistatin dramatically reduced paw swelling (a measure of inflammation) and joint destruction in rats with AA. The dramatic effects in preventing joint destruction and reduced inflammation after sodium narcistatin treatment suggests that sodium narcistatin could be developed as a treatment for RA. These findings also suggest that Amaryllidaceae alkaloids have a modulating activity against inflammatory responses. This notion is supported by studies indicating the narciclasine inhibits lipopolysaccharide (LPS)- or bacteria-induced production of tumor necrosis factor (TNF)-α. by macrophages. TNF-α. is recognized as a pivotal cytokine that regulates inflammation and has a major role in disease pathology in RA. Inhibition of macrophage TNF-α. production could explain the decrease in disease severity observed after treatment with narciclasine as dramatic effects in reducing inflammation and joint destruction after treatment with anti-TNF-α. therapies have been observed in collagen-induced arthritis in mice, transgenic mice that over-express TNF-α., and RA patients. Current treatments for RA do not adequately prevent or halt the joint destruction that leads to joint deformities and disability. Therapeutic results with the current gold standard anti-rheumatic treatment, methotrexate, have been disappointing. Recently, the FDA approved two anti-TNF-α therapies for the treatment of RA. While these treatments have been reported to have dramatic effects in a fair number of patients, some patients show no benefit from the treatment. Additionally, these therapies have several disadvantages. They are extremely expensive, and they are administered either by infusion or by injections, not orally. Recent reports indicate there may be serious complications with anti-TNF-α treatments. Current anti-TNF-α. therapies also are not a cure. They remove TNF-α. from the joints and circulation, they do not reduce TNF-α production; thus, if patients are taken off the drug, circulating TNF-α levels again rise and the disease process progresses. Additionally, this treatment reduces anti-inflammatory production leaving patients with less ability to dampen effects of pro-inflammatory cytokines still being produced after treatment is terminated. Research into alternative approaches must

continue. Sodium narcistatin could potentially be developed into an alternative therapeutic that has numerous advantages over current treatments of RA. Since two of the most concentrated geriatric communities in the world are in the state of Arizona, a significant proportion of this population has RA. This represents a significant health problem for many Arizona residents.

The goals of this research are 1) to explore whether Amaryllidaceae alkaloids can be used as therapeutics for treatment of RA to prevent joint destruction and 2) to determine whether these compounds alter joint destruction by reducing pro-inflammatory cytokine production, increasing anti-inflammatory cytokine production and/or reduce macrophage populations. The proposed studies will be completed in a well-established animal model frequently used to test efficacy of drugs in treating RA, AA in Lewis rats.

Emmanuelle J. Meuillet, Ph.D.

University of Arizona
Award Amount FY05: $164,500

Novel inhibitors of Akt as Anticancer Drugs

A defining feature of cancer cells is their ability to survive under adverse conditions where normal cells will die through a process of programmed cell death. This characteristic allows cancer cells to thrive where normal cells cannot and also makes cancer cells resistant to cell killing by chemotherapy. Inhibiting the signaling pathways that promote cancer cell survival offers a rational and attractive way of selectively inhibiting cancer growth. The protein Akt is a key player in this cell survival signaling pathways in cancer cells. It, therefore, 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. The goal of the work is to identify a lead compound for development as a cancer drug.

The hypothesis upon which our work is based is that the PH domain of Akt provides a novel target for the development of drugs to selectively inhibit cancer growth and to increase the activity of existing anticancer drugs. This double activity makes Akt particularly attractive as a cancer drug target. In the previous grant period, we have obtained and published proof-of-principle studies that inhibitors of Akt PH domain prevent Akt activation and have antitumor activity. These compounds are pharmacological probes and do not have drug-like properties. Thus, we have used the X-ray crystal structure of the PH domain of Akt to perform *in vitro* screening of a library of over 200,000 compounds and have identified and tested a lead compound with high Akt PH domain binding affinity. Using principles of rational drug design and an iterative process of chemical synthesis, testing, and modeling, we will develop analogues with increased potency, selectivity, and drug-like properties and test them as potential antitumor agents. The goals of the study are to obtain data that will allow us to obtain national funding for preclinical and clinical development of this novel class of Akt inhibitors as antitumor agents. The objectives of our work are (1) to use molecular modeling to rationally design small molecule inhibitors of the PH domain of Akt based on a lead compound we have already identified. The modeling will be optimized by an iterative process involving information obtained from objectives 3 and 4 below; (2) to chemically synthesize analogues of the Akt PH domain inhibitor having improved potency, selectivity and drug like properties; (3) to conduct enzymatic and cancer cell survival studies to confirm the biological activity and selectivity

of the compounds synthesized in objective 2 above, and finally (4) through an iterative process involving objectives 1, 2, and 3, to produce lead compounds that will be subjected to *in vivo* testing as potential anticancer drugs. The studies will be carried out by an interdisciplinary research team with expertise in protein structure, computer-assisted protein modeling, chemical synthesis, enzyme biochemistry, and cancer biology. This team has previously worked together and is responsible for much of the current knowledge about the role of Akt and the impact of the inhibition for such survival pathway. The goal of our studies is to identify an Akt inhibitor as a candidate for clinical development as an anticancer drug.

Marek J. Romanowski, Ph.D.

University of Arizona
Award Amount FY05: $50,000

Contrast Agents for Optical Coherence Tomography

Optical coherence tomography (OCT) is a biomedical imaging technique that allows nondestructive visualization of living tissues to a depth of approximately 2 mm. Applications of this imaging modality encompass a surprisingly broad range of pathologies including arteriosclerosis, the leading cause of death in the United States; macular degeneration, the leading cause of blindness in people over 55; and many carcinomas such as that of colon, ovary and skin. Beyond its potential as a diagnostic tool, OCT provides researchers with a unique capability to monitor the *in vivo* development of diseases in animal models.

OCT uses near-infrared light backscattered from the tissue to create cross-sectional images, similar to the way ultrasound uses backscattered acoustic waves. While OCT images have proven to be very useful, they are low-contrast, noisy, and can be difficult to interpret. A general or selective contrast agent would be useful to detect subtle changes and better differentiate tissue types with similar scattering properties. OCT contrast agents have the potential to revolutionize this fledging imaging modality much as microbubbles have opened new diagnostic avenues for ultrasound or gadolinium has increased opportunities for magnetic resonance imaging. In addition to the improved visualization of pathologic changes, one can contemplate the attachment of disease-specific targeting molecules to enable precise localization of malignancy, or the use of such contrast agents for controlled delivery of therapeutic agents. Recent experiments indicate that certain metallic nanostructures, *i.e.*, structures smaller than 100 nm, can provide contrast in OCT. This contrast arises from optical resonances in nanoparticles of metal, a phenomenon already known for the intense red color of colloidal suspensions of gold. Only recently however, metallic nanostructures in a variety of shapes and sizes have become available, where optical resonances can be tuned to cover large regions of electromagnetic spectrum including the near infrared operating range of OCT. This discovery opens new possibilities for the design of OCT contrast agents for biological and medical applications.

Skin cancer research and noninvasive diagnosis of skin lesions exemplify the need for contrast enhanced OCT imaging. Incidence of skin cancer in Arizona is higher than in any other state. Arizonans are three to seven times more likely to develop non-melanoma skin cancer, and they develop melanomas twice as often as residents of other states. When applied to skin, the penetration depth of OCT covers epidermis and dermis making it an appropriate tool for observation of areas affected by early stages of skin cancer in either animal models or in a human subject. Unfortunately, conventional OCT lacks subcellular resolution, and thus cannot directly determine the degree of cellular atypia. While excisional biopsy

remains the standard for skin cancer diagnosis, OCT with a contrast agent may be capable of noninvasive identification of pre-malignant changes in the tissue.

The overall goal of this bioengineering project is to develop a novel class of biocompatible contrast agents for OCT. These agents will be fabricated using the idea of self-assembly, *i.e.*, a non-covalent association of individual molecules that yields a construct of predefined size, shape and optical properties. To accomplish the overall goal of this project, dense arrays of gold nanoparticles will be formed on a self-assembled biocompatible template, such as the liposome. The research plan is designed to test the following hypotheses:

1. Assemblies of biological molecules (e.g., liposomes) can support dense arrays of gold nanoparticles.
2. Optical properties (absorption and scattering) of this construct can be tuned within the near-infrared part of electromagnetic spectrum.
3. These agents improve quality of OCT images obtained in animal models of cancer while administered at safe doses.

This new class of contrast agents will enable researchers to develop insight into a wide variety of diseases, including skin and other cancers, and will potentially have significant impact on development of diagnostic and treatment modalities.

Joyce A. Schroeder, Ph.D.

University of Arizona
Award Amount FY05: $49,718

Molecular Therapeutic 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. We propose the development of a targeted anti-cancer therapy that inhibits the growth of metastatic cancer cells while having no effect on normal tissue. Specifically, the tumor antigen MUC 1 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 propose to utilize this discovery to create a molecularly targeted therapy that would specifically target metastasis and prevent cancer progression.

We hypothesize that by blocking the interaction between MUC1 and β-catenin, we can prevent metastatic invasion in breast cancer. To block this interaction, we propose the following experiments:

I. To determine if MUC1/j3-catenin interactions are essential to cellular invasion, we will design and test MUC 1-mimetic peptides and assay for the ability to inhibit cellular invasion by blocking MUC 1/β-catenin interactions. This aim will allow us to test small proteins (mimetic peptides) for their ability to inhibit the MUC1/β-catenin interaction, thereby preventing MUC1 and β-catenin from promoting invasion(metastasis).We will perform these experiments on human breast cancer cells growing in the laboratory.

II. We will utilize established invasion-inhibiting MUC1-mimetic peptides to treat MMTV-pyMT transgenic mice, a model of invasive breast cancer. We will then analyze the effects of peptide treatment on spontaneous tumor growth and metastasis formation. This aim will use mouse models in preclinical trials to determine how our inhibitory peptides will affect spontaneously occurring tumors. Utilizing the preclinical mouse model is an important step that will allow us to determine if our inhibitory peptides work in a whole-animal system before moving our therapy into human patients.

David J. Segal, Ph.D.

University of Arizona
Award Amount FY05: $44,671

Protein Interactions Mediated by Cys2-His2 Zinc Finger Domains

Protein interactions underlie many important cellular processes such as signal transduction and gene regulation. Dysfunction of these processes has been linked to a variety of diseases including developmental defects and cancer. A better understanding of how protein interactions occur might provide insights into the etiology of diseases such as cancer, as well as suggest candidate drug targets for therapeutic intervention. Cys2-His2 zinc finger domains constitute the most common protein fold in the human genome with over 4,500 examples identified. Proteins containing these domains typically contain multiple repeats of the domain (up to 37 copies), each differing slightly in sequence composition. Zinc fingers are best known for mediating protein-nucleic acid interactions, but usually only 3-4 domains are involved in DNA or RNA recognition. We hypothesize that many non-nucleic-acid-binding zinc finger domains will mediate protein interactions. Several limited studies from other groups support our hypothesis..

The immediate goal of this proposal is to develop assays that can identify protein-interacting zinc finger domains and their binding partners. Future studies will apply these assays to examine uncharacterized zinc finger domains, as well as investigate their structural basis of protein recognition. The ultimate goal of this research is to increase our understanding of the function of one of the largest superfamilies of proteins in the genome, which includes a majority of transcription factors. Lessons learned should be broadly applicable to studies of disease etiology and intervention. Furthermore, studies of DNA-binding zinc finger domains have led to a technology for creating custom DNA-binding proteins which are now being exploited commercially as sequence-specific molecular tools and therapeutics. By analogy, the potential to create similar tools based on protein recognition would have far-reaching impact.

Michael Sierks, Ph.D.

Arizona State University
Award Amount FY05: $121,369

Morphology specific antibodies for treating Parkinson's Disease

The protein α-synuclein has been strongly correlated with Parkinson's Disease (PD) since it is a major component of Lewy Body aggregates, mutations in the α-synuclein gene associate with early onset cases of PD, and transgenic animal models expressing α-synuclein develop PD symptoms. α-synuclein is a natively unfolded protein, however it can adopt a number of different folded conformations including the b-sheet form which facilitates formation of numerous aggregated morphologies, including large fibril structures found in Lewy Bodies, spherical protofibril structures, and smaller aggregates or oligomers. While all these structures occur, their respective roles in the progression of PD are not known. Identifying the toxic form of α-synuclein is essential for developing a successful therapeutic. A critical need exists to develop a tool whereby we can control formation of different morphologies of α-synuclein and study how presence of the individual morphologies affect the progression of PD. The long-term objective of this proposal is to generate antibody fragments that can be used to control folding of α-synuclein *in vivo*. These antibodies can be used in conjunction with other therapeutics as part of a non-invasive long-term treatment for PD and other related disorders.

Our hypothesis is that misfolding of α-synuclein into specific toxic morphologies is a fundamental step in the progression of PD, and that antibody based proteins can be expressed intracellularly to control formation of the individual α-synuclein morphologies providing a means to identify the toxic α-synuclein forms and serving as part of a potential therapeutic treatment for controlling PD. In order to test this hypothesis, we will utilize a novel spectroscopic technique. Single Molecule Immuno-Selection, that we have developed, will allow us to both visualize individual α-synuclein morphologies and to recover individual antibody fragments that bind specifically to a particular morphology.

Steven P. Stratton, Ph.D.

University of Arizona
Award Amount FY05: $164,366

Phase II Trial of Topical Perillyl Alcohol in Sun-Damaged Skin

Skin cancer is by far the most common cancer (with an estimated 1.3 million new cases diagnosed annually in the U.S.) and is a tremendous public health problem, especially in Arizona and the southwestern United States where sun exposure is highest. As reported by the Southeastern Arizona Skin Cancer Registry, rates of non-melanoma skin cancer in Arizona are among the highest in the world and are 4-6 fold higher than in the general US population. Incidence rates for melanoma, the most deadly form of skin cancer, are rising faster than almost any other cancer. This problem will continue to worsen as the population growth in Arizona continues to rise at one of the highest rates in the country. The current primary methods for skin cancer prevention, including behavioral modification and the use of sunscreens, have not proven sufficient to protect against the rise in skin cancer incidence. Therefore, we propose to develop topically administered chemopreventive drugs that actually stop or reverse the growth of precancerous lesions in the skin. Perillyl alcohol is a molecule found in the essential oils of lavendin, 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 non-melanoma 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.

Our goal is to prevent skin cancer in high-risk populations by developing safe and effective therapies for clinically imperceptible precancerous lesions (intraepithelial neoplasia) in sun-damaged skin. This proposal will help support the necessary clinical research required to advance this goal. The objective of this proposal is to perform a Phase II clinical trial of topical perillyl alcohol in subjects with moderate to severe sun damage in their skin. The hypothesis being tested in this proposal is that topical perillyl alcohol, when applied twice daily for three months, can successfully reverse sun damage to skin in a dose-dependent manner, as measured by histopathology in skin biopsy tissue. As a secondary endpoint, we will also determine if topical perillyl alcohol can significantly alter surrogate biomarkers of abnormal skin cell growth, using the latest high-tech microscopic and non-invasive imaging techniques. Establishment of new and valid biomarkers is essential for demonstrating the activity of this and other drugs in future studies. Safety, tolerance, skin uptake, and stability of the perillyl alcohol cream will also be monitored. Ultimately, positive results (i.e. evidence of skin sun damage reversal with excellent skin tolerance) from this Phase II trial could lead to a definitive, NIH-sponsored Phase III trial of topical perillyl alcohol as a skin cancer preventive agent.

Timothy L. Vail, Ph.D.

Northern Arizona University
Award Amount FY05: $44,992

Paramagnetic Nanoparticle Immunoassay for Food Pathogen Detection

Recent and potential future national outbreaks of food borne illnesses demonstrate a need for rapid, sensitive, and specific methods to test for the presence of disease-causing bacteria in food supplies. Although such tests exist, their sensitivity comes at the cost of increasing the time to get a result. Conversely, rapid assays (less than 10 minutes to result) typically lack sensitivity. Sensitivity is a major testing criterion because of the nature of bacterial growth. Under the appropriate conditions, several organisms can multiply into millions in several hours time. Thus, there exists a need for the continued development of highly sensitive and accurate assay technology. This technology should also be cost effective and potentially allow the user to perform sample testing at the source of contamination, including remote rural areas of the state. Further, the assay technology should lend itself to adaptation to other forms of testing, including medical and veterinary diagnosis, environmental (e.g. water), and bio-defense testing.

This proposal seeks to further the state of the art of assay technology through the research and development of a prototype rapid assay using nanometer-sized paramagnetic particles onto which fluorescent dyes have been bound. Through a multi-step process of chemical modification, the magnetic particles will be coated with a thin layer of silica (glass) into which a series of fluorescent dyes have been added. Also, the silica coating will provide a means of chemical modification so that antibodies that are specific for the causative agent of one type of food poisoning (Listeria monocytogenes) can be permanently attached. Once fully functionalized, these magnetic nanoparticles will be utilized in a new type of assay system that will exploit their properties of magnetism, fluorescence, and small size. This prototype system will provide a means by which further modifications and miniaturization (outside the scope of this proposal) can take place. The ultimate goal is a miniaturized assay system that will probe for multiple types of disease-causing organisms simultaneously and provide testing results in a very short time. This system is intended to be adaptable to a wide variety of public health, medical, veterinary, environmental, and bio-defense applications.

Section F

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Principal Investigators, New Awards and Continuing Contracts