ARIZONA BIOMEDICAL RESEARCH COMMISSION ANNUAL REPORT 2006–2007

Janet Napolitano, Governor

David Landrith, M.P.A., Chairman

COMMISSION MEMBERS

General Public
David Landrith, M.P.A.
David Jerman, M.B.A.
Gregorio M. Garcia, J.D.

Medical Community
Colleen Brophy, M.D.
Barbara Wuebbels, R.N., M.S.
Eve Shapiro, M.D.

Scientific Community
Manuel Modiano, M.D.
Thomas "Lon" Owen, Ph.D.
Joan Rankin Shapiro, Ph.D.

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January 2008

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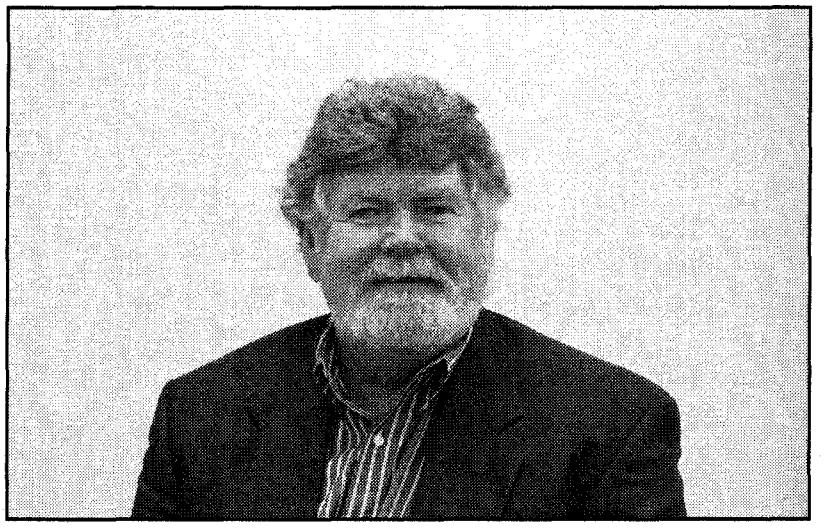


David Landrith, M.P.A.

Message from the Chairman

Fiscal Year 2007 was a year of increasing activity in both basic research and translational research. The Arizona Biomedical Research Commission continued its pivotal role in advancing biosciences in Arizona.

The Commission awarded twenty-eight new scientific research contracts this year. There will be a total of seventy-five research projects under contract with the Commission beginning in FY2008. The Annual Report contains abstracts of all 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 enabled many Arizona researchers to prove their investigative concepts and go on to obtain additional funding at the national level. The Commission through its statutory authority continues its technology transfer efforts.

Translational research expanded through continuing contracts addressing Parkinson's disease, Alzheimer's disease, cancer, bioengineering, bioimaging, and innovative approaches to research in Native American communities. The Commission sponsored AzTransNet (Arizona Translational Research Network) conducted workshops and meetings focused on tissue issues, intellectual property, business practices, institutional review boards, and clinical research practices.

The Commission worked with The Flinn Foundation sponsoring symposia, convening meetings, and advancing the promise of the Arizona Bioscience Roadmap. The Commission in concert with the Arizona Board of Regents was actively involved in supporting the development of the Arizona application to the National Institutes of Health Clinical and Translational Science Award. The final award decision is to be made in 2008.

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

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 2005–2006 are presented below.

General Public

Gregorio M. Garcia, Esq.

Shughart, Thomson & Kilroy, P.C.

Commissioner Garcia received his undergraduate and graduate degrees from Arizona State University. He holds a Juris Doctorate and Master of Business Administration. He is currently pursuing a Master of Laws (LL.M.) in Biotechnology and Genomics. Commissioner Garcia is an attorney and practices with the firm of Shughart, Thomson & Kilroy, P.C. He sits on the board of directors for Arizona's largest legal aid law firm, Community Legal Services, and has held leadership positions within the State Bar of Arizona and other legal organizations. Commissioner Garcia was appointed by Governor Napolitano in 2006.

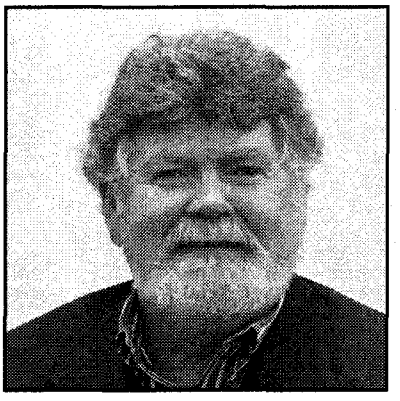
David Jerman, M.B.A.

Administrative Director
Arizona Alzheimer's Research Center and Arizona Alzheimer's Disease Institute

Commissioner Jerman received his undergraduate accounting and Masters of Business Administration in finance degrees from the University of Utah. He has extensive experience in the pharmaceutical industry and in technology transfer issues. Commissioner Jerman is the Administrative Director of the Arizona Alzheimer's Disease Institute located within Banner Healthcare system. The Arizona Alzheimer's Research Center is a statewide research consortium composed of ASU, UA, TGen, Banner, Mayo Clinic Scottsdale, Sun Health Research Institute, and Barrow Neurological Institute. Commissioner Jerman is also Chairman of the Board of Directors of Frontier Scientific Incorporated. Jerman was appointed by Governor Napolitano in 2005.

David Landrith, M.P.A.

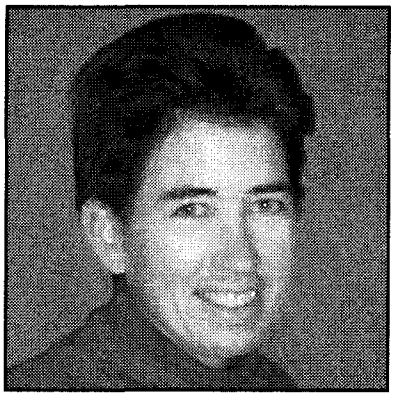
Vice President of Policy and Political Affairs, Arizona Medical Association



Commissioner Landrith is the Vice President of Policy and Political Affairs at the Arizona Medical Association. His undergraduate studies were in political science at Arizona State University. He received a Masters of Public Administration degree at Harvard University and accomplished summer studies at Oxford. Commissioner Landrith is the Chairman of the Board of Arizona Health-e Connection and serves on the steering committee of The Arizona Partnership for Immunization. He has served as co-chairman of the ASU Dean's Advisory Council, a member of the Arizona Town Hall Board of Directors, member of the ST. Vincent De Paul Free Medical and Dental Clinic Endowment Committee, Director of the Arizona Bioethics Network, and Past Chairman and Executive Secretary of the Arizona Council of Governments Directors' Association. He has received the Partnership Award from the Arizona Chapter of the American Academy of Pediatrics, and the Presidential Award for the Arizona State Association of Physician's Assistants. Commissioner Landrith was appointed by Governor Napolitano in 2004.

Colleen Brophy, M.D.

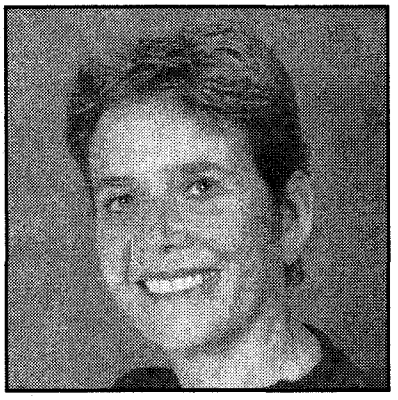
Chief of Vascular Surgery
Carl T. Hayden VAMC



Dr. Brophy is a vascular surgeon, scientist, and entrepreneur. She is currently Research Professor of Kinesiology at the Center for Metabolic Biology, Adjunct Professor of Bioengineering, and Adjunct Professor of Cellular and Molecular Biology, Arizona State University; Chief of Vascular Surgery at the Carl T. Hayden VA Medical Center in Phoenix; and a Clinical Professor of Surgery at the University of Arizona. Brophy received both her B.S. and M.D. from the University of Utah. She was a surgical resident at Yale University and a vascular fellow at Harvard University. She has received the National Institutes of Health (NIH) National Research Service Award; the American College of Surgeons Faculty Fellowship Award; the SVS/ISCVS Lifeline Foundation Award; the Clinician Scientist Award from the American Heart Association and the Von Leibig Foundation Award for Early-Career Academic Surgeons, for her investigative research. She has been continuously supported by the NIH and VA (Merit Award) for over 15 years. She has over 70 publications in peer reviewed journals and has edited a textbook in vascular surgery. Dr. Brophy has served on the Executive Councils for the Association of Academic Surgery, Society of University Surgeons, and the Lifeline Foundation Board of Directors. She is an associate editor for the Journal of Surgical Research and has served on the editorial board of Surgery. She served as an active member of the Bioengineering, Biotechnology, and Surgical Sciences (BTSS) and the Cardiovascular Devices (SBTS) study sections of the NIH. She is currently serving on the Executive Committee of the Surgical Research Committee of the American College of Surgeons and was recently appointed Chair of this committee. Dr. Brophy is the Chair of the Young Surgical Investigators course for the American College of Surgeons. She has been Chair of the Committee on Women's Issues for the Society for Vascular Surgery. She was appointed in 2002 and 2006 by Governor Napolitano.

Eve Shapiro, M.D., M.P.H.

Orange Grove Pediatrics



Commissioner Shapiro received her undergraduate degree from Brandeis University and her M.D. from SUNY Upstate Medical Center in1976. She did a pediatric residency at Strong Memorial Hospital in Rochester, N.Y. and Montefiore Hospital, Bronx, N.Y. She completed an adolescent medicine fellowship at Mt. Sinai Hospital in NY in 1982. She received an MPH from the University of Arizona in 2001. Dr. Shapiro is a pediatrician in private practice in Tucson, specializing in adolescent medicine. She has been involved in legislative and voter initiatives to increase access to care for the uninsured and improve health care funding. She is on the boards of the Pima County Medical Society, Arizona Medical Association and Pima County Pediatric Association and has been on the executive committee of the Arizona chapter of the American Academy of Pediatrics. She was appointed to the commission in 2006 by Governor Napolitano to compete the term of Gary Krahenbuhl.

Barbara Wuebbels, R.N., M.S.

Director of Clinical Education
BioMarin Corporation

Commissioner Wuebbels received her Bachelor of Science in Nursing from St. Louis University, her Master of Science in Business Administration from the University of Phoenix, and her Master of Science in Adult Health Nursing from Arizona State University. Prior to joining BioMarin, she served as Director of Clinical Education at Medicis Pharmaceutical Corporation, as research coordinator at Maricopa Medical Center, and as Director of Clinical Affairs at Vivra Health Advantage in Brentwood, Tennessee. Commissioner Wuebbels has presented at various national conferences and she has published on nursing research, spinal cord stimulation, and wound care in the long term care setting. Wuebbels was appointed by Commissioner Napolitano in 2006.

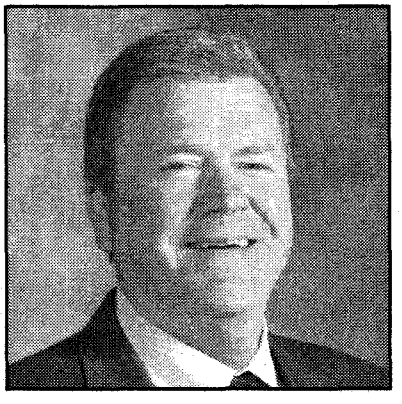
Manuel Modiano, M.D.

Arizona Oncology Associates

Commissioner Modiano obtained his Bachelor of Science degree from Colegio Collumbia in Mexico City. He received his M.D. with high honors from the Universidad Nacional Autonoma de Mexico. He completed post graduate education at the University of Wisconsin, Mount Sinai Medical Center, and the University of Arizona – Arizona Cancer Center. Commissioner Modiano has published numerous peer reviewed articles and has served as Principal Investigator in numerous clinical research studies. Dr. Modiano is Director of Research for Arizona Oncology Associates, Medical Director of the Arizona Clinical Research Center, Past Chief of Hematology and Oncology and Past-President of the Medical Staff at Carondelet St. Mary's Hospital and Medical Center, and Past President of the Arizona Clinical Oncology Society. He was appointed by Governor Napolitano in 2006.

T. Lon Owen, Ph.D.

Professor of Medical Anatomy and Physiology
Northern Arizona University



Commissioner Owen received his B.A. in Zoology from the University of California, a Master's Degree in Biology from California State University at Sacramento, and his Ph.D. in Physiology from U.C. Davis. He was an NIH Postdoctoral Fellow at Michigan State University and Visiting Associate Professor in the Pharmacology Department of the University of Arizona College of Medicine. He is a member of the American Physiological Society and has chaired the Research Committees of the American Heart Association at both the Arizona Affiliate and Southwestern Regional levels. His publications are in the areas of cardiovascular, aging, and environmental physiology. He has taught physiology and pathology at Northern Arizona University since 1974. He is a member of the Translational Genomics Research Institute Board of Directors. Commissioner Owen was appointed to the Commission by Governor Hull in 1998 and 2001. His term expired in 2004, and he was reappointed by Governor Napolitano in 2006.

Joan Rankin Shapiro, Ph.D.

St. Joseph's Hospital and Medical Center

Commissioner Joan Rankin Shapiro is a human geneticist who received her M.D., Ph.D. from Cornell University Medical College in 1979. Her initial research was in human birth defects at Rockefeller University. She began her cancer career at Memorial Sloan-Kettering Cancer Center, New York. In September,1989, she relocated to the Barrow Neurological Institute (BNI) of St. Joseph's Hospital and Medical Center, Phoenix, Arizona, where she became the Director of Neuro-Oncology Research. Her research involved the characterization of genetic abnormalities associated with central nervous system malignancies. Since 1979 her grant awards have totaled more than fourteen million dollars. In November 2007 she received a Life Time Achievement Award from the Society of Neuro-Oncology for her contribution to the field. She served thirteen years as an NIH Reviewer on Pathology A and on Experimental Therapeutics study sections. In 2001 Dr. Shapiro retired from the laboratory and has assumed the role as V.P., Research and Development at St. Joseph's Hospital and Medical Center. She is the Past President of the National Organization Women in Cancer Research. Dr. Shapiro has also retained a strong commitment to community education. She has developed and continues to teach numerous school enrichment programs. In conjunction with the American Academy of Neurology, she conducted K-12th grade neuroscience enrichment training workshops for physicians and scientists. She is the past Chairman of the National Neuroscience prize for high school students. She remains at St. Joseph's Hospital and Medical Center as a Consultant for research activities. She was appointed by Governor Napolitano.

Summary of 2006–2007 Commission Activities

The Commission administered 72 contracts worth over $6 million with medical researchers in Arizona. The Commission continued its commitment to individual investigators, investigator collaborations, and expansion into translational research. 21 new contracts and $2.2 million were directed toward assisting individual investigators in developing proof of their research concepts, collecting preliminary data, and in continued support of translational research. Inter-institutional collaborators have formed working relationships to better address more complex research problems.

A special effort is being made to ensure the success of TGen. The Translational Genomics Research Institute receives $5,000,000 per year for a period of five years plus a $500,000 annual award for a period of ten years.

Section headings in this report list each program and whether the project is in its first, second, or third year of funding. Research abstracts outlining the progress made during the year are contained in Sections A-C. Citations for scientific publications and abstracts arising out of the research are also listed. Section D provides information on new contracts awarded beginning July 2007 (FY2008).

Over 1,000 Requests for Proposals (RFPs) for 2006-2007 awards were mailed to potential applicants in September 2006. The amount of funding available for new unrestricted medical research was approximately $2.0 million. In response to the RFP, the Commission received 113 unrestricted medical research proposals.

ABRC Projects Submitted/Accepted FY 2007

Institution	Submitted	Accepted	Percent Accepted	Amount $	Percent of Total $
Arizona State University	12	3	25	250,000	13
Northern Arizona University	5	2	40	88,606	4
University of Arizona	73	17	23	1,196,321	60
Sun Health Research Institute	3	1	33	50,000	3
Barrow's Neurological Institute	12	4	33	299,973	15
Mayo Clinic	1	1	100	110,279	5
Others	7	00	0	0	0
Total	**113**	**28**	**25**	**1,995,179**	**100**

In November and December the medical research proposals received were sent to a panel of national and international scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more than 170 out-of-state peer reviewers. Three reviews were sought for each proposal. In the spring and summer of 2006 the Commission selected 28 proposals for funding. During 2006-2007 the ABRC managed a total of 72 basic research contracts.

ABRC Total New and Continuing Project Contracts 2007

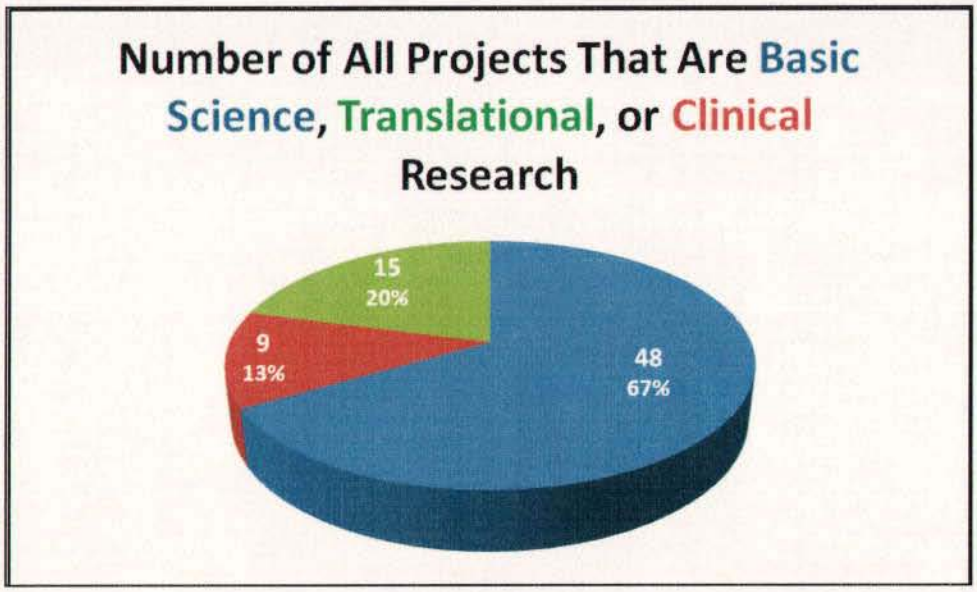
Institution	Award	% of Total Awarded
Arizona State University	8	11
Northern Arizona University	4	6
University of Arizona	41	56
Sun Health Research Institute	5	7
Barrow's Neurological Institute	7	10
TGen	2	3
Mayo Clinic	2	3
Others	3	4
Total	**72**	**100**

In May of 2007 the Commission awarded 21 new research contracts for a total of $1,999,440. The contracts were effective on July 1, 2007. Progress on these projects will be reported in the next Commission Annual Report.

ABRC Projects Submitted/ Accepted FY 2008

Institution	Submitted	Accepted	Percent Accepted	Amount $	Percent of Total $
Arizona State University	24	3	12.5	350,000	18
Northern Arizona University	2	1	50	300,000	15
University of Arizona	76	14	18.4	999,440	50
Sun Health Research Institute	6	1	17	150,000	7
Barrow's Neurological Institute	10	1	10	50,000	3
Translational Genomics Institute	6	1	17	150,000	7
Others	5	0	0	0	0
Total	**129**	**21**	**100**	**1,999,440**	**100**

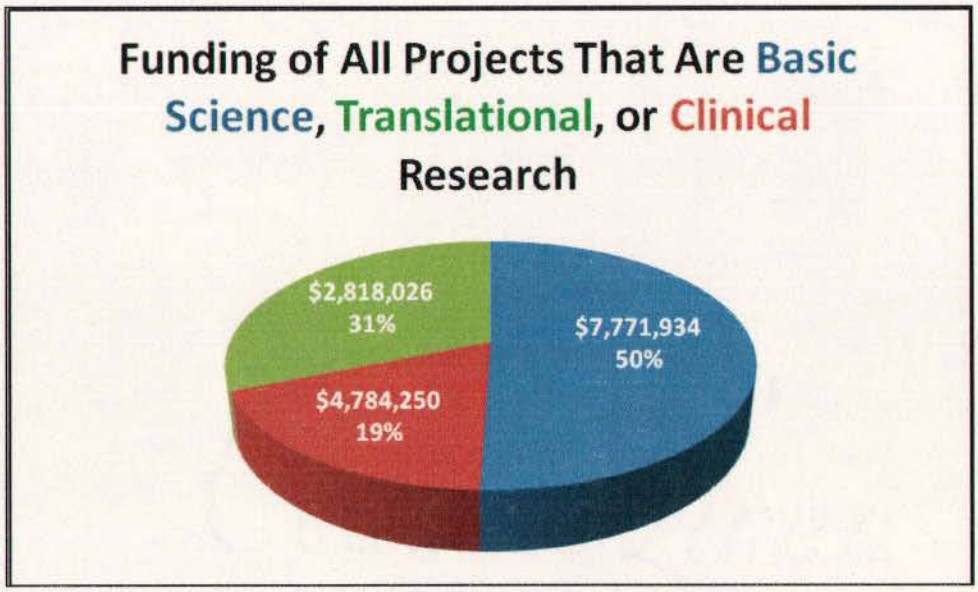
The Commission remains committed to making the results of scientific discovery more readily available to health care providers. The Commission currently has 15 translational research projects underway. The Commission has sponsored workshops and symposia examining translational issues such as the appropriate treatment of biospecimens. Institutional barriers are being addressed by the Commission sponsored Arizona Translational Resource Network (AzTransNet). Workshops, model documents, and consulting services relating to Institutional Review Boards, collaborative agreements, intellectual property contracts, and clinical trial networks have been developed and delivered by AzTransNet. The Commission is confident that that these translational projects will result in more rapid deployment of medical therapies to Arizonans.



Basic Science Research: Scientific studies that increase knowledge of basic life processes.

Translational Research: Medical research that attempts to more directly connect basic research to patient care.

Clinical Research: The study of drugs, biologics, or devices in human subjects with the intent to discover potential effects and/or determine safety or usefulness.



Section A

Continuing Contracts

Medical Research

Year Three

FY 2007

Burris Duncan, M.D.

University of Arizona
Award Amount FY05: $172,841

Acupuncture as Complementary Therapy for Cerebral Palsy

The conclusion of this collaborative investigation between the Department of Pediatrics at the University of Arizona and the Beijing Children's Hospital has resulted in a number of very interesting findings that may have far reaching implications for children with cerebral palsy not only for those born in Arizona, but wherever they may live.

- The feasibility of initiating and completing a trans-cultural study involving investigators on three continents was confirmed.
- The project was able to assess a combined east-west intensely administered therapeutic approach (physical, occupational, and hydro-therapies plus acupuncture) for a condition where the results are less than satisfactory when the standard-of-care used in the U.S. is administered.
- One hundred and sixteen children were enrolled in the study with 83 completing the 28-week trial and 8 more completing at least 12 weeks of the trial, making this one of the largest investigations ever conducted by U.S. researchers assessing therapeutic interventions for cerebral palsy.
- Preliminary analysis of the first 76 children who completed the trial clearly shows that the combined east-west package of the care when initiated early and intensely is far superior to any report published in the western medical literature regardless of the intervention, including invasive surgical procedures.
- Although not conclusive, it appears that acupuncture does play a positive role in the improvement seen in these children.
- The project has generated one letter to the editor (Journal of the American Medical Association); two articles submitted for publication; three abstracts submitted to the Society Acupuncture Research recently held in Baltimore, one accepted for oral presentation and one accepted as a poster presentation; three research projects submitted to foundations or governmental agencies in China; a project to be submitted to the United Cerebral Foundation in the U.S. with committed financial assistance from the Tucson Medical Center, and a large research proposal to be submitted to the National Institutes of Health to continue this investigation that the ABRC had the vision to fund. The NIH grant will involve 240 children and will include an exploratory phase using imaging techniques to determine if any structural/functional changes occur in the brain as a result of these therapies.

Caspi O, Duncan B, Han T, Zou L. MRI Findings and Cerebral Palsy. **JAMA** 297:466, 2007.

Wu Y, Jin Z, Li K, Han T, Zheng H, Caspi O, et al. Ya-wei Acupoint-specific fMRI Patterns in Child Brains. **Journal of Alternative & Complimentary Medicine**. Submitted for publication.

Caspi O, Zou L, Han T, Duncan B. Ethical and Methodological Challenges in Conducting International Acupuncture Research. **Society for Acupuncture Research**. Presentation Nov. 2007.

Robert P. Erickson, M.D.

University of Arizona
Award Amount FY05: $131,535

Identification of Genes Involved in Lymphedema by Single Nucleotide Polymorphism Mapping

During the year we extended the clinical base for studies on the genetic causes of lymphedema. We were able to identify a five generation family with most of the members in the Phoenix area. We preformed physical examinations and obtained DNA from twenty-two members of this family. In addition, we used the most sensitive method of delineating and characterizing the lymphatics of patients, lymphangioscintograms, on several members of the family. This allows us to compare the specific kind of lymphatic abnormality particular to this family to the kind of abnormality in other families. We also added more individuals, more DNA samples, and lymphangioscintograms in three other Arizona families. The refined linkage analyses on chromosome 3 were performed but the data have not yet been analyzed (due to illness/pregnancy/termination of key personnel at the Translational Genomics Institute).

Brent Gendleman

5AM Solutions
Award Amount FY05: $100,000

5AM Illumine

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

Eric J. Guilbeau, Ph.D.

Arizona State University
Award Amount FY05: $50,000

Biosensor for Measurement of Breath Acetone

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

Ahmad L, Dodt S, Guilbeau E. The Development of a Non-invasive Breath Ketone Biosensor for Obesity Management. Abstract, Biomedical Engineering Society, Philadelphia, October 2004.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY05: $164,500

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

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

Cherry L. Herald, Ph.D.

Arizona State University
Award Amount FY05: $164,500

Preclinical Development of Three Anticancer Drugs

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

Pinney K, Jelinek C, Edvardsen K, Chaplin D , and Pettit G. The Discovery and Development of the Combretastatins: Emphasis on combretastatin A-4 (CA4). **Anticancer Agents from Natural Products**, Cragg, Kingston and Newman eds. CRC Press, Taylor & Francis, Boca Raton, FL, 2005.

Pinney K, Jelinek C, Edvardsen K, Chaplin D , and Pettit G. Combretastatin A-1 (CA1). **Anticancer Agents from Natural Products**, Cragg, Kingston and Newman eds. CRC Press, Taylor & Francis, Boca Raton, FL, 23-46, 2005.

Monk K, Siles R, Hadimani M, Mugabe B, Ackley J, Studerus S, Edvardsen K, Trawick M, Garner C, Pettit G, Pinney K. Design, Synthesis and Biological Evaluation of Combretastatin Nitrogen-containing Derivatives as Inhibitors of Tubulin Assembly and Vascular Disrupting Agents. **BioOrg. Med. Chem.** 14: 3231-34, 2006.

Steven Hoffman, Ph.D.

Arizona State University
Award Amount FY07: $50,000

Autoantibodies in CNS Lupus

The overall goal of this project has been to understand how mental disorders can occur in the autoimmune disease, systemic lupus erythematosus. Significant progress has been made by showing that there are correlations between brain-reactive autoantibodies (BRAA) and behavior in mouse models of autoimmune disease. We have also shown that there is evidence for increased blood-brain barrier (BBB) permeability, through elevated MHC class I levels, as a mechanism to allow access of these autoantibodies into the brain. Furthermore, we are showing how these BBB permeability changes can occur involving the neuropeptide substance P. We have also made good progress toward developing a phage-display technique to identify the molecular specificity of autoantibodies reactive with brain and producing immortalized cell lines of some of the BRAA. These advances are instrumental in allowing us to develop diagnostic and therapeutic techniques in the future for these diseases.

Laurence Hurley, Ph.D.

University of Arizona
Award Amount FY05: $49,746

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

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

Qin Y, Rezler E, Gokhale V, Sun D, Hurley L. Characterization of the G-quadruplexes in the Duplex Nuclease Hypersensitive Element of the *PDGF-A* Promoter and Modulation of *PDGF-A* Promoter Activity by TMPyP4. **Nucleic Acids Res.** In press.

Tamara King, Ph.D.

University of Arizona
Award Amount FY05: $164,500

Model of Bone Metastasis Pain and Phenotype

We have successfully characterized a new murine model of bone cancer pain in which breast cancer cells are injected and sealed into the femur. This model of bone cancer pain is novel in that 1) it models bone cancer pain from breast cancer, and 2) it models bone cancer pain from mixed osteoblastic/osteolytic reactions within the femur. This model will allow analysis of potential differences in mechanisms underlying bone metastasis pain between pure osteolytic reactions as modeled with the osteosarcoma cells or the human prostate cell lines, and mixed osteolytic/osteoblastic as observed in this new breast cancer cell line. This model of bone cancer induced pain using breast cancer cells may lead to novel pain management treatments for women suffering from bone pain due to breast cancer metastases. We also demonstrated that noproxen (i.e. Aleve) reduced sarcoma-induced bone loss and attenuated pain behavior in this osteolytic bone cancer.

Douglas Lake, Ph.D.

University of Arizona
Award Amount FY05: $39,600

Dendritic Cells and Immunity of Valley Fever

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

Dionne S, Podany A, Ruiz Y, Ampel N, Galgiani J, Lake D. Spherules Derived from *Coccidioides posadasii* Promote Human Dendritic Cell Maturation and Activation. **Infection and Immunity**. April: 2415-22, 2006.

Dianne Lorton, Ph.D.

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

Sodium Narcistatin in Treatment of Rheumatoid Arthritis

Narciclasine, an isocarbostyrils, is an active component of Narcissus species and is reported to have anti-inflammatory properties and to significantly reduce the soft tissue swelling of arthritic joints in an adjuvant-induced arthritis (AA) rat model for rheumatoid arthritis (RA). In this series of studies we demonstrated 1) that sodium narcistatin, a pro-drug of narciclasine, was the most effective of three potential anti-proliferative drugs for treating the inflammation and bone loss that characterizes RA, 2) the most effective dose range for the narcistatin in preventing inflammation and bone loss, 3) assessed narcistatin's bone marrow, cardiac, kidney and liver toxicity, 4) completed preliminary screens of secondary immune organ cytokine and immune cell profiles for potential mechanisms of action, and 5) completed a head to head comparison of the narcistatin with the current gold standard RA treatments, anti-TNF-a and methotrexate for efficacy and toxicity. These studies support narcistatin is a safe and effective candidate for use in RA treatment.

Schaller L, Meldoy J, Pettit G, Lorton D. Sodium Narcistatin Reduces Inflamation and Bone Destruction of Arthritic Joints in a Rat Adjuvant-induced Arthritis Model. **J. Rheumatol.**

Emmanuelle Meuillet, Ph.D.

University of Arizona
Award Amount FY05: $50,000

Novel Inhibitors of Akt as Anticancer Drugs

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

Marek Jan Romanowski, Ph.D.

University of Arizona
Award Amount FY05: $50,000

Contrast Agents for Optical Coherence Tomography

The overall goal of this project is to develop a new class of contrast agents for cancer research. The proposed contrast agent will be used in conjunction with optical coherence tomography (OCT), a biomedical imaging technique for visualization of living tissues. We seek to develop this contrast agent by forming dense arrays of gold nanoparticles on the surface of liposome, a biocompatible sphere of diameter ca. 100nm. In Year 3 of this project we successfully generated and characterized liposome-supported gold nanoshells, a new class of structures strongly interacting with light in the near-infrared spectral range in a tunable manner. These plasmon resonant structures are supported on biodegradable soft template appropriate for applications *in vivo*. We demonstrated optical tunability of these structures, their biodegradation, and safe use in animal models of cancer.

Troutman T, Barton J, Romanowski M. Optical Coherence Tomography with Plasmon Resonant Nanorods of Gold. **Optics Letters**. 32-11: 1438-40.

Troutman T, Barton J, Romanowski M. Gold-coated Liposomes - Biodegradable Plasmonic Nanostructures. Submitted to **Nano Letters**. August 2007.

Joyce A. Schroeder, Ph.D.

University of Arizona
Award Amount FY05: $49,718

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

Breast cancer is the third leading cause of cancer-related deaths in Arizona. The majority of these patients die when breast cancer spreads from the breast to distant sites in the body (metastasis). While current chemotherapeutic treatments are making small gains against this devastating disease, the need for a treatment that targets a large number of patients is needed. We have identified a molecular event that occurs in a high percentage of transformed cells, but not in normal, non-cancerous cells. Specifically, the tumor antigen MUC1 becomes highly expressed (greater than 90% of patients analyzed have increased MUC1 levels) and interacts in a novel way with the adhesion protein β-catenin and oncogene EGFR during breast cancer growth and metastasis. Experimental evidence indicates that by preventing this interaction, breast cancer cells lose the ability to progress. We have developed a targeted anti-cancer therapy that inhibits the growth and spread of metastatic breast cancer cells while having no side-effects on normal tissue. We have discovered that this drug significantly inhibits breast cancer growth and spread in relevant mouse models. We are very hopeful that this drug will have similar strong efficacy in treating breast cancer patients, and we will soon be applying for funding to perform clinical trials.

Michael R. Sierks, Ph.D.

Arizona State University
Award Amount FY05: $121,369

Morphology Specific Antibodies for Treating Parkinson's

We have shown that scFv fragments directed toward α-synuclein can alter α-synuclein folding and toxicity *in vivo*. Here we have developed the protocols needed to generate and image α-synuclein morphologies, to isolate morphology specific scFv's, to characterize binding and specificity of the scFv's, and to improve affinity of the isolated scFv's. We have also demonstrated that scFv's expressed intracellularly as intrabodies can provide protection against α-synuclein induced toxicity. We have shown that the scFvs can label specific folded morphologies of α-synuclein *in vivo* in cell culture models and the morphology specific scFvs can also label specific protein morphologies in brain tissue samples obtained from human PD patients. These results hold great promise for developing a potential therapeutic for Parkinson's Disease.

Barkhordarian H, Emadi S, Sierks M. Isolating Recombinant Antibodies Against Specific Protein Morphologies Using Atomic Force Microscopy and Phage Display Technologies. **PEDS**. 19: 497-502, 2006.

Emadi S, Barkhordarian H, Wang M, Sierks M. Isolation of a Human Single Chain Antibody Fragment Against Oligomeric Alpha-synuclein That Inhibits Aggregation and Prevents Alpha-Synucelin Induced Toxicity. **J Mol Biol**. 368: 1132-44, 2007.

Zhou C, Emadi S, Sierks M, Messer A. A Human Anti-alpha-synuclein Intrabody Interferes with Pathogenic Cellular Effects of Overexpressed Synuclein. **Molecular Therapy**. 10: 1023-31, 2004.

Shlyakhtenko L, Yuan B, Emadi S, Lyubchenko Y, Sierks M. Single Molecule Selection and Recovery of Structure Specific Antibodies Using Atomic Force Microscopy. **Nanomedicine**. 2007. In press.

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 more than 1.3 million new cases expected in 2003 in the U.S.), and is a tremendous public health problem, especially in Arizona and the southwestern United States where sun exposure is high. As reported by the Southeastern Arizona Skin Cancer Registry, rates of nonmelanoma skin cancer in Arizona are among the highest in the world and are 4–6 fold higher than in the general US population. Incidence rates for melanoma, the most deadly form of skin cancer, are rising faster than almost any other cancer. Topically administered chemopreventive drugs that actually stop or reverse the growth of precancerous lesions in the skin may reduce this burden. Perillyl alcohol is a molecule found in the essential oils of 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 nonmelanoma skin cancers. We have recently performed a Phase I clinical trial of a cream formulation of topical perillyl alcohol developed by our group. Results of this study indicate that this formulation is safe when applied twice daily for 30 days. Further clinical testing is now warranted to determine if perillyl alcohol applied directly to sun-damaged skin can reverse such damage. The objective of this research is to perform a randomized, placebo-controlled, double-blind, Phase 2a dose-finding clinical trial of topical perillyl alcohol in subjects with moderately to severely sun-damaged skin. The hypothesis being tested is that topical perillyl alcohol, when applied twice daily for three months, can successfully reverse sun damage in skin in a dose-dependent manner as evidenced by histopathologic normalization. As secondary endpoints, we will also determine if topical perillyl alcohol can significantly alter previously-studied surrogate endpoint biomarkers of neoplastic changes, including optical coherence tomography (OCT) of skin; as well as p53 expression, c-Fos expression, and apoptosis (as measured by expression of activated caspase-3) in skin biopsy tissue. In addition, karyometric analysis of nuclear chromatin patterns in skin biopsy tissue will be measured. Establishment of valid biomarkers is vital for demonstrating the activity of this and other drugs in future studies. Safety, tolerance, absorption, and formulation stability will also be monitored. Ancillary studies dependent on performance of this trial (but funded from other sources) will include comparative genomic hybridization analysis of skin biopsy samples for gene copy number changes (in collaboration with the Translational Genomics Research Institute (TGen) in Phoenix, Arizona) and nutritional correlates and dietary assessment. Due to unexpected delays regarding FDA approval of experimental use of topical perillyl alcohol in the proposed clinical trial, this project has been extended while we complete additional preclinical dermal toxicology studies. These studies have been completed (December 2005) and we began enrolling patients beginning in January 2006. The protocol has been approved by the University of Arizona Institutional Review Board (IRB) and enrollment will commence once final FDA approval is granted.

Timothy L. Vail, Ph.D.

Northern Arizona University
Award Amount FY05: $44,992

Paramagnetic nanoparticle Immunoassay for Food Pathogen Detection

Recent national outbreaks of food borne illnesses demonstrated 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. Thus, there exists a need for the continued development of highly sensitive and accurate assay technology. This research 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. To date, we have demonstrated the ability to create silica-coated paramagnetic nanoparticles with incorporated fluorescent dye (FITC), or quantum dots, through a multi-step process of chemical modification. We have attached a linker protein (avidin) to the surface of the particles and have shown increased stability via chemical cross-linking. Current experiments underway include the incorporation of fluorescent quantum dots as an alternative fluorescent marker. We have initiated a series of prototype immunochromatographic assays to demonstrate the functionality of the nanoparticles. The next steps will include the creation of functional particle that are specific for the causative agent of one type of food poisoning (Listeria monocytogenes). This system is also being used as a means to create quantitative, rapid assays that are being tested for the detection of endocrine disrupting compounds in wastewater. We have filed a provisional patent with the USPTO on the design of a hand-held, field portable integrated assay and radial cassette reader. 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, and environmental applications.

Section B

Continuing Contracts

Medical Research

Year Two

FY 2007

Charles H. Adler, M.D., Ph.D.

Mayo Clinic Scottsdale
Award Amount FY06: $250,000

Arizona Parkinson's Disease Center

The overall goals of the APDC are to develop clinical biomarkers for Parkinson's disease and PD with dementia (PDD) as well as find targets for novel treatment strategies. To this end the program is divided into a clinical core (which prospectively examines PD and control subjects enrolled in the brain donation program), a neuropathology core that performs the autopsies and provides CSF and brain tissue to the laboratory scientists, and 4 projects investigating the pathophysiology of PD and PDD. To date > 5,000 clinical evaluations of >1,000 subjects have occurred. In the past year 15 PD/ parkinsonism and 15 control subjects came to autopsy. Projects 1 and 2 have found changes in the BDNF and DJ-1 proteins, respectively. Project 3 has found dysregulation of multiple sets of genes when comparing brain tissue of control, PD, and PDD cases. Project 4 has developed the methodology for CSF protein analysis. The second year's goals of our program have been met with plans for the next year of funding being on target. Eight papers have been published, four others have been submitted, and five presentations made. Additional funding from the Michael J. Fox Foundation has been obtained.

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Beach T, Adler C, Sue L, Peirce J, Bachalakuri J, Lue L, Caviness J, Conno D, Sabbagh M, Walker D. Reduced Striatal Tyrosine Hydroxylase in Incidental Lewy Body Disease. **Acta Neuropathol.** In press.

Beach T. Sue L, Walker D, Roher A, Lue L, Vedders L, Connor D, Sabbagh M, Rogers J. Sun Health Research Institute Brain Donation Program: Description and Expertise, 1987-2007. **Cell & Tissue Banking**. In Press.

Caviness J, Driver-Dunckley E, Sabbagh M, Connor D, Noble B, Evidente V, Shill H, Adler C. Defining Mild Cognitive Impairment in Parkinson's Disease (PD-MCI). **Mov. Disorders.** 22:1272-77, 2007.

Caviness J, Hentz J, Evidente V, Driver-Dunckley E, Samanta J, Mahant P, Connor D, Sabbagh M, Shihll H, Adler C. Both Early and Late Cognitive Dysfunction Affects the Electroencephalogram in Parkinson's Disease. **Parkinsonism and Related Disorders.** 13:348-54, 2007.

Sabbagh M, Lahti T, Connor D, Caviness J, Shill H, Mahant P, Samanta J, Burns R, Evidente V, Driver-Dunckley E, Reisberg B, Bircea S, Adler C. Functional Decline Correlates with Cognitive Impairment in Alzheimer's Disease and Parkinson's Disease. **Dementia, Geriatric, Cognitive Disorders.** 24:327-34, 2007.

Shill H, Adler C, Sabbagh M, Connor D, Caviness J, Hentz J, Beach T. Pathological Findings in Prospectively Ascertained Essential Tremor Subjects. **Neurology**. In press.

Adler C, Grover A, Sabbagh M, Caviness J, Connor D, Beach T. Clinical and Pathologic Findings in PD with LRRK2 Mutations: 2 Cases with Mild Cognitive Impairment and Small Amplitude Myoclonus. **Mov. Dis.** 21(Suppl 15): S583, 2006.

Beach T, Connor D, Sue L, Caviness J, Sabbagh M, Adler C. Motor and Cognitive Findings in Incidental Lewy Body Disease. **J. Neuropathol. Exp. Neurol.** 66(5): 422, 2007.

Leslie V. Boyer, M.D. **University of Arizona**
Award Amount FY06: $150,177

Scorpion Treatment and Imaging of Neurotoxicity Group (STING)

Neurotoxicity from scorpion sting is rare, but it may be life-threatening, especially in young children. This study addresses (1) the shortage of an effective treatment severe scorpion neurotoxicity and (2) the lack of a validated clinical assessment tool with which to further new drug development and train emergency providers. The infrastructure for providing experimental antivenom as therapy for significant scorpion stings, established in the last reporting period, was expanded and refined. A total of 16 rural and urban hospitals in Arizona are approved to treat patients and have treated over 250 patients ranging in age from 2 months to 80 years since the start of this study. This successful treatment has meant shorter hospital stays, fewer admissions to the Pediatric and Adult Intensive Care Units and increased safety for children and adults stung and treated in Arizona.

Danny Brower, Ph.D.

University of Arizona
Award Amount FY06: $49,995

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

The primary goal has been to develop a system that can identify specific genetic lesions that sensitize cells to the effects of particular anti-cancer drugs. This system takes advantage of the ease with which individual genes can be neutralized in cells from the fruit fly, Drosophila. To date, we have established procedures for eliminating hundreds of genes, one at a time, and asking if this sensitizes cells to die when a drug is added, and tested the assay using established anti-cancer drugs. We are now proceeding to use the system to test novel compounds that target pathways that potentially promote abnormal cell growth or survival in tumor cells and will attempt to uncover previously unknown drug/ genetic interactions.

David J. Duggan, Ph.D.

TGEN
Award Amount FY06: $50,000

Genetic Basis of Auriculo-condylar Syndrome in 2 Arizona Families

At the conclusion of year 1, a disease-causing locus had been localized to chromosome 20. Within the critical region of interest a candidate disease-causing gene, JAGI, was identified. In year 2, the complete coding sequence of this candidate gene JAGI was resequenced. Four novel DNA sequence variants were identified. One of the variants, a T-to-C nucleotide change, segregated with the disease phenotype at least for the 4 affected individuals that were resequenced. Further testing of the identified and putative disease-causing T-to-C change revealed that the base change did not segregate with the disease phenotype when the entire pedigrees from both families were tested. Additionally, genetic markers in the form of microsatellites were subsequently genotyped. These data were integrated with the genome-wide linkage data from the Affymetrix 10Kstudy (year 1) and the resequencing data (year 2). Haplotype analysis of the combined data revealed that we had successful narrowed the region of interest to a minimum 2.3Mb region flanked by markers rs771943 and 26B-2. In the final year (year 3), we intend to (1) prioritize the candidate genes in this newly defined minimal region of interest, (2) resequence the candidate gene(s), (3) confirm the identification of a putative causative variant in all members of the pedigrees, (4) confirm the identified variant is not an unreported polymorphism, and (5) determine the functional significance of the identified variant(s).

Dr. Jason Johnson (UA Med. Sch. 2006) was part of the group that published the hearing finding on individuals from these families (Storm et al. 2005). Dr. Johnson has also presented three oral presentations and or posters at national and international meetings on this subject matter.

Katerina Dvorak, Ph.D.

University of Arizona
Award Amount FY06: $153,922

Barrett's Esophagus Esophageal Adenocarcinoma and Apoptosis

About 10% of patients with chronic heartburn have Barrett's esophagus (BE), a premalignant condition associated with a 30-fold increased risk for the development of esophageal cancer (EC).

The major focus of our studies is to identify molecular pathways that are responsible for the development of BE and for the progression from BE to EC. Bile acids and gastric acid are two major components of refluxate. We found that the combination of gastric acid and bile acids induce oxidative stress and DNA damage leading to increased mutations, activation of anti-apoptotic pathways and an increase in cells with damaged DNA. We used several different experimental approaches on tissue samples and cell lines obtained from normal, BE and EA patients. The results from these studies resulted in new NCI/NIH funding (Specialized Program of Research Excellence), presentation at national and international meetings, and publications in high ranking journals.

Dvorak K, Payne C, Chavarria M, Ramsey L, Dvorakova A, et al. Bile Acids in Combination with Low pH Induce Oxidative Stress and Oxidative DNA Damage: Relevance to the Pathogenesis of Barrett's Oesophagus. **Gut.** 56: 763-71, 2006.

Dvorak K, Chavarria M, Papyne C, Ramsey L, Crowle-Weber C, Dvorakova K, et al. Activation of the Interleukin-6/Signal Transducers and Activators of Transcription 3 Antiapoptotic Pathway in Esophageal Cells by Bile Acids and Low pH: Relevance to Barrett's Esophagus. **Clin. Cancer Res.** In press.

Dvorak K, Garewal H. Synergistic Interaction of Low pH and Bile Acids. **OESO Congress Book**, Guili ed. In press.

Dvorak K, Garewal H. How Might GERD Promote Carcinogenesis in Barrett's Esophagus? **OESO Congress Book.** Guili ed. In press.

Dvorak K. Are Genetic Alterations Observed in Non-dysplastic Barrett's Epithelium? **OESO Congress Book.** Guili ed. In press.

Dvorak K, Bernstein H, Payne C, Bernstein C, Garewal H. Bile Acid Induction of Apoptosis in Relation to Gastrointestinal Cancer. **Bile Acids: Toxicology and Bioactivity**, Jenkins and Hardie eds. RSC Publishing.

Scot W. Ebbinghaus, M.D.

University of Arizona
Award Amount FY06: $50,000

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

Our major goals are to understand how an important leukemia gene called MYB is controlled and discover drugs that could be used for leukemia treatment by shutting off MYB expression. We found that the on-off switch for MYB expression contains a repetitive DNA sequence, a GGA repeat region, which controls MYB expression, and we discovered that a protein called MAZ binds to the GGA repeat region to repress MYB expression. MYB expression is repressed by MAZ when leukemia cells are treated with Gleevec®, a targeted therapy for leukemia. This finding suggests that drugs that bind directly to the GGA repeat region could be used to silence MYB expression to treat leukemia, and we are testing drugs that can bind to a unique DNA structure, discovered in our laboratory, formed by the GGA repeat region of the MYB gene.

Mohamed Gaballa, Ph.D.

Sun Health Research Institute
Award Amount FY06: $50,000

Human Umbilical Progenitor Cell Based Therapy for Myocardial Infarction

Intramyocardial and intravenous delivery of Human umbilical cord blood cells (hUCBCs) improves vascularization (the # of blood vessels) and heart function after myocardial infarction (M, heart attack). The effects of intracoronary delivery of hUCBCs on vasculogenesis induction and its mechanisms in the ischemia-reperfusion model remain unclear. Ischemia was produced in immune compromised rats by ligation of the left anterior coronary artery for 60 minutes, followed by reperfusion with either 150 ml of media containing one million stem cells or media only. Animals were studied 3 weeks later. Compared to media only, left heart muscle contractile pressure increased and left heart size was decreased, in animals treated with stem cells. The improvement in heart function is more likely due to the increase in the number of new blood vessels after stem cells delivery. In conclusion, intracoronary delivery of hUCBCs improved heart function after an acute heart attack.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY06: $50,000

Ultra Miniature Endoscopes for Biomedical Imaging

The general objectives of this research are 1) to build an ultra-miniature endoscope with simultaneous diffuse white-light reflectance imaging and fluorescence imaging capability and 2) to demonstrate unique scientific and clinical applications for the instrument. Ultra-miniature endoscopes with total diameters of 0.5mm have been successfully built and evaluated exploiting the concept of numerical aperture sharing. With this technique, illumination and imaging can be done down the same fiber optic imaging bundle channel. Excellent suppression of background from the proximal end of the imaging catheter has been achieved via this approach. Distal end reflection from the GRIN lens surface is now the performance limiting aspect of the numerical aperture sharing system. A design for a multi-modal system with white-reflectance and fluorescence imaging channels has been completed and is under construction. Small scale (less than 1mm) catheters have also been built with more conventional separated illumination and imaging channels, and these have been used in imaging studies of mouse models of Barrett's esophagus. Mouse model imaging using the various ultra-miniature endoscopes will continue to be used to test and validate the systems that have been constructed.

Kano A, Gmitro A. Ultrathin Fiberscope Utilizing a Single Channel for Both Illumination and Imaging. **Frontiers in Optics**. FTuG4, 2005.

Kano A, Koshel R, Gmitro A. Broadband Endoscopic Imaging Through a Single Fiberoptic Channel. **SPIE Optics & Photonics**. 6668-7, 2007.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY06: $150,000

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

The overall goal of this inter-institutional multidisciplinary project is to investigate Sonoran desert organisms for novel cell motility (migration) inhibitors and to conduct structure-activity relationship (SAR) studies of beauvericin, a fungal metabolite encountered in a previously funded ABRC project, with the broad long-term objective of discovering and developing novel anticancer agents to treat solid tumors. During the course of the second year of this project, screening of plant and microbial extracts for inhibition of cell-motility and precursor-directed biosynthesis, and *in vivo* and *in vitro* mutasynthesis of beauvericin analogs for SAR studies were continued. In addition, the most active analog of streptimidone isolated from Streptomyces albofaciens, namely dihydrostreptimidone, obtained by semi-synthesis was subjected to gene expression profiling in brain, bladder, and pancreatic cancer cell lines. Studies on other compounds inhibiting cell migration are currently in progress. We are hopeful that this project would provide lead compounds that can be further studied and developed into natural product-based non-toxic drugs that can be used to treat metastatic solid tumors. Such anticancer drugs might eventually alleviate the suffering of many people in Arizona and rest of the world.

Xu Y, Zhan E, Wijeratne E, Burns A, Gunatilaka L, Molnar I. Cytotoxic and Anti-haptotactic Beauvericin Analogues from Precursor-directed Biosynthesis with the Insect Pathogen Beauveria bassiana ATCC 7159. **Journal of Natural Products**. 70: 1467-71, 2007.

Bashyal A, Burns M, Liu M, Paranagama P, Seliga T, Turbyville E, Wijeratne E, Zhan J, Gunatilaka M, Arnold A, Faeth S, Whitesell L, Gunatilaka L. Discovery of Small Molecule Bioactive Agents from Endophytic Fungi of the Sonoran Desert. Proc. 6th Internat. Symp. Fungal Endophytes of Grasses. 211-14, 2007.

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY06: $183,010

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

The overall objective of this proposal is to characterize the structure of the i-motif in the silencer element of the c-MYC promoter and also its drug complexes, which will then be used as basis for drug design and development. The long-term objective is to identify a small molecule that will selectively modulate c-MYC gene expression and then work to identify a clinical candidate molecule.

During the time period covered by this report, we have made progress on specific aims 2–4. The specific aims are:

2. to define the structure of the biologically relevant i-motif in the promoter region of c-MYC and its drug complexes by calorimetry,
3. to define the overall structure of the silencer element in the c-MYC promoter and its complexes with agents that modulate c-MYC gene expression,
4. lead identification and subsequent optimization and drug development.

In addressing specific aim 2, we have characterized the thermal stability of at least two equilibrating species in a variety of solution environments. In addressing specific aim 3, molecular models have been created for the c-MYC nuclease hypersensitivity element III, from the two 39-base complementary sequences. Finally, in addressing specific aim 4, we have identified a number of lead compounds for further optimization.

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Buscaglia R, Freyer M, Cashman D, Blynn M, Hurley L, Lewis E. Structural Stability and the Porphyrin Binding Interactions of a Mutant Construct of the i-Motif Forming Sequence in the Human c-MYC NHE III. **Nucleic Acids Research**. 2007. Submitted.

Cashman D, Freyer M, Dettler J, Hurley L, Lewis E. Molecular Modeling and Biophysical Analysis of the C-MYC NHE III Silencer Element. **Journal of Molecular Modeling**. 2007. In press.

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Edwin A. Lewis, Ph.D.

Northern Arizona University
Award Amount FY06: $149,920

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

BCL-2 protein functions as an inhibitor of cell death and has been found to be aberrantly expressed in a wide range of human diseases. Approximately one third of all new U.S. cancer incidences each year involve BCL-2 over expression. Preclinical studies of anti-BCL-2 agents have demonstrated improved antitumor activity. The final goal of this research is to identify lead compounds for the development of drugs that will turn off BCL-2 expression. During the time period covered by this report, we have characterized the thermal stability of the BCL-2 model quadruplex using DSC, fluorescence and Circular Dichroism. Using TMPyP4 as a model drug to probe the structure, we have discovered that the BCL-2 quadruplex structure and small molecule binding processes are almost exactly the same as previously determined for the c-MYC proto-oncogene. In spite of having a more complex series of guanine runs than c-MYC, the wild-type BCL-2 oligonucleotide forms fewer different quadruplex conformers than expected.

John Lewis, M.D., MPH

Inter Tribal Council of Arizona
Award Amount FY07: $150,000

Promoting Tribal Community Participation in Biomedical Research

Arizona's Bioscience Roadmap describes a comprehensive strategy to promote bioscience research in the State of Arizona. Implementation strategies focus on building partnerships and collaborations among the State's public and private sectors. Platform Committees were developed to identify specific collaboration issues, and among these issues is a recommendation to establish a "Special Population Alliance" in Arizona. The committees recognize that Arizona has a significant population of American Indians, and that Tribes are sovereign entities. Investigators need to understand that each Tribe has its own procedures for reviewing research proposals, and investigators need to adhere to these procedural systems to appropriately engage Tribes in the research process and to ensure inclusion in Arizona's Roadmap.

Unfortunately, there is currently no single document that outlines the research review processes for each Tribe in Arizona. Additionally, most Tribes have not been engaged by the research community in such a way as to develop their own research agendas. Therefore, specific aims of the proposed research are 1) identify and describe the research review processes for each of the 22 American Indian Tribes in Arizona; 2) develop decision-making approaches and identify priorities for establishing Tribal Research Agendas; and 3) develop a Regional Tribal IRB and/or Tribal Research Review Technical Assistance Program based on the findings in specific aims 1 and 2.

The objectives of this project include the following:

- describe the salient processes for researchers to follow in order to appropriately engage Tribal communities;
- streamline the processes for effective communication between Tribal communities and the research community;
- create a document, Inventory of Tribal Research Review Processes in the State of Arizona, that clearly describes these processes;
- work in partnership with Tribes to develop decision-making approaches for establishing their own research agendas and create a document outlining these approaches; and
- identify the need for and develop a Regional Tribal Institutional Review Board and/or a Tribal Research Review Technical Assistance Program as determined by the Tribes.

Rena Li, M.D., Ph.D.

Sun Health Research Institute
Award Amount FY06: $50,000

Role of Estrogen in BACE Regulation *in Vitro* and *in Vivo* Systems

Increasing evidence indicates that postmenopausal women have a higher risk of developing Alzheimer's disease (AD) than do age-matched men. In some postmenopausal women β-secretase (BACE) enzymatic activity is significantly increased and this elevation is correlated with amyloid load in AD brains. This project examines the role estrogen plays in β-secretase (BACE) activity.

Data collected during the project year demonstrates that BACE activity is significantly altered in APP transgenic mice with genetic deletion of aromatase (APP/ArKO) and that the BACE activity in the APP/ArKO mice appears earlier and creates more AD neuropathology. In addition, we have identified an estrogen-binding site in our recently cloned BACE promoter.

Lonnie P. Lybarger, Ph.D.

University of Arizona
Award Amount FY06: $50,000

CD8 T Cell Priming Using Engineering MHC Class I Molecules

The goal of this research project is to evaluate the utility of a promising new vaccine technology for the generation of anti-cancer immune responses. Cancer is a leading health concern in Arizona, with approximately 24,000 new cases in Arizona in 2004. The immune system, through MHC class I molecules, can detect and eliminate tumor cells. Here, we have developed a novel strategy to engineer MHC class I molecules to be especially potent in terms of their ability to stimulate anti-cancer T cells. This project will compare the ability of these engineered molecules to prime anti-cancer immune responses versus more conventional vaccine approaches in a mouse model that mimics human cancers. We have generated a series of reagents for these experiments and have begun testing their efficacy in vaccine trials in mice.

T. Philip Malan, M.D.

University of Arizona
Award Amount FY06: $50,000

Long-term Activation of Pain-enhancing Systems Following Short-term Opioid Use

Opioids, drugs like morphine, are the most effective drugs used for the treatment of severe pain. However, they also have properties that may worsen pain, a highly undesirable side effect. We are studying the mechanisms by which short-term opioid administration, such as is used to treat postoperative pain, produces a delayed and long-lasting increase in pain sensitivity. Previously, we found that selected sensory pathways leading from the spinal cord to the brain, pain-regulating pathways descending from the brain to the spinal cord, and activity of pain-enhancing molecules are necessary for opioid-induced pain hypersensitivitity. This year, we enhanced our data regarding the effects of opioids after surgical incision, to prepare for publication of these results. In addition, we discovered that the central amygdala is an essential component of the descending pain facilitating system that is activated by opioids. These findings give important insights into this unwanted side effect of pain-treating drugs.

Istvan Molnar, Ph.D.

University of Arizona
Award Amount FY06: $49,885

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

The objective of the project is to discover novel natural products in a unique collection of bacteria at the Natural Products Center, University of Arizona for the development of anticancer drugs. These bacteria originate from the rhizosphere of desert plants growing under extreme environmental conditions. We have characterized 470 bacterial strains by molecular taxonomy and selected 107 for fermentation in 4 culture media. The resulting 428 extracts were assayed in a mechanism-based and a general cytotoxicity assay. Hits from these assays were fermented in liquid culture. Scale up yielded 8 bacteria reproducibly producing 15 extracts with cell proliferation inhibitory activity. Prioritization of positive extracts from the first project year provided an additional 6 bacterial strains producing 14 extracts with cell proliferation inhibitory activity. These extracts could contain novel cytotoxic metabolites that might be used for anticancer chemotherapy, improving the health prognosis of cancer patients in the state of Arizona and worldwide.

Jitendran Muthuswamy, Ph.D.

Arizona State University
Award Amount FY07: $150,000

Microactuated Microeletrodes to Access Cortical Roles in Memory Deficits

During FY06, progress was made in the following three critical areas 1) successfully developed an optimal set of polysilicon fabrication conditions necessary for one-way communication with neuronal tissue in the brain, 2) identifying an effective hydrophobic, resealable, sealant that will keep the micromotors in the implanted chip isolated from the surrounding brain fluids, and 3) preliminary demonstration of a novel packaging strategy for MEMS based brain implants.

Identifying the optimal processing conditions for polysilicon will impact the successful development of both *in vitro* and *in vivo* brain implants that are used in advanced prosthetic devices for the brain. The novel silicone gel reported and characterized by our study will provide an attractive alternative for neurosurgical procedures that require repeated brain penetrations by implants. Lastly, the flip-chip on flex approach developed by our lab for implantable microdevices will have significant impact on how future microdevices are packaged for implantation.

John J. Osterhout, Ph.D.

University of Arizona
Award Amount FY06: $49,999

Structure and Mechanism in Alzheimer's Disease

The objectives of this research are 1) to study the structure of fibrils and aggregates of Aβ(1-42) and 2) to determine the interaction of Aβ(1-42), its aggregates and fibrils with membranes. Fibrils of Aβ(1-42) have been formed by standard methods. Fibril dissolution and peptide separation studies have been performed. Cross-linking studies are now beginning. Plasmon waveguide resonance (PWR) has been used to measure the interactions of Aβ(1-42) with membranes. Interestingly, the interaction seems to proceed in two phases which are now attributed to binding the elongation of the peptide from within the membrane. Our efforts are now aimed at detecting amyloids fibrils in membrane vesicles to confirm the presence of amyloid fibril formation. This work bears directly on the underlying mechanism of Alzheimer's disease, and so is of great significance to the people of Arizona.

Alyssa Panitch, Ph.D.

St. Joseph's Hospital
Award Amount FY07: 250,000

Biomimetic Scaffolds for Spinal Cord Regeneration

A therapeutic approach that combines the mechanical support of scaffolding with molecules that are present during development, and molecular cues to the regenerating axons, will improve the potential for functional recovery after spinal cord injury. The hypothesis of this investigation is that complementing biologically relevant developmental scaffolds with cell signaling peptides will improve neurite outgrowth from primary cortical neurons and improve rate and number of regenerating neurons after spinal cord injury. Progress over the past year has demonstrated that insertion of hyaluronic acid gels of appropriate concentration into the injured spinal cord decrease the volume of injured tissue. It is anticipated that coupling the gels with regeneration signals will improve recovery after spinal cord injury. Progress has been made in incorporation of the neurotrophic signals and the function of the improved materials is being tested.

Joseph Rogers, Ph.D.

Sun Health Research Institute
Award Amount FY06: $225,000

Translational Research on Aβ Metabolism, from Synthesis to Clearance

This multi-institutional, multi-disciplinary research program seeks to discover new diagnostic and treatment methods for Alzheimer's disease based on mechanisms for synthesis and clearance of amyloid β peptide (Aβ), a molecule that forms millions of toxic deposits in the Alzheimer's brain. Project 1 and Project 3 are collaboratively developing methods to lower Aβ production so that toxic Aβ deposits never form. Project 2 has shown that Alzheimer's patients have a deficit in clearing Aβ from the body, and that this defeat, which can be measured in a blood sample, may be diagnostic for Alzheimer's. In addition, based on the findings, Project 2 and Project 3 are collaboratively developing methods to clear Aβ from the body at a faster rate, thereby increasing Aβ clearance from the brain. Arizona has some of the world's most concentrated Alzheimer's populations. This project may provide new treatments and the first viable, inexpensive diagnostic for the disorder.

Adrienne C. Scheck, Ph.D.

Barrow Neurological Institute
Award Amount FY06: $50,000

Molecular Analysis for the Diagnostic Identification of Clinically Aggressive Meningiomas

Meningiomas are the most commonly reported brain tumor in the US, accounting for ~27% of all primary brain tumors. They are typically considered benign tumors that can be cured by complete surgical removal; however, a percentage of patients have recurrent disease after apparently complete removal of a low grade tumor. The development of diagnostic tools that identify molecularly-defined subsets of meningiomas that will behave aggressively would allow the use of additional therapy prior to recurrence, resulting in an improvement in survival and quality of life. We are doing gene expression analysis of a large group of meningiomas to identify genetic markers of aggressive tumors. To provide a more accurate list of potential markers, we have increased the representation of aggressive tumors in our sample set. We have also added proton magnetic resonance spectroscopy to our analyses. This is a non-invasive procedure that can be done in patients prior to surgery.

Jiaqi Shi, M.D., Ph.D.

University of Arizona
Award Amount FY06: $50,000

Deregulation of Translation Initiation by eIF3f in Melanoma

The hypothesis of this project is that eIF3f is a translation inhibitor, and that disruption of eIF3f function contributes to melanoma tumorgenesis by deregulating translation and apoptosis. One specific aim is to evaluate eIF3f gene abnormalities in melanoma. We have finished the analysis of the loss of eIF3f gene in 24 melanoma samples by LOH using three different microsatellite markers. We also performed mutation analysis of eIF3f gene in melanoma tissue samples. Decreased eIF3f protein expression in melanoma was demonstrated by IHC. Another specific aim is to characterize the mechanism of eIF3f-mediated regulation of translation and apoptosis. We used proteomics approach to identify proteins associated with eIF3f. This analysis revealed that hnRNP K increased association with eIF3f during apoptosis. Interestingly, hnRNP K is implicated in regulating rRNA and mRNA stability. We then confirmed that during apoptosis there is a significant increased association between endogenous eIF3f and hnRNP K by co-IP. These results support our hypothesis that eIF3f regulates rRNA degradation, translation and apoptosis through interaction with hnRNP K, which will be further investigated in Aim 2.

Shi J, Kahle A, Hershey JWB, Honchak B, Warneke J, Leong S, Nelson M. Decreased Expression of Eukaryotic Initiation Factor 3f Deregulates Translation and Apoptosis in Tumor Cells. **Oncogene**. 25: 4923-36, 2006.

Daekyu Sun, Ph.D.

University of Arizona
Award Amount FY06: $48,984

Targeting Tumor Angiogenesis Using G-quadruplex Interactive Ligands

The characterization of secondary DNA structures in the promoter regions of cancer-related genes could result in the evolution of an entirely new approach to anticancer drug design and development through the drug targeting of these secondary DNA structures. The main objective of this project was to explore a new therapeutic strategy aimed at preventing the growth of new blood vessels during tumorigenesis by targeting i-motif structures formed by the C-rich sequence formed in the promoter region of VEGF gene with small molecules to repress the transcription of this gene. As a direct consequence of this work, we have made the important observation that the C-rich strand of the double-stranded polyguanine/polycytosine sequence of the VEGF promoter can spontaneously convert to i-motif structures in a cell-free system, while a guanine-rich sequence of the same region can be readily converted to a kinetically flavored parallel G-quadruplex, which could potentially silence gene expression. To investigate the formation of i-motif structures by C-rich strands, we employed circular dichroism (CD) in combination with a Br2 chemical footprinting technique. Interestingly, there is also evidence that hnRNP K, which binds to the C-rich element in the single-stranded DNA form, also interacts with Pol II via TBP to initiate transcription of the VEGF gene, suggesting drug binding to the i-motif could inhibit conversion to the single-stranded DNA form and prevent hnRNP K binding to the C-rich element, effectively repressing VEGF transcription. On the basis of our accumulated data, we could further exploit a new approach by targeting the i-motif, which is a critical component of the silencer element controlling transcription of VEGF. If successful, this strategy will lead to additional first-in-class drug molecules and a new molecular targeted approach in cancer therapeutics.

Tom Tsang, Ph.D.

University of Arizona
Award Amount FY06: $50,000

Cancer Immunotherapy by TCR-modified HSC Transfer

About ten thousand people in Arizona die from cancer annually. Therefore, new cancer treatments are urgently needed. We are attempting to combine three highly promising new therapies—gene therapy, immune therapy and non-embryonic stem cell therapy—into one novel cancer fighting strategy. Specifically, we aim to create new genes that can recognize and destroy cancer cells, put them into immune system stem cells and then allow the stem cells to form an entirely new immune system. The new immune systems, in theory, would only recognize and destroy cancer cells. The first step, which is constructing a new cancer fighting gene, is now complete. This gene has been inserted into a replication defective HIV virus so that it can be transferred into the appropriate stem cells. Next, these stem cells will be tested for their ability to form new immune systems and for the immune system's ability to reject cancer cells.

Johanna K. Wolford, Ph.D.

Translational Genomics Research Institute
Award Amount FY06: $50,000

Diabetic Kidney Disease in American Indians

Diabetes is the leading cause of kidney failure in developed countries, and people with diabetic kidney disease are often disabled or die prematurely. Inherited (genetic) factors appear to strongly influence the risk of developing diabetic kidney disease. The overall goal of this study is to identify the genetic determinants of diabetic kidney disease in Native Americans, who have very high rates of this complication of diabetes. Studies conducted in one group of Native Americans, the Pima Indians, suggested that genes on chromosome 3 increase susceptibility to diabetic kidney disease. In year 1 of this study, we initiated investigations of genes mapping to this region of linkage and found substantial evidence for association between variants in the succinate receptor gene (SUCNR1) and diabetic nephropathy in Pima Indians. These results strongly support a role for SUCNR1 in the development of diabetic kidney disease in this population.

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

St. Joseph's Hospital
Award Amount FY07: $49,271

Nicotine Acetylcholine Receptors in the VTA and Nicotine Dependence

Nicotinic acetylcholine receptors (nAChRs) expressed in the brain reward center (ventral tegmental area, VTA) contain devise subunit compositions and contribute generally to pleasure, reward and drug reinforcement, but the pharmacological properties are largely unknown. We systematically evaluated nAChR pharmacological properties using patch-clamp recordings in single DA neurons freshly dissociated from rat VTA. We clarified three functional subtypes (ID, IID and IIID) of nAChRs based on their pharmacological features. ID neurons were sensitive to α4β2-nAChR agonist, RJR-2403 and antagonist; dihydro-β-erythroidine (DhβE)' IID neurons were sensitive to selective α7-n-AChR agonist choline and antagonist, methyllycaconitine (MLA); while IIID neurons were sensitive to β4-containing nAChR agonist cytisine and sensitive to mecamylamine (MEC), suggesting that ID, IID, or IIID neurons predominantly express α4β2-, α7-, or α3β4-containing nAChRs. Collectively, in single VTA DA neurons, we have characterized pharmacological features of three functional subtypes of nAChRs which may contribute to different roles in nicotine dependence.

Section C

Continuing Contracts

Medical Research

Year One

FY 2007

Craig A. Aspinwall, Ph.D.

University of Arizona
Award Amount FY07: $48,323

Stabilized Polymer Phospholoid Imaging Probes

For project year 1 (2006–2007 of ABRC contract number 0701), we have successfully developed a new class of nanometer-sized, biomimetic chemical labels that may prove useful for *in vivo* fluorescence, imaging of small changes in cellular mass associated with Type I diabetes as well as a number of other disease states, including cancers. The nanoprobes possess a highly fluorescent core coated with a cross linked phospholipid bilayer membrane. During year one, we investigated a number of fluorescent core materials including silica sol-gel and polyacrylamide polymer nanoparticles doped with fluorescent indicators. Key demonstrated advantages of this novel fluorescent probe include a) markedly increased higher sensitivity; b) enhanced biocompatibility, i.e. reduced biofouling; c) improved chemical and environmental stability; and d) improved selectivity (e.g. lower non-specific adsorption). With continued development and characterization, this probe geometry should prove useful for disease diagnosis, prognosis and early detection of disease states.

Senarath-Yapa M, Phimphivong S, Coym J, With M, Aspinwal C, Saaverdra S. Preparation and Characterization of Poly(lipid)-coated, Fluorophore Doped Silica Nanoparticles for Biolabeling and Cellular Imaging. **Languir**. In press.

Yongchang Chang, M.D., Ph.D.

St. Joseph's Hospital
Award Amount FY07: $49,973

Mechanisms of p1 GABAc Receptor Activation and Antagonism

Last year, with the support of ABRC grant 0702, we finished two sub-projects and submitted an abstract to the Annual Meeting of Society for Neuroscience. Now, we are preparing two manuscripts 1) Agonist and Antagonist Induced Opposite Conformational Change of the Loop F in the p1 GABA Receptor; and 2) Structural Basis for Distant GABA, and GABAc Receptor Antagonist Pharmacology. In the first manuscript, we identified structural elements in a region of GABAc receptor that is important in receptor function. In the second manuscript, we have identified key structural elements in GABAc receptor underlying its antagonist properties distinct from GABAa receptor. Our results will help to design new GABA receptor subtype specific compounds to selectively target specific subtypes of GABA receptors for drug development to treat neurological and psychiatric disorders with GABA receptor dysfunction such as sleep disorders, seizures, depression, anxiety, and schizophrenia, which affect many Arizonians.

Richard J. Coast, MS., Ph.D.

Northern Arizona University
Award Amount FY07: $43,130

Fatigue and Training of Respiratory Muscles via Non-respiratory Activity: Implications for Rehabilitation

This project was designed to evaluate whether core exercises could fatigue the respiratory muscle and whether those activities could be used to train the respiratory muscles. We have performed studies that show subjective indices of respiratory muscle fatigue give the same results as more objective indices and are easier to perform. We have also shown that non-respiratory exercises (sit-ups) can cause fatigue of the respiratory muscles, and that other, easier, non-respiratory exercises such as toe-touches activate the respiratory muscles. We are now using those exercises to train the respiratory muscles. If these training studies proceed as we suspect, we will find exercises that are easy to perform that can train the respiratory muscle. If such exercises increase the strength and fatigue resistance of respiratory muscles in pulmonary disease patients, they may prove to be good for training these subjects. We will work with another researcher to study this aspect of training should the current studies be positive.

Paul Coleman, Ph.D.

Sun Health Research Institute
Award Amount FY07: $50,000

Development and Validation of a Blood Diagnostic for Alzheimer's Disease

Four and a half million Americans are now estimated to have Alzheimer's disease. We also know that even more people have early stages of disease, but have not yet been diagnosed because their symptoms are not yet severe. In fact, we now know that the disease starts decades before it is clinically diagnosed, so that the disease has decades to damage the brain before the effects of disease become severe enough to come to medical attention. The fact that the disease starts decades before it is diagnosed dictates two major goals for Alzheimer's disease research: 1) early detection, before the brain has been overwhelmed by disease and 2) effective treatment to halt or slow progression of the disease so that people who have the disease can live the rest of their lives free of the symptoms of disease, as many people with high blood pressure can, with effective treatment, live normal lives. In our project "Development and Validation of a Blood Diagnostic for Alzheimer's Disease" we are working on the first goal of early detection of Alzheimer's disease by a blood test. We already have evidence that examination of the expression of a limited number of genes can distinguish people already diagnosed with AD. Our goals now are to validate this in a larger sample of people already diagnosed with AD and also to extend our studies to people at risk for developing Alzheimer's disease to see whether we can, with our blood test, detect the disease early by predicting those who will be diagnosed with AD several years later. The support of the people of Arizona in making this work possible is very much appreciated.

John K. DiBaise, M.D.

Mayo Clinic Scottsdale
Award Amount FY07: $136,322

Transmucosal Delivery of Erythromycin to Treat Gastroparesis

Gastroparesis, a condition in which an abnormal delay in stomach emptying occurs, is commonly associated with diabetes mellitus and results in numerous gastrointestinal (GI) symptoms and inconsistent delivery of medications. This is an important problem for Arizona because of the high incidence of diabetes, particularly among the Native American population. A method to deliver medications that improve stomach emptying that bypasses the GI tract may provide more consistent levels of these drugs and result in improvement in gastroparetic symptoms, diabetes control and quality of life. The immediate goal of this research study is to develop a sublingual system that is able to deliver therapeutic levels of erythromycin, a potent stimulant of stomach emptying. During the first year of this study, we have successfully demonstrated the *in vitro* release of erythromycin from Carbopol, developed a reliable test to measure erytromycin levels, and have begun preparing for the sublingual rat pharmacokinetic studies.

Kathleen Dixon, Ph.D.

University of Arizona
Award Amount FY07: $149,822

Imaging of Markers for Skin Cancer Risk

Arizona has one of the highest rates of skin cancer in the world. Exposure to UV radiation in sunlight is a major risk factor for skin cancer development. We have established a statewide multidisciplinary collaboration for the image analysis of cellular responses to UV radiation. This work focuses on the identification of skin cancer susceptibility factors and the development of chemopreventive agents. This collaborative project involves investigators at two major Arizona universities (University of Arizona and Northern Arizona University) including mathematicians and statisticians from UA and biomedical scientists from UA colleges of Medicine and Science, the Arizona Cancer Center, and the NAU college of Engineering and Science. The ultimate goal of this work is to provide tools that can be used in a clinical setting to monitor skin cancer susceptibility, progression, and responses to prevention/intervention strategies.

Graeme J. Dougherty, Ph.D.

University of Arizona
Award Amount FY07: $49,535

Molecular Therapy of Bladder Cancer

Bladder cancer is a common malignancy. In the state of Arizona alone, there are over 1000 new cases diagnosed each year and this number is expected to rise as the population ages. The goal of the present study is to explore a novel approach to the treatment of bladder and other cancers that employs a genetically-engineered DNA construct, the product of which can trigger tumor cell death upon the binding of a soluble molecule that is differentially produced within the tumor microenvironment. Studies carried out to date have helped define the precise nature of the signals that can activate the tumor cell killing activity of this "molecular medicine" as well as certain of the processes employed by tumor cells to avoid such killing. It is hoped that these studies will help define the patient group most likely to benefit from this novel form of therapy.

Lars Ewell, M.D., Ph.D.

University of Arizona
Award Amount FY07: $50,000

Diffusion-weighted MRI and Magnetic Resonance Spectroscopy to Differentiate Radiation Necrosis and Recurrent Disease in Gilomas

We are pleased with the progress that we have achieved in the first year of our contract. The funds allocated allowed us to hire Naren Vijayakumar, a graduate student in Electrical and Computer Engineering. Utilizing his computer programming skills, he has written image analysis programs (mainly in Interactive Data Language (IDL®)) that have allowed us to analyze MRI scans from the 11 patients currently enrolled in our imaging protocol. We presented some of his results at this year's American Association of Physicists in Medicine (AAPM) conference in Minneapolis, 7/22/07. This work enabled us to more accurately measure an Apparent Diffusion Coefficient (ADC) for a disease site in the brain. The ADC allows for physicians and researchers to monitor treatment for gliomas (brain tumors). Having an indication of how effective a given treatment is, can allow for clinical decisions to be made that can result in better quality of life for patients being treated in the Department of Radiation Oncology at the University of Arizona Medical Center.

Robert J. Gillies, Ph.D.

University of Arizona
Award Amount FY07: $250,000

Multimeric Ligands for Targeting Cancer for Imaging and Therapy

A goal for diagnosis and treatment of cancer is to target the diagnostic imaging or therapeutic agents directly to the cancer without cross-reacting with other cells or tissues. Cell surface receptors are attractive as targets because the targeting agents do not have to cross the cell membrane. This proposal seeks to develop agents that can discriminate these targets cells from normal cells in humans using heteromultivalent ligands (htMVLs) nanoparticles that contain different ligand binding motifs attached to a common linker. htMVLs can cross-link two or more different receptors, leading to extremely high binding specificity. This proposal is focused on Pancreatic Cancer, which is an area of strength in Arizona, at the Mayo, UA and TGen. This technology was invented at the UA and Tgen which remain leaders in this field.

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John G. Hildebrand, Ph.D.

University of Arizona
Award Amount FY07: $49,999

Kissing Bugs in Southern Arizona Potential Risks for Human Health and Development of Tools for Monitoring and Control

We have successfully established a colony of T. rubida, the most abundant species around Tucson. Wild T. rubida was found widely distributed in and around Tucson, and surprisingly, 6 insects were captured in Central Tucson. Every specimen is being analyzed for the presence of T. cruzi, a parasite that can cause Chagas Disease. The parasite is transmitted through the insect feces. Our study of the defecation patterns of T. rubida indicates that the juveniles are not very effective in transmitting the parasite, whereas adult females could potentially be good transmitters (vectors). We are testing a trap for T. rubida. Even when we are able to trap the bugs, our present prototype needs improvement to increase trapping. We conducted activities that increased public awareness of the risks attributable to these insects. We developed a website, organized public outreach activities, and our research was featured by the media (press and TV).

James B. Hoying, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Genetics Diagnostics of Angiogenesis

The overall goal of this project is to identify genes that when "on" or "off" are 1) indicative of small blood vessels (microvessel) health and 2) potential targets for new therapies. Our efforts this first year have resulted in the development and characterization of an ideal system, or model, necessary for our studies. This model recreated all aspects of a growing microvasculature in the body and allows us to make clear measurements of gene expression/activity. With this model, we have generated very good biological data on how networks of blood vessels form during tissue healing and corresponding genetic data. Our results show that microvessels can exist in three distinct health conditions (something which was not described before). In addition, gene expression during this process is highly dynamic providing a good foundation for identifying new diagnostic genes. This work has been submitted to the scientific journal Physioloical Geneomica for publication.

Richard D. Lane, M.D., Ph.D.

University of Arizona
Award Amount FY07: $150,000

Neural Basis of Vagal Tone Dyregulation in Depression

Depression is a major public health problem in Arizona. Major depressive disorder (MDD) is a common and disabling condition for which treatment exists, however, many patients do not respond and most do not recover fully. The purpose of the research is to examine how patterns of brain activity interact with physiological responses (particularly heart rate variability [HRV] in patients with MDD, as compared to healthy volunteers. If HRV is an indicator of brain activity, clinicians could use HRV to help diagnose and treat depression which would ultimately lead to reductions in the prevalence of depression in Arizona.

Three depressed patients have enrolled so far (one has completed the study and two are currently active). Ten fMRIs have been obtained, and HRV and clinical data have been collected at all patient visits. Analysis of imaging, HRV and clinical data is being conducted as the information is obtained.

Ana Maria Lopez, M.D.

University of Arizona
Award Amount FY07: $150,000

Expedited Breast Cancer: A New Model In Breast Health

Patients requiring breast biopsy were approached sequentially for participation at a community hospital and a mammography center. Following biopsy, tissue underwent ultra-rapid processing. Slides were reviewed by telepathology. Time assessments were collected. Patients from the mammography center received their results as per usual care. Patients from the surgical clinic received their results via video conference by a teleoncologist and completed a satisfaction survey.

We processed 153 patients in the mammography center and 38 in the surgical clinic. On average tissue processing and telepathology interpretation was competed in <5 hours (19% 37/ 191) presented difficult diagnoses and took longer (+24 hours) to diagnose). The 38 participants from the clinical center reported overall satisfaction with EBC, indicated they would seek EBC again, and expressed relief at receiving prompt results.

This preliminary data indicate that EBC can reduce the wait for breast biopsy results for patients. EBC may help ameliorate health care disparities in many regions of Arizona.

Stephen L. Macknik, Ph.D.

St. Joseph's Hospital
Award Amount FY07: $50,000

Blood Flow Measurement During Ictal Events: Implications For Neuroprotective Therapies

One of the crippling effects of epilepsy is the progressive development of neural sclerosis. Excessive magnitudes of local hyperperfusion or hypoperfusion caused by seizure-related vasospasms could potentially contribute to this debilitating problem. However, *it is not known if ictal blood flow dynamics are truly abnormal*; to quantify the degree of abnormality, ictal blood flow must be compared directly to normal peak functional blood flow within the same capillaries, which has not been done. If seizures do cause either hypoperfusion, hyperperfusion, or both, we expect their long-term cumulative degenerative effects to be similar to those caused by iterative mini-strokes. In such case, therapies already developed to protect stroke victims from ischemic cell death may also provide neuroprotection for epileptics. Our hypothesis is that seizures cause neural damage through abnormal blood flow. The significance of the project is that it will provide the basis to develop neuroprotective treatments from vasospasms in epilepsy. Our work thus far has been to acquire and setup the supplies and equipment for the project, and to calibrate the imaging system quantitatively in living animals. It is going well, and the setup stage, which involves doing experiments that serve as calibration controls for the main experiments later this year, are expected to be complete within the next 6 months. The current work involves insuring that the combined neurophysiology and *in vivo* two-photon imaging studies that are proposed in the project will come off without a hitch. The initial seizure imaging experiments have been scheduled for August–October of 2008.

Lawrence J. Mandarino, Ph.D.

Arizona State University
Award Amount FY07: $150,000

Metabolic Syndrome and Inflammation

Insulin resistance is a reduced response of tissues to insulin. Insulin resistance in muscle can result in type 2 diabetes mellitus, a public health concern. Recent experiments suggest that chronic inflammation may be responsible for insulin resistance, and thus type 2 diabetes. However, it is not known what causes the inflammation. We hypothesize that, because insulin resistance is often found in obesity, an oversupply of fat leads to inflammation and insulin resistance. The oversupply of fat acts as an "insult" to tissue such as muscle, producing inflammation. We want to see if fat oversupply precedes muscle inflammation. A marker of inflammation in muscle is IkB, a protein involved in inflammation. Our studies show that obese individuals have lower IkB protein than lean individuals, indicating muscle inflammation. Our next step is to produce an experimental fat oversupply by infusing fat into lean individuals and determining if IkB protein decreases.

John J. Marchalonis, Ph.D.

University of Arizona
Award Amount FY07: $150,000

Modulation of Autoimmune Disease by Autoantibodies and Immunopeptides

The immune system is disrupted in autoimmune diseases resulting in damage of organs and tissues. We have been characterizing autoantibodies (antibodies reactive with self components) directed at receptors of immune cells that are responsible for most autoimmune diseases. We propose that these antibodies are beneficial and function to control potential auto destructive cellular reactions. We have now developed methods for the production of sufficient quantities of pure autoantibody preparations for testing our hypothesis using *in vitro* assays and mouse models of human disease. Recently we discovered new types of autoantibodies directed at opioid receptors, which are mainly known for the relief of pain. We have found that these autoantibodies can disrupt the delicate balance of cytokine profiles in the immune system, thus causing exacerbation of symptoms in autoimmune disease. Our work should lead to treatment protocols for the significant relief of suffering in autoimmune disease such as RA and SLE.

Jesse D. Martinez, Ph.D.

University of Arizona
Award Amount FY07: $49,999

Nutritional Modulation of Colorectal Cancer Risks

Colorectal cancer is second only to lung cancer in causation of death due to cancer, and epidemiological studies have implicated diet as the most important factor in determining risk for this disease. Our studies have shown that bile acids which are produced by the liver and which help in the absorption of dietary fats can increase the risk for developing colon cancer. Our most recent progress indicates that bile acids can stimulate cell growth which in turn is a precursor condition to tumor development. Importantly, our most recent studies indicate that polyunsaturated fatty acids (PUFAs) can counteract this activity at the molecular level. Hence, fish oils which have long been known to have a beneficial effect on general health and are associated with reduced risk of colon cancer may exert their effects by antagonizing the cell growth stimulated by bile acids and other factors in the diet that promote colon tumor development.

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

University of Arizona
Award Amount FY07: $50,000

Translational Regulation of Protein Expression in Prostate Cancer Progression

Mortality from prostate cancer is primarily due to metastasis to distant sites. Each year in Arizona approximately 4300 new cases of prostate cancer are diagnosed and 500 men die due to prostate cancer. Therefore, understanding the molecular mechanisms of prostate cancer metastasis could provide great therapeutic potential. Laminin-332 provides stable adhesion structures in normal prostate and prevents cellular invasion and metastasis. Its expression is lost in prostate cancer progression and we have been working on determining the cause of this loss. Here we have demonstrated that LM-332 loss in prostate cancer is not due to alterations in translational regulation. This finding has led us to investigate other possible mechanisms of LM-332 loss, such as targeted protein degradation.

Kremer C, Klein R, Mendelson J, Browne W, Samadzedh L, Vanpatten K, Highstrom L, Pestano G, Nagle R. Expression of mTOR Signaling Pathway Markers in Prostate Cancer Progression. **The Prostate.** 66: 1203-12, 2006.

Mark Preul, M.D.

St. Joseph's Hospital
Award Amount FY07: $150,000

A Novel Peptide Mimetic for the Immunotherapy of Brain Tumors

Direct activation of phagocytic cells—the final effectors of the immune response against tumors—is a promising approach to cancer treatment. Endogenous human serum macrophage activation factor (MAF), which produces phagocytic activation, is efficacious against certain tumors. However, MAF has not been studied extensively because its source—human blood—precludes its widespread experimental use. Furthermore, MAF has not been assessed in the setting of brain cancers. We have recently developed a novel synthetic peptide mimetic of MAF, L4MAF, which can activate microglia—the cells responsible for the cellular immune response in the brain—*in vitro*. L4MAF can be mass produced and is comparatively protected against enzymatic degradation in the bloodstream. In this project, we attempt to establish the efficacy of L4MAF at reducing tumor growth in animal models of brain cancer. Our results to date include:

- We used GL261 malignant glioma brain tumor model in C57BL/6 mice, which is widely employed in the study of immunotherapeutic approaches to brain cancer. With a stereotactic neurosurgical protocol, we implant 105 GL261 cells intracranially into the right frontal lobes of experimental mice. In our hands there is 94% MRI-confirmed rate of tumor formation, which compares favorably

with the existing literature.

- We have established the pharmacokinetic profile of L4MAF following its administration in C57BL/6 mice. Following subcutaneous injection, L4MAF is detectable in serum up to 4 hours after treatment, ensuring appropriate length of exposure for immunostimulatory action. No toxicity has been observed at treatment-level doses.
- We have demonstrated that L4MAF is efficacious as a radiation sensitizer for glioma. Tumor-bearing mice receiving 4 Gy of whole brain radiation, combined with L4MAF administered at 1 nanomole/g on alternate days, had median survival of 29 days compared to 21.5 days in mice receiving L4MAF alone, 20.5 days in mice receiving radiation alone, and 20 days in untreated control mice. This survival increase was statistically significant. Furthermore, mean tour cross-sectional area measured on MRI, 2 weeks post-implantation was significantly reduced in the L4MAF + radiation group.
- We have found that, to a point, L4MAF administered at lower doses may be effective in slowing glioma growth even without added radiation. Groups of tumor-bearing mice receiving progressively lower L4MAF doses had correspondingly longer median survival and smaller tumor cross-sectional areas. This trend was lost below 0.1 nanomole/g. We suspect that this result is explained by the deleterious inflammation within the brain which accompanies larger L4MAF doses.
- We are presently examining brain tissue from tumor-bearing mice treated with L4MAF. Preliminary analyses demonstrate, as hypothesized, microglial sequestration and activation within tumor. Cytokine assays on serum from these animals suggest that L4MAF also induced a systemic cell-mediated immune response.

In vivo data to date show that direct phagocytic cell activation, achieved by administering a novel peptide mimetic of MAF, improves survival in a murine model of glioma. This anti-tumor effect is enhanced by combination with whole brain radiation.

Naomi E. Rance, M.D., Ph.D.

University of Arizona
Award Amount FY07: $49,644

Effects of Estrogen Withdrawal on Hypothalamic Thermoregulation

Hot flushes represent a disorder of the brain's regulation of body temperature (thermoregulation) caused by the menopausal loss of estrogen. Hot flushes may last for up to 5 years and have a negative impact on the quality of life in many individuals. Despite the vast numbers of individuals experiencing hot flushes, there is little understanding of what causes hot flushes and few laboratories devoted to studying this phenomenon in animal models. Our goal is to provide basic information on how estrogen withdrawal affects the regulation of body temperature. In the past year, our studies have shown effects of estrogen on the thermoneutral zone of the rat. Our results support the hypothesis that estrogen withdrawal leads to an increase in the sensitivity of thermoregulatory networks. These studies provide insights into the possible etiology of hot flushes. Understanding of the cause of hot flushes is necessary for the design of appropriate therapies.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY07: $50,000

Sulfonium-salt Suicide Inhibition (SSSi) of Cancer Cell Division

Cancer cells disable processes that are designed to make abnormal cells self-destruct, allowing growth and spread of the cancer cells. Cancer cells also turn on growth-stimulating processes that should not be active. We devised, made, and tested new chemical compounds for possible treatment of cancer. Some of these compounds were designed to be turned into a highly reactive, cell-killing form by proteins associated with the abnormal processes in the cancer cell. When such a compound was tested with a protein analogue, the expected conversion to a highly reactive form occurred. We also tested these compounds for their ability to kill cancer cells grown in culture and they were able to do so at low doses. Others of our compounds, which interfere with cell division, were found to induce self-destruction of cancer cells in culture. These studies further the development of anticancer drugs for the benefit of Arizona cancer patients.

Philip M. Service, Ph.D.

Northern Arizona University
Award Amount FY07: $47,535

Genetics of Aging: Fine-scale Mapping of Life Span Genes in Drosophila

During the first year of our project, we have made progress in three related areas. First, using the Drosophila (fruit fly) genome project, we have successfully located many of the genetic markers that we need in order to map genes that influence life span in our fly populations. Second, we have obtained evidence from our long- and short-lived flies that a specific gene, Dopa decarboxylase (Ddc), may explain a portion of the variation in life span that we see in our flies. The enzyme DDC is used in the synthesis of the neurotransmitters dopamine and serotonin which are also present in humans. Third, we have implemented a set of computer programs that will allow us to assess the power and precision of our genetic mapping experiments.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY07: $50,000

Non-nucleotide Inhibitors of IMP Dehydrogenase

The cancer cell must synthesize cofactors and DNA components at a rapid pace in order to spread through out the host. The enzyme IMP dehydrogenase (typeII) plays a key role in this regard and is often present at elevated concentrations in cancer cells. Many of the candidate IMP dehydrogenase inhibitors are nucleotides that can interfere with other aspects of purine metabolism. Our strategy for designing non-nucleotide inhibitors of IMP dehydrogenase is to design a purine ring mimic tethered to amino acid residues that binds to type II enzyme.

In the past year, we accomplished the following:

- synthesis of two libraries of candidate non-nucleotide inhibitors of IMP dehydrogenase using models of the enzyme's active site;
- screening of these libraries at the National Cancer Institute;
- elucidation of a Structure Activity Relationship from these screening results;
- animal screening of an active analog; and
- provisional Patent in preparation.

Lucy Treiman, Ph.D.

St. Joseph's Hospital
Award Amount FY07: $50,000

How Do Febrile Seizures Cause Epilepsy: Possible Role of Gene Expression

Febrile seizures in young children appear to be an important risk factor for developing subsequent chronic epilepsy, especially when the seizures are prolonged. Anything that can be done to reduce the incidence of epilepsy in Arizona will be important to the health of its people and to the reduction of health care costs. We are using an animal model to study the consequences of prolonged neonatal febrile seizures. We are investigating whether or not febrile seizures result in deficits in visual-spatial learning, which may correlate with severity of the seizures and with greater susceptibility to develop of seizures in adulthood. We are in the process of identifying differences in gene expression that may explain these results. One of our important observations is that maternal care reduces the morbidity and mortality of prolonged febrile seizures in rat pups. This may have important implications for the management of human febrile seizures.

Tsu-Shuen Tsao, Ph.D.

University of Arizona
Award Amount FY07: $49,999

Biological Function and Chemistry of Adiponectin Oligomerization

The overall objective of this grant is to understand the role of impaired adiponectin oligomerization in the pathogenesis of type 2 diabetes mellitus. To that end, we have accomplished the goals set forth in Aim 1 to define the oligomerization state of the largest isoform of circulating adiponectin. Previously described simply as higher molecular weight (HMW) adiponectin, we have during the past year established the HMW adiponectin to consist of 18 identical subunits (18mer). This finding has allowed us to further define the composition of two minor adiponectin species in human serum to be 9mer and 12mer. The 18mer can be collapsed into 6mers by treating it with mild acid. Together, these results have led us to advance testable models of adiponectin multimerization pathways and potential mechanisms that govern the formation of different adiponectin oligomers. This will allow us to answer why diabetic patients lack adiponectin 18mer.

Marlys H. Witte, M.D.

University of Arizona
Award Amount FY07: $49,676

Massage Therapy in Childhood Lymphedema

The swollen or unequal legs or arms of children are most commonly due to birth defects of the lymphatic drainage system (termed congenital lymphedema) or complications of cancer treatment ("acquired lymphedema"), which slows down the removal of excess tissue fluid (lymph). These children often suffer severe physical and psychological disabilities. An estimated several hundred Arizona children, occasionally with multiple other family members, suffer from childhood lymphedema (CLE). We are testing a simplified method of CLE management, namely a specialized massage therapy (manual lymph drainage-MLD) in growing children to determine responsiveness to a short application and maintenance MLD regimen, compliance, and long-term outcomes. During the project's first year, we have trained personnel, finalized protocols, obtained Institutional Review Board approval, and begun subject enrollment. To accrue more subjects, we have identified Arizona-wide and national pediatric and cancer centers, physicians, therapists, and other health care personnel who care for CLE; contacted known Arizona and out-of-state CLE families, and are developing Telemedicine links with world-renowned CLE centers of excellence for multi-institutional collaboration.

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Noon A, Hunter R, Witte M, Kreiderman B, Bernas M, Rennels M, Percy D, Enerback S, Erickson R. Comparative Lymphatic, Ocular, and Metabolic Phenotypes of FoxC2, Haploinsufficient and AP2-FoxC2 Transgenic Mice. **Lymphology**. 39: 84-94, 2006.

Witte M, Jones K, Wilting J, et al. Structure Function Relationships in the Lymphatic System and Implications for Cancer Biology. **Cancer Metastasis Rev**. 25: 159-84, 2006.

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Hunter R, Witte M, Dellinger M, et al. The Generalized Hypo-dysplastic Lymphatic-lymphedema Phenotype of Angiopoietin-2 Knockout Mice Persists Throughout Adulthood and Is Fully Rescued by Angiopoietin-1 Knock-in. **Lymphology**. Andrade and Witte eds. Lymphology 40 (Suppl), 2007.

George T. Wondrak, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Melanoma Cell Survival Signaling by Glycolytic Intermediates

The rising incidence of skin cancers in the State of Arizona is a public health problem of increasing concern. In the State of Arizona, melanoma accounts for only 5% of all skin cancers, but causes almost 80% of all skin cancer deaths. Metastatic melanoma is a highly aggressive tumor that originates from pigment producing cells in human skin and poses a formidable therapeutic challenge with poor treatment options. Based on my ABRC-sponsored research (7-2006 until 6-2007) I have examined the mechanism of action of two novel classes of prototype drugs [called methylglyoxal-antagonists (MG-antagonists) and phenothiazinium redox cyclers (PRC)] that effectively kill melanoma cells without harming normal cells. The novel molecular mechanism by which these agents kill melanoma cells involves the inactivation of cancer cells survival factors called heat shock proteins and has been partly elucidated using advanced tools of melanoma cell protein analysis and mass spectrometry.

Wondrak G. NQO1-activated Phenothiazinium Redox Cyclers for the Targeted Bioreductive Induction of Cancer Cells Apoptosis. **Free Radical Biology & Medicine**. 43: 178-90, 2007.

Yitshak Zohar, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Novel Applications of Nanotechnology: Microdevices for Capture and Analysis of Circulating Tumor Cells

The goal of this project is to use biology-based technology to create microdevices that can capture specific populations of cells from complex suspensions, such as blood, for analysis of their molecular profile. So far we developed the methodology to produce a model system of cells to evaluate cell sorting techniques based on specific adhesion molecules on the cell membrane, as well as a protocol to detach the cells for subsequent manipulation in microfluidic systems. In parallel, we developed the technology to fabricate microchannel-based systems with patterned bio-active coatings to facilitate the specific interaction between the coated surface and the model cells. Furthermore, we started investigating the nature of target-particle/coated-surface interaction as well as the behavior of liquid flow suspended with particles in microchannels. The results will allow us to design microsystems for manipulating biological cells with adhesion properties similar to cancer cells to evaluate our proposed cell sorting.

Iwama R, Lee L, Cho E, Zohar Y. Fabrication of Microchannels with Patterned Bioactive Layers. Proc. 20th Int. Conf. Micro Electro Mechanical Systems. MEMS'07, 333-36, 2007.

Cho E, Lee L, Baygents J, Guzman R, Heimark R, Zohar Y. Particle/surface Interaction in a Microchannel. Submitted to 21st Int. Conf. Micro Electro Mechanical Systems. MEMS'08, 2008.

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Section D

New Contract Awards

Beginning in FY 2008

Emmanuel T. Akporiaye

University of Arizona
Award Amount FY07: $150,000

Chemo-immunotherapy of Adenomatous Polyps in a Mouse Model of Polyposis

Familial adenomatous polyposis (FAP) is a hereditary disease in which patients develop multiple intestinal polyps in the duodenum, colon and rectum that frequently progress to invasive cancer. Colorectal cancer is the third most prevalent cancer and the second leading cause of cancer deaths in the U.S. The American Cancer Society estimates that 106,680 new cases of colon cancer and 41,930 new cases of rectal cancer will be diagnosed in 2006 in the US and that 55,170 individuals will die from the disease. Of every 100,000 people in Arizona, 75 will be diagnosed with coorectal cancer of which 30 will die. Despite the surgical removal of polyps and parts of the colon to control the disease, a significant proportion of patients develop new cancers in the upper gastrointestinal tract including the duodenum with fatal consequences. Currently, celecoxib (Celebrex) is the only viable treatment option to prevent cancer-related deaths in this patient population. When administered over a long period, celecoxib reduces the size and number of intestinal polyps in FAP patients without the gastrointestinal toxicity that develops with use of the non-steroidal anti-inflammatory drug, sulindac. Celecoxib use is not without its own limitations, however. Firstly, celecoxib treatment does not completely eliminate polyps, and if discontinued, new polyps arise. Second, long-term use of celecoxib may increase the risk of heart attacks in some patients. With the goal of treating this form of cancer with minimum side-effects, we propose to combine short-term celecoxib treatment with immunotherapy in which the body's immune system is stimulated to act in its own defense. For this approach to work, specialized cells of the immune system known as cytotoxic T cells must recognize specific parts of the tumor known as antigens. Upon stimulation with the tumor-specific antigen, the T cells are activated to destroy cancer cells while sparing normal healthy cells. In this project, we will target a cancer-associated protein, MUC1 that is often found on the surface of human polyps and colon cancer. The goal of this project is to test the effectiveness of short-term celecoxib treatment plus MUC1-specific cancer vaccines in preventing and treating polyps in a unique mouse model that mimics humans with FAP. This approach reaps the benefits of celecoxib treatment but avoids its side-effects because only short-term celecoxib therapy is needed. Furthermore, the T cells will destroy the MUC-expressing polyps and provide protection from future tumors. If successful, this approach can be readily moved to the clinic for treating FAP patients.

The hypothesis to be tested is that the combination of the COX-2 inhibitor, celecoxib plus MUC1-specific vaccination will result in prevention or abrogation of established non-chlorine adenomas with a considerably reduced likelihood of polyp recurrence. A specific aim of the project is to prevent the development of MUC1-expression adenomas in MUC1.Tg/MIN mice using a combination of limited dietary celecoxib plus MUC1 peptide-based vaccination. The mice will receive non-toxic doses of celecoxib in their food and will be vaccinated with a vaccine cocktail consisting of two MUC1-specific MHC class I-restricted peptides (APGSTAPPA, SAPDTRPAP) and a MHC class II-restricted pan helper peptide (Hepatitis B core antigen peptide, TPPAYRPPNAPIL) in incomplete Freund's adjuvant

(IFA) plus the biological adjuvants, CpG ODN and GM-CSF, to amplify the T cell and dendritic cell immune compartments. The treated mice will be evaluated for suppression or abrogation of polyps in the gastrointestinal tract. We will also assess proliferation, apoptotic cell death and angiogenesis of adenomas to identify potential mechanisms of anti-tumor activity of this chemo-immunotherapeutic. A second specific aim of the project is to characterize the immunologic responses in treated mice to determine if a positive correlation exists between the anti-tumor immune response and clinical outcome. Treated mice will be evaluated for the development of humoral (antibody) and cellular (cytotoxic T lymphocyte activity, TH and TH2 cytokine secretion) immune responses to MUC1. Sera and lymphocytes (spleen and draining lymph nodes) will be isolated from experimental and control animals and evaluated for the presence of anti-MUC1 antibody and MUC1-specific T cell reactivity respectively.

Klaus Beyer, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Membrane Interactions and Neurodegeneration in Parkinson's Disease

This is a pilot project that is designed to elicit extramural support from the U.S. Public Health Service for research involving neurodegenerative diseases. With continued rapid aging of the general population, we can expect much increased demand on the health care infrastructure of the U.S. in coming years. Apart from cancer and cardiovascular disease, Alzheimer's Disease and Parkinson's Disease are two of the most prevalent diseases of an aging population. A common trait in neurodegenerative diseases, which include Alzheimer's Disease, Parkinson's Disease, and many other diseases, is the deposition of misfold and insoluble protein aggregates in the brain and other neural tissues. One example of such aberrant protein folding involves Lewy bodies, which are characteristic features of sporadic and hereditary variants of Parkinson's disease and of other neurological disorders such as Dementia. The proposed studies are required for a rational design of medicines as a possible therapy for Parkinson's disease and other neurodegenerative disorders. The preliminary data obtained from the proposed studies will be used to obtain funding from the National Institute of Health and should ultimately provide a significant health benefit to the people of Arizona.

In Parkinson's disease the major component of the intracellular deposits is the protein α-synuclein. Our goals are to understand now aggregation of α-synuclein is implicated in neuronal membrane damage. The project will pursue research on the biomembrane interactions of this hallmark protein of Parkinson's disease. A specific hypothesis is that aggregation of α-synuclein is affecting nerve signal transmission which involves cellular membranes. We specifically plan to study the interaction of α-synuclein with membranes in order to elucidate the relationship between binding and protein folding to nerve function and dysfunction. An additional aspect is to study how the fusion of small membrane vesicles involved in nerve impulses is affected by the properties of α-synuclein. Last, we propose to study lipid models mimicking the composition of neuronal membranes to gain a better understanding of the factors affecting nerve degeneration. Our objective is to discover how membrane interactions involving α-synuclein and membrane lipids are implicated in the onset and progression of Parkinson's and possibly other diseases of the nervous system. These studies of α-synuclein and its membrane interactions are needed for the development of medicines aimed at treating Parkinson's disease.

Christopher A. Buneo, Ph.D.

Arizona State University
Award Amount FY07: $50,000

Contribution of Posterior Parietal Areas to State Estimation

As humans we demonstrate a seemingly effortless ability to perceive the world around us and to move through it. This ability is remarkable considering that each of our senses provides information about our location in the world, as well as the locations of all other objects, with respect to very different landmarks or "frames of references." For example, our eyes provide information about the visual motion of a fly with respect to where we are currently looking, while our ears relay complementary information, the buzzing sound generated by the flapping of the fly's wings, with respect to our heads. In other words, the eyes and ears, and the associated parts of the brain to which they are connected, use different 'language' to describe the location of objects in the world. The fact that the senses provide many simultaneous descriptions of the world is apparently not a challenge but a boon to the brain, as humans show an immense degree of flexibility in their abilities to perceive and move under varied and constantly changing conditions.

If we want to reach out and swat away a fly that is buzzing around us, how does the brain combine information from our senses in a way that is useful for moving the arm, which speaks yet another language altogether? Problems of this nature have challenged neuroscientists since the 19th century. Clearly some sort of 'interpreter' is required to allow the eyes, ears, and arms to work together to solve this task. It is currently believed that the posterior parietal cortex of the brain may serve this role, but precisely how this is accomplished is unclear. The importance of the problem is immediately apparent, however, when someone suffers damage to this part of the brain. When this happens, the affected individuals often (but not always) are still able to move their arms and legs, as well as see and hear, but they may have difficulty moving their arms and eyes accurately, or may even 'neglect' entire body parts or parts of space near their body. Rehabilitation of these motor problems is often difficult and incomplete.

The research described in this proposal is aimed at understanding how the brain combines information provided by the senses and uses it to help plan and modify our movements. Although examination of patients that have had cerebrovascular accidents or 'strokes' affecting the posterior parietal cortex suggests this area plays an important role in this function, neurological diseases such as Parkinson's disease, suggest the involvement of other brain areas as well. In addition, this function can be affected by the normal aging process and certain developmental abnormalities. Thus, the proposed work touches on several key biomedical issues facing the citizens of Arizona including aging, cerebrovascular disease, developmental disorders, as well as basic neuroscience and neurological diseases. It is hoped that the information obtained in this series of studies will allow treatment efforts to be better tailored to meet the needs of affected individuals. This may be accomplished through improved rehabilitation protocols or through the development of assistive technologies that directly interact with or bypass affected parts of the brain. Thus, there is a potential bioengineering link to this proposal work as well.

The overall goal of this research is to improve our understanding of the role of three separate subdivisions of the posterior parietal cortex (areas 5,7a, and 7b) in combining sensory and motor information during movement. The specific objectives are to determine whether each area processes only sensory information, only motor related information, or both types of information. Based on what is known about how these different areas are connected to other parts of the brain, as well as what happens when these different areas are damaged, it is hypothesized that these different subdivisions process sensory and motor information differently. Specifically, it is hypothesized that areas 7a and 7b process only sensory information during movement, while area 5 processes and combines both sensory information and 'copies' of motor commands generated elsewhere in the brain.

Setsuko K. Chambers, M.D.

University of Arizona

Award Amount FY07: $50,000

Regulation of c-fms Proto-oncogene Related Breast Cancer Metastasis

Breast cancer, a common malignancy in women in the United States, is often a disease which has spread beyond the breast, even where the tumor is of a very small size. Unfortunately, patients with a cancer that has invaded normal breast tissue and has spread to other organs have only a 25% chance of surviving 10 years. Metastasis to the bone occur in over 70% of patients with breast cancer spread, resulting in debilitating symptoms such as severe bone pain and fractures. It is in this stage where breast cancer cells have invaded the bone that the cancer is virtually incurable. Specific genes within different breast cancers may make them more likely to spread. If approaches can be developed to inactivate these genes, this may improve the outcome for these breast cancer patients. RNAs, essential building blocks which are created from genes, along with the proteins which bind to the RNAs, have the capacity to activate some genes and by so doing, can control tumor behavior. Our laboratory is studying the role of a gene called c-fms which is found in abundant amounts in breast cancers but not in normal breast tissue. Large amounts of c-fms can turn normal breast cells into cells capable of producing tumors and spreading to distant organs. We found that glucocorticoid steriod hormones, which are present in all individuals and act on normal breast tissue, elevate the levels of c-fms in breast cancer cells. Elevated levels of c-fms lead in turn to an increased capacity of the breast cancer cells to invade normal tissues and to spread to other organs. We have identified a short stretch of RNA sequences within the tail end of c-fms, described below, which binds the protein HuR, best known for binding sequences not present in this stretch of RNA. We found that glucocorticoid stimulation of c-fms RNA and protein is largely dependent on the presence of HuR protein. We have also recently identified another protein, β-actin, which also binds the same stretch of c-fms RNA. β-actin has multiple roles in the cell, one of them being to help the cell move in a directional manner. Studies such as this which identify and test novel regulators of c-fms proto-oncogene could have a great impact on breast cancer metastasis since the majority of breast cancers are capable of responding to glucocorticoid hormones.

In our search for how levels of c-fms rise in response to glucocoticoid hormones, we have recently discovered that there is a unique short stretch of RNA sequences at the tail end of c-fms which we found unexpectedly binds two proteins: HuR and β-actin. This set of RNA sequences is unique because it does not contain any other known sequence or motifs which are recognized to bind regulatory proteins. We found that silencing HuR significantly reduced the ability of glucocorticoids to stimulate c-fms levels. We hypothesize that his novel c-fms motif regulates c-fms levels by interaction with β-actin and HuR, and thereby, modulates breast cancer behavior. In this proposal we aim to define the regions within this unique motif important to HuR and/or β-actin binding. We also aim to study whether β-actin acts to help c-fms, or whether HuR and β-actin compete for binding within this short stretch of c-fms RNA sequences and have opposing effects on c-fms. We will then study the effect of mutations in these binding regions on c-fms and related breast cancer behavior such as invasiveness and metastasis. We will use a model to study the spread of breast cancer cells to bone, since breast cancer cells are bone-seeking and bone is replete with CSF-1, which is the cytokine that binds c-fms. Bone metastases are very common in metastatic breast cancer, and they cause significant pain in patients. Thus, we propose to understand the pathway through which glucocorticoid hormones act on elevating c-fms in breast cancer cells and augment aggressive tumor behavior. These studies are necessary in order to design scientifically based, molecular therapies for patients to decrease c-fms levels in their tumors, and thereby, inhibit the spread of breast cancers. A greater understanding of the molecular mechanisms underlying why breast cancer cells seek and spread specifically to bone has the additional potential to improve the quality of life of breast cancer patients. Funding for this research will enable us to obtain the preliminary data necessary to be competitive for extramural funding.

Susana Martinez-Conde, Ph.D.

St. Joseph's Hospital
Award Amount FY07: $50,000

The Effect of Progressive Supranuclear Palsy on Microsaccade Dynamics During Visual Fixation

Eye disease leading to the loss of sight is a growing problem in Western society. One of the main reasons for this growth is the increase in the proportion of elderly people in the population. This issue is of special significance to Arizona, the home to a disproportionate population of elderly people.

When we explore a visual scene, we spend 80% of the time fixating our gaze on the various points of interest of the image. However, our eyes are never completely still. Even during visual fixation, we constantly produce tiny involuntary eye movements called "fixational eye movements." If fixational eye movements are suppressed or defective, visual perception fades due to neural adaption. Therefore, patients with dysfunctional fixational eye movements experience visual deficits during fixation. In summary, our visual system has a built-in paradox: We must fixate our gaze in order to inspect the minute details of our world, yet if we were to fixate perfectly, the entire world would fade from view. Due to their role in counteracting adaptation, fixational eye movements are an important tool to understand how the brain makes our environment visible.

One type of fixational eye movement is called a "microsaccade." Microsaccades are similar to the large voluntary eye movements we make to inspect the world around us, except that they are much smaller, they occur involuntarily, they occur several times each second, and we are completely unaware that we make them. Our previous research has shown that microsaccades generate neuronal firing in various visual areas of the brain and that microsaccades serve to counteract perceptual fading in the periphery of our vision during fixation. Here, we aim to determine the mechanisms by which microsaccades counteract visual fading in a variety of visual tasks and conditions. We will, moreover, determine the dynamics of microsaccades and their functional role in progressive supranuclear palsy (PSP) and cardiac patients, who suffer from impaired vision and who are known to produce pathologically slow saccades.

Impaired fixational eye movements are observed in patients with a variety of central and peripheral pathologies of the nervous system. Although we spend about 80% of our waking lives fixating gaze, the contribution of impaired fixational eye movements to vision loss is generally overlooked clinically. This gap in knowledge has prevented the field from developing new treatments and early diagnostic tools to ameliorate visual deficits that are contributed to by impaired fixational eye movements. Progressive supranuclear palsy (PSP) and cardiac patients often exhibit slow saccades (that is, saccades of normal magnitude but pathologically slow velocities). Our preliminary data suggest that microsaccades in PSP and cardiac patients are also pathologically slow. However, it is unknown whether pathologic microsaccades contribute to the vision loss associated with PSP and cardiac patients. This gap in knowledge has prevented the field from developing new treatments and early diagnostic tools to ameliorate those visual deficits that are due to impaired microsaccades. The goal of the proposed research is to determine the impact of impaired microsaccades in the visual deficits exhibited by PSP

and cardiac patients. This determination will provide critical information for the differential diagnosis of visual disorders associated with abnormal microsaccades. This result will be moreover critical to determining the best way forward with the development of microsaccadic replacement therapeutics in PSP patients and others with abnormal microsaccades.

Harinder Garewal, M.D., Ph.D. **University of Arizona**

Award Amount FY07: $150,000

Unique, Hypothesis-driven Biomarkers of Colon Cancer Risk

In Arizona, each year about 1000 people die from colon cancer, the second leading cause of cancer deaths in the state. Improvements in prevention and treatment,to reduce colon cancer mortality will likely depend on better early detection and increased understanding of the causes of colon cancer. Since the cure rate for metastatic colon cancer is dismal, early diagnosis and the identification of subjects at high risk for colon cancer, to be screened aggressively and have pre-cancerous lesions removed, is urgently needed in Arizona.

The main objective of our research for over a decade has been to develop biomarkers that would identify patients at increased risk for colon cancer. One of our major findings is that resistance to programmed cell death (apoptosis resistance) in stressed human tissue samples is a key characteristic of the colon of patients at high risk of developing colon cancer. Apoptosis resistance increases survival of cells with DNA damage, thereby increasing genomic instability, and is considered to be a major factor in causing colon cancer. However, measuring apoptosis resistance is not practical on a routine basis in a clinical setting.

In recent work we identified three proteins present at abnormal levels in the colon, associated with apoptosis resistance, and with the potential of becoming unique biomarkers for identifying increased risk of colon cancer. These potential biomarkers are 1) reduced or absent expression of cytochrome c oxidase subunit 1 (CcO1), 2) increased expression of inducible nitric oxide synthase or (NOS2), and 3) reduced expression of a dual role DNA mismatch repair/apoptosis protein called Pms2. These proteins participate in separate pathways controlling apoptosis. In our pilot studies, aberrant expression of CcO1, NOS2 and Pms2 were clearly and positively associated with colon precancer and cancer growths. Each of these proteins can be assessed using standard procedures on routinely prepared human biopsies, potentially making the analysis of colon cancer risk a relatively simple assessment.

We and others found that CcO1 is a key molecular switch for introduction of apoptosis. We and others also found that NOS2 inhibits stress-induced apoptosis. Absence of Pms2 in mice leads to an approximate 100-fold increase in mutagenesis in the colon, and Pms2 also plays a key role in inducing apoptosis

when DNA damage is excessive, so that low Pms2 also has a likely role in causing colon cancer.

In light of our preliminary findings, we plan to evaluate 200 patients, half in low risk and half in high risk categories, to establish these protein expression alterations as a unique and practical set of biomarkers for assessing colon cancer risk with high specificity and high sensitivity. In a published study from our laboratory, we also identified "field defects" (areas of the colon with patches of apoptosis resistance) that were far removed from the location of the cancer. Therefore, we also plan to determine whether aberrancies in markers in the sigmoid colon, reachable in a doctor's office by flexible sigmoidoscopy, can reliably indicate the likelihood of aberrancies 4 or 5 feet away along the length of the colon, in the proximal colon reachable only by a complete colonoscopy. Further, we plan to assess whether aberrancies in our selected protein markers are associated with measurable histopathologic or microarchitectual alterations in the colonic mucosa, to add to the accuracy of biomarkers used to detect increased risk of colon cancer. The identification of a set of biomarkers that are specific, sensitive and mechanistically based is crucially needed to target individuals for more frequent screening colonoscopies with removal of pre-cancerous growths. This would also help identify a high risk cohort of subjects for preventive intervention studies such as diet and lifestyle changes or potential chemopreventive agents.

Hypotheses to be tested are 1) assessment of biopsies from the non-tumor segments of the colon for aberrant expression of CcO1, NOS2 and Pms2 and any associated histopathologic/microarchitectual alterations should yield a practical molecular mechanism-based set of biomarkers of colon cancer risk and 2) aberrant protein levels in the easily accessible part of the colon will indicate aberrancies in harder to reach parts of the colon. Our goal is better prevention of colon cancer. This requires early detection and increased colonscopies, with removal of pre-cancerous growths, for individuals at high risk.

Our specific objectives are to 1) compare expression of proteins CcO1, NOS2 and Pms2 in colons of those at low risk for colon cancer to their expression in non-tumor areas of colons of those at high risk, to confirm that when these proteins have aberrant expression then they are early indicators and specific hypothesis-driven biomarkers of colon cancer risk, 2) determine if aberrant CcO1, NOS2 and/or Pms2 expression is associated with altered histologic findings in the non-neoplastic colons of patients at high risk of colon cancer, to add histologic indicators of risk, and 3) determine whether aberrant expression of CcO1, NOS2, and/or Pms2 in the non-neoplastic flat mucosa of the sigmoid colon (easily obtained) reliably indicates aberrant expression in the cecum, and thus, likely indicates colon-wide risk.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY07: $150,000

Withaferin A Analogs Targeting Annexin II as Novel Drugs for Pancreatic Cancer

Cancer accounts for a vast number of deaths and suffering. Each year 6.5 million people are diagnosed with cancer worldwide, and in the U.S. alone more than 10 million people are living with a history of cancer. According to the American Cancer Society about 1,399,790 new cases are expected to be diagnosed this year and 564,830 Americans are expected to die of this disease. Arizona's desirable climate attracts many elderly retirees, and thus, the population of cancer patients in the state has been steadily increasing. In 2004, cancer was responsible for 9,710 deaths and 23,560 newly diagnosed cases in Arizona. It is estimated by the American Cancer Society that in 2006 cancer will be responsible for 10,270 deaths and 25,450 newly diagnosed cases in Arizona.

The majority of anticancer drugs in use today are from natural resources. Our preliminary studies with the natural product withaferin A, isolated from the desert medicinal plant *Withania somnifera* (winter cherry), has led to the discovery of the protein Annexin II as its target. As the next critical step in our efforts to exploit the unique potential of Annexin II as a novel anticancer target, this multi-institutional and interdisciplinary project purposes to evaluate the activity of naturally-occurring and semi-synthetic analogs of withaferin A against pancreatic cancer, the fourth leading cause of cancer deaths in the U.S. and the one having the worst survival rate.

Withaferin A and some its naturally-occurring analogs will be obtained from *Withania somnifera* that has been grown aeroponically, a newly developed bio-agriculture technique. Other analogs will be obtained starting from withaferin A by chemical synthesis and microbial biotransformation. All analogs will be evaluated in assays relevant to Annexin II and pancreatic cancer.

The proposed research will lead to testing of our hypothesis that the diverse biological and medicinal activities that have been attributed to the withanolides, especially withaferin A, result in large part from their disruption of one or more of Annexin II's multiple cellular functions and that manipulation of some structural features of withaferin A will result in more potent analogs which can be used to treat pancreatic cancer and other solid tumors.

We are hopeful that this project involving two niche areas of Arizona Biosciences Roadmap, cancer and medically-oriented bio-agriculture, will provide an appropriate candidate for further development as a mechanistically unique and clinically effective drug to treat pancreatic cancer.

Melissa D. Halpern, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Enterohepatic Circulation of Bile Acids in Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is a life-threatening gastrointestinal disease of premature infants. "Necrotizing" means tissue death, "entero" refers to the small intestine, "colo" refers to the large intestine, and "itis" means inflammation. Therefore, NEC is an inflammatory disease that can cause destruction of the intestines. Damage to intestinal tissues can allow bacteria to leak into the abdomen and cause infection. In its severest form, NEC can result in surgical removal of portions of the affected intestine. It is a potentially life-threatening disease that affects approximately 500 premature infants in Arizona each year; 20–50% of these babies will die. Over the last decade, the number of prematurely born infants in Arizona and nationwide has steadily increased.

Consequently, the number of babies with NEC has also increased. Statewide, NEC is responsible for up to 5% of all neonatal intensive car unit admissions and is associated with high medical costs. The exact cause of NEC is unknown, and currently, there are no specific treatments for this devastating disease. Infants that require surgical removal of intestine may experience lifelong digestive difficulties. Thus, the search for the causes of NEC is of critical importance in both Arizona and the rest of the nation.

Using a neonatal rat model of NEC, our laboratory was the first to show that elevated levels of bile acids in the ileum (the site of NEC injury) contribute to intestinal damage during NEC. However, the mechanisms by which bile acids accumulate and cause tissue injury have not been clarified. The goals of this proposal are to systematically examine the mechanisms involved in bile acid induced tissue injury during disease development using neonatal rat and mouse models of NEC. The goals of this proposal will be achieved using the following specific aims testing the hypothesis that:

1) bile acids accumulate in the ileum because of an inability to regulate ileal bile acid transport,
2) changes in production and or transport of bile acids in the liver contribute to NEC pathogenesis, and
3) bile acids disrupt intestinal barrier function and affect disease development during experimental NEC.

Clarification of the mechanisms involved in bile acid accumulation and bile acid-induced barrier dysfunction in experimental NEC will provide crucial data regarding this devastating disease. These data will allow examination of bile acids in human infants with NEC and may ultimately lead to the use of elevated bile acids as a predictive test of disease risk in the clinical setting.

Ronald P. Hammer, Jr., Ph.D.

University of Arizona
Award Amount FY07: $49,995

Brain Dopamine, Glutamate and Cortical Activity: A Rodent Model of Schizophrenia

Schizophrenia affects about 1% of the population, or more than 60,000 persons, in Arizona. The cause of the disorder is unknown, but one prominent hypothesis postulates that overactivity of the brain chemical, dopamine, contributes to the symptoms. These symptoms cause patients to perceive sensation such as sounds or voices that are not really there (hallucinations). In fact, drugs that increase brain dopamine levels can produce schizophrenia-like symptoms, as do drugs such as phencyclidine (PCP or angel dust) which block a particular type of glutamate receptor. Furthermore, a large multi-site study recently showed that 74% of patients with schizophrenia discontinue their medication within 18 months due to inefficacy or intolerable side effects. Therefore, more effective and tolerable treatments are urgently required. The proposed studies will use sophisticated cellular and molecular imaging methods to investigate a novel hypothesis as to how dopamine or PCP alters a specific brain circuit to stimulate regions related to sound perception. This approach will help to explain the brain mechanisms underlying schizophrenia symptoms and to develop new therapeutic targets.

Schizophrenia is a chronic, debilitating disorder with onset frequently observed during adolescence and symptoms lasting throughout life. Thus, the medical and economic cost is devastating to patients and their families. Patients are often unable to hold a job or continue normal social relationships, and the burden to taxpayers is enormous. A better understanding of the biological basis of this disease will help define its causes and focus our efforts to develop effective treatments. Funding of this pilot project will provide the resources necessary to obtain federal research funding to continue these efforts.

This objective of the research is to determine whether pharmacological manipulation of a particular brain circuit arising in a region called the caudate nucleus in rats can produce cortical activation in a pattern resembling that present in humans experiencing auditory hallucinations. We will also compare the resulting pattern of brain activity to that produced by actual sound stimulation in experimental rat subjects. The brain chemistry, circuitry and response to dopamine and glutamate in the caudate is nearly identical in rats and humans, enhancing the predictive value of this experimental model. In fact, a parallel brain circuit (in the putamen nucleus) is responsible for producing stereotyped patterns of motor activity, limiting movement in Parkinsonism when too little dopamine is present. However, much less research has been conducted to examine the influence of excessive dopamine or NMDA blockade in caudate on patterns of cognitive activity.

The hypothesis is that selective regional drug infusion into the caudate nucleus in rats will produce aberrant activity in the auditory cortical region similar to that produced by sound. Normal function of caudate neurons is modulated by both dopamine and glutamate. Although we cannot determine whether the experimental animal subject "perceives" sound, we will examine the precise localization of nerve cell activity and compare this pattern to that produced by actual auditory stimulation in

additional rat subjects. In fact, schizophrenia patients experiencing auditory hallucinations exhibit a pattern of auditory cortex activation resembling that produced by sound in imaging studies of brain. Thus, this experimental approach can provide a proof of concept for the involvement of caudate in a novel mechanisms of schizophrenia symptoms, providing potential regional pharmacological targets for future drug development.

Paul Keim, Ph.D.

Translational Genomics Research Institute
Award Amount FY07: $150,000

Novel Genomic Analysis of *Coccidioides Posadasii* Isolates for Molecular Epidemiology and Population Characterization of Valley Fever in Arizona

Valley Fever (VF) is an increasingly important infectious disease in Arizona. More cases of VF occur in Maricopa County than anywhere else in the world, and the number of reported cases is increasing each year at an alarming rate. Public health officials are limited in their ability to prevent or respond to outbreaks because of a lack of appropriate molecular epidemiology tools needed to genetically link cases. The primary reason for a lack of tools is that researchers have not been able to properly genetically characterize this pathogen, thereby preventing any informative populations analysis.

Previous researchers have been able to develop genetic tools to separate the California species (*C. immitis*) from the non-California species (*C. posadasii*), as well as to separate out isolates from geographically diverse areas (Arizona versus Mexico versus South America). These studies have involved primarily the use of microsatellite markers (repeated DNA units that provide information about the relatedness of isolates). These markers, however, do not provide much population information at smaller geographic scales (e.g., isolates collected across an individual state, such as Arizona). This is likely because Coccidioides had been reported to genetically recombine (where different isolates swap pieces of their DNA), causing confusion regarding the genetic relatedness of isolates of Coccidioides.

The need exits, therefore, for newer highly-refined genetic analysis schemes to better understand this pathogen. Such a system will allow public health officials to better understand the transmission of VF, including the identification of genetically linked cases and potentially pin point sources of infection for case clusters and outbreaks. It is proposed here to use recent advances in pathogen genomic technology, used previously here in Arizona, for population analysis of other important pathogens.

The outcome of this research will provide much needed genetic information regarding the cause of Valley Fever (VF) in Arizona, *Coccidioides posadasii*. Our proposed work will use previously proven analytical processes and will build upon an existing and proven collaboration between TGen-North and the Arizona Department of Health Services (AZDHS).

The hypothesis to be tested by this research is that newer genomic techniques provide the power necessary to identify informative population structures for *C. posadasii*. Additionally, it is hypothesized that these tools will be useful for public health officials in Arizona to contact DNA fingerprinting and genetically link cases that may be involved in an outbreak of VF, thereby leading officials to potentially determine the source of an infection or outbreak.

The primary goals of the proposed research will be to develop and validate two genetic analysis techniques that have not been previously described for VF research. These techniques include enhanced multi-locus sequences typing (eMLST) and whole genome single nucleotide polymorphism (SNP) analysis. These techniques have been used previously by the researchers to develop informative population structures for other pathogens of public health importance. Following the development of these techniques, the researchers will validate them using a large repository of isolates (over 150) of Coccidioides, collected from around Arizona.

Pawel Kiela, DVM, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Modulation of Neutrophil Function by Curcumin in Inflammatory Bowel Diseases

Human Inflammatory Bowel Disease (IBD) is a chronic disorder of the digestive tract characterized by inflammation of the bowel. The most common types of IBD are Ulcerative Colitis (UC) and Crohn's Disease (CD). Both environmental factors and genetic predispositions have been implicated in the pathogenesis of IBD. IBD has not only a great influence on the quality of life of the affected individuals, but also a significant economic impact in the United States and in other developed countries. At least 1 million of Americans suffer from IBD, with approximately 30,000 new cases diagnosed each year. Adolescents and young adults are more susceptible to IBD. The total costs (medical and disability costs) of IBD in the US totals more than 2.6 billion every year. Complete medical cure remains a challenge and the probability of relapse is over 70%. Therefore, new treatment modalities are still in high demand. The precise causes of IBD remain mostly indeterminate and may be due to an inappropriate and persistent immune response against the non-pathogenic intestinal bacterial flora. The balance between an appropriate immune response against invasive pathogens and a controlled-immune response against harmless pathogens is required to maintain intestinal integrity. Neutrophils (PMN) constitute the "first line" of defense of the mucosal immune system, but a dysregulation in PMN recruitment and functions may increase intestinal inflammation. While major proinflammatory cytokines regulating T cell activation, such as TNFα IFNy, and IL12, have been recently the center of attention for pharmacological interventions, neutrophil chemotaxis is increasingly recognized as a potential therapeutic target in the treatment of chronic inflammatory diseases including IBD. Considerable resources are currently being devoted in the pharmaceutical industry to development of small molecules which would interfere with neutrophil migration, particularly targeting major receptors for chemoattractants such as CXCR2 and CXCR.

As a result of recent advances in phytochemical research, several botanicals have been considered to treat human diseases. The dried rhizome of *Curcuma longa Linn.*, also called tumeric, is described as a powerful anti-inflammatory agent. Curcumin has been identified as the most active constituent of tumeric and is also defined as an anti-inflammatory, anti-oxidant, anti-carcinogenic, pro-apoptotic and anti-proliferative agent. The effect of curcumin on the immune responses (both innate and adaptive) has been a subject of attention in the past 10 years. Dietary curcumin was proven efficacious in mouse IBD models. Results obtained in our laboratory in chemically-induced mouse model of colitis indicate that dietary curcumin significantly increases survival, protects from weight loss, and normalizes the indices of the disease. Genome-wide microarray analysis of colonic gene expression in this model identified a significant number of genes related to neutrophil function as targets of curcumin-mediated modulation. Subsequently obtained preliminary data presented in this proposal point to significant effects of curcumim on multiple aspects of neutrophil biology and prompted us to hypothesize that part of the beneficial effects of this phytochemical in IBD may be due to its effects on neutrophil functions.

George R. Pettit, Ph.D.

Arizona State University
Award Amount FY07: $150,000

Molecular Targeting of Prostate Cancer Vasculature: A New Approach to Treatment

Over one million American families are vitally affected and impacted by prostate cancer. One in six men in the United States will be diagnosed with prostate cancer in their lifetime. In Arizona in 2004, some 4,000 new cases of prostate cancer were detected and about 600 men died from the disease. Prostate cancer is the most commonly diagnosed non-skin cancer and the second most common cancer killer of American men. Recent (2005) statistics indicate some 240,000 new cases diagnosed and 30,000 deaths. The generally tragic and all-too-frequently lethal outcome for prostate cancer victims will not be alleviated until treatment approaches are greatly improved by introduction of new and more generally curative anticancer drugs for controlling prostate cancer. Unfortunately, curative therapy in the form of radical surgery or radiotherapy requires the disease be confined to the prostate. Metastatic prostate cancer is usually incurable and most men diagnosed with metastatic disease die over a period of months to years. To further complicate the treatment problem, prostate cancer is not a homogenous disease at the molecular level. In addition, no treatment regimen has been proved to provide a substantial improvement in survival time, and many are quite detrimental to the quality of life. Our research group has pioneered the discovery and development of new cancer vascular targeting drugs/prodrugs, and we will extend this very successful research focus to making improvements in the treatment of human prostate cancer.

The proposed new anticancer drug and prodrug structures have been designed whereby the active anticancer drug should be released at the sites of prostate cancer tissues by means of reaction with the serine protease PSA and/or prostate phosphatases. The lead structures for syntheses of the prodrugs bearing an octapeptide containing a serine unit known to be cleaved by PSA have been chosen from three lead groups of plant and marine invertebrate anticancer drugs with powerful antiangiogenesis/cancer vascular targeting properties. The new antiangiogenesis/cancer vascular targeting drugs will be utilized in a new molecular targeting of prostate vascular systems to prevent blood delivery to the tumor and regrowth of the vasculature. In addition, as noted above, certain of the prodrugs have been designed to contain phosphate esters and amides that will allow activation by one or more of the three enzyme systems - PSA, phosphatases, and/or amidases. Those potentially multiple systems of enzymatic activation should lead to at least several really promising anticancer drug candidates for advancement to phase I human cancer clinical trials against refractory prostate cancer.

Richard Posner, Ph.D.

Northern Arizona University
Award Amount FY07: $296,695

Modeling Vulnerability of Cancer

Successful patient tailored personalized medicine will involve the use of genetic, genomic, or proteomic information to guide the selection of specific therapies to treat a disease in a particular individual. While physicians have employed individualized treatments for years, the recent advances in the development of target-based cancer treatments provide motivation for more precisely identifying which patients will benefit from various treatment options. This proposal is aimed at combining experimental profiling techniques with sophisticated mathematical analysis to elucidate optimal treatment strategies for individual cancer patients.

As our basic understanding of molecular cell biology has grown, it has become increasingly clear that the underlying cause of many serious health problems is cellular signaling pathways that have gone awry. As a result, these pathways, in which protein-protein interactions play a central role, have become a focus of intense investigation. As the aberrant proteins within these networks have been identified, a new generation of pharmaceuticals that are designed to specifically target and modulate their behavior are being developed. For example, in many forms of cancer, the tumor often exhibits an over abundance of the epidermal growth factor receptor (EGER). Over abundance of EGFR has been observed in solid tumors, such as colorectal, lung, head and neck, as well as brain tumors. Since EGFR provokes strong responses in cells, and since EGFR is over abundant in cancer cells, drug discovery efforts to inhibit EGFR signaling is a step towards highly specific anti-cancer therapies (examples of drugs that block EGFR activation are Erbitux or Tarceva). There may also be biochemical reactions in the downstream pathway of EGFR which could be very effective targets for new drugs, with fewer side effects and greater cancer specificity. While these pharmaceuticals have tremendous potential, for most cancer patients a single therapeutic agent is generally inadequate to control the disease. For such individuals, it is likely that a "cocktail" approach which combines two or more therapeutics may be the best solution. A mathematical model of the different signal transduction pathways in cancer cells assembled from actual biochemical measurements of these reactions would greatly assist in discovering and prioritizing where in the pathway new treatments should be directed.

Every year about 10,000 Arizonans succumb to cancer and an even larger number are diagnosed. This project, which is a collaborative effort between a computational biologist at NAU and genomics & proteomics investigators at TGen, seeks to develop novel therapeutic approaches for the treatment of this devastating disease.

The goal of this project is to combine experimental studies and sophisticated computer modeling techniques to develop a quantitative model EGFR signaling that can be translated directly into the clinic. Such a model offers the promise of 1) understanding the mechanistic details of disease and drug action, 2) elucidating potential targets for new therapeutics, 3) predicting side effects of various treatment

regimens, 4) finding optimal dosages and combinations of therapies, and 5) informing the development of treatment schedules for new targeting agents.

Donato F. Romagnolo, Ph.D.

University of Arizona
Award Amount FY07: $49,445

Epigenetics of Breast Cancer and Chromatin Remodeling of the BRCA-1 Gene

One of the puzzles in cancer predisposition is that women carrying inherited mutations in the BRCA-1 gene preferentially develop tumors in estrogen (E2)-responsive tissues such as the breast. This occurs in spite of the fact BRCA-1 is involved in biological functions such as DNA repair that are universal to all cells. Moreover, sporadic breast tumors, which represent ~90% of all breast cancer cases, contain lower levels of BRCA-1 in the absence of BRCA-1 mutations. The evidence suggests that loss or reduced expression of BRCA-1 renders breast epithelial cells particularly susceptible to neoplastic growth. This application addresses the environmental mechanisms that lead to repression BRCA-1 transcription. In year 2005, ~3,000 new cases of breast cancer were diagnosed for the State of Arizona. However, only a small fraction (~10%) are related to mutations in the BRCA-1 gene. This proposal seeks to investigate whether the exposure to environmental pollutants alters normal BRCA-1 levels, thus predisposing to sporadic breast cancer.

The long-range goal of this project is to identify the factors that increase the risk of sporadic breast cancer and develop dietary strategies that prevent the onset of this malignancy. Reduced levels of wild-type BRCA-1 protein have been detected in a large percentage of sporadic breast tumors suggesting that silencing of the BRCA-1 gene may be a contributing factor in the onset of breast cancer. The central hypothesis of this proposal is that the activation of the AhR by dietary and environmental AhR-ligands silences the BRCA-1 gene; whereas, this repressive effect can be prevented by dietary compounds.

Joyce A. Schroeder, Ph.D.

University of Arizona
Award Amount FY07: $49,999

A Mechanism of CD44-HA Mediated Inhibition of Breast Cancer Metastasis

Breast cancer is the third leading cause of cancer-related deaths in Arizona. The majority of these patients die when breast cancer spreads form 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.

One protein highly produced by cancer is CD44, a protein that controls how cells adhere and migrate through their tissue environment. Through studies in our laboratory, we have found that when CD44 interacts with certain tumor associated sugars, it inhibits cancer progression. We hypothesize that CD44 engagement is a natural response of the body to prevent metastasis, and we propose to determine how this is occurring.

To investigate how CD44 expression is inhibiting tumor metastasis, we will perform experiments to determine how activation of CD44 affects the ability of breast cancer cells to metastasize. One main goal of this proposal is to design proteins that may be able to mimic the anti-metastatic effects of CD44, with the goal of designing an anti-cancer therapeutic drug.

Ornella Selmin, Ph.D.

University of Arizona
Award Amount FY07: $49,954

Folate as a Nutrient Competitor Against Environmental Exposure to Trichloroethylene (TCE)

Congenital heart defects (CHD) are the No. 1 occurring birth defect. It is estimated that each year in the United States 40,000 babies are born with one of the many known heart defects. Over a million children and adults in the United States are living with a CHD. Each year nearly twice as many children die from heart defects than all childhood cancers combined. However, for every dollar provided by the National Institute of Health (NIH), only one penny is provided for pediatric research, and only a portion of that penny goes to support research on heart defects.

Exposure to environmental contaminants has been associated to increased numbers of CHD in both humans and laboratory animals. Trichloroethylene (TCE) is a man-made compound which is used as a solvent in the manufacturing of house-cleaning products, cosmetics, and paints. It is a ubiquitous pollutant present in air and water worldwide. TCE metabolites such as trichloroacetic acid (TCAA) are present in municipal water as by-products of chlorination. Non-occupational exposure to TCE in

humans is primarily through inhalation, water ingestion, and skin absorption. In Arizona, previous epidemiological studies have shown that exposure to TCE-contaminated water increased the number of newborns carrying cardiac malformations. Because of careless disposal of TCE in the past, and current use of TCE in industrial settings, our desert region is at risk, in particular in those areas that rely on groundwater wells as a source of drinking water. The relevance of the health issue represented by TCE contamination in Arizona has been recently highlighted in the October issue of the Phoenix Magazine in an article titled: "Trouble in the Tap," by Kelly Kramer. This article reports that as of today, there are 6 areas in the Maricopa County listed as National Priorities List, 5 of which are contaminated with TCE. Additionally, of the 21 sites in Arizona listed as Superfund Sites, 15 are contaminated with TCE at levels much higher than those allowed by federal standard. Research conducted in recent years by our laboratory and others indicated that TCE alters crucial cellular processes required for normal heart development. However, the biochemical and molecular mechanisms through which TCE induces these changes remain largely unknown. This proposal offers the opportunity to clarify some of these mechanisms and develop preventive strategies based on specific nutrients which may be administered during pregnancy to the mother.

The objective of this study is to assess the effects of folic and deficiency and supplementation, created by diet, on alteration of phenotype and gene expression induced by maternal exposure to trichloroethylene (TCE). The hypothesis is that a reduction of folate in the diet will decrease the amount of methyl groups available for DNA methylation and consequently affect normal heart development. On the other hand, folate supplemented diet may help to overcome the effects of TCE exposure during development. The effect of folate deficiency and interactions with TCE exposure will be determined in the Sprague-Dawley rats, using two different approaches. In the first one, we will analyze individual embryos (at embryonic day 22) from dams receiving a diet containing low, normal, or high folate, and drinking water with or without TCE. We expect that addition of folate will ameliorate or prevent the negative effects of TCE on heart development, and therefore, will reduce the number of cardiac defects observed in the newborn. In the second approach, we will test the hypothesis that exposure to TCE will affect the level of gene expression in the embryonic heart. Alteration of expression of genes that are important during heart development will eventually lead to heart defects; therefore, these genes can be used as markers of TCE-induced heart malformations. According to our hypothesis, a low folate diet will induce similar changes in the expression level of the selected maker genes; whereas, folate supplementation will reduce or suppress changes in their expression level. Two genes (vimentin and Serca2a) were selected as markers of exposure to TCE in previous studies. Changes in their expression levels will be determined by real-time PCR and methylation analyses. We expect that folate supplementation will reverse or ameliorate the inhibitory action of TCE on expression level of the marker genes. Results from this study will be important for the design of strategies aimed at protecting or preventing the onset of heart defects in environmentally exposed populations.

Larry D. Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY07: $150,000

CSF Copper and Cognitive Performance

Cognitive dysfunction (dementia) is one of the most devastating possibilities facing Arizona's aging population. Discovering a biomarker predictive of impending cognitive dysfunction would aid in developing better medications to treat and medically follow disorders causing dementia such as Alzheimer's disease (AD) and Parkinson's disease(PD). In a study using autopsy brain and cerebrospinal fluid (CSF), samples we found that reduced levels of copper in the brain and the CSF (as a surrogate marker of brain levels) were significantly related to changes in cognitive performance. High levels of brain and CSF copper were found in cognitively normal controls and cognitively normal PD patients. Very low levels of brain and CSF copper levels were identified in demented AD and PD patients, while intermediate brain and CSF copper levels were found in patients with mild cognitive impairment (no longer considered control, but not sufficiently severe to be diagnosed as dementia). Although a bio-diagnostic for dementia itself might have limited utility, a biomarker that tracks and/or predicts cognitive decline would be extremely useful in clinical trials and patient management. Because the preliminary data were obtained using autopsy tissues, it is imperative that we replicate these findings among living individuals and demonstrate that reductions in CSF copper precede changes in cognitive function before this may be deemed a prognostic biomarker of future dementia. A three-year longitudinal assessment, with CSF collection and neurologic evaluation at one-year intervals, is therefore proposed to test the hypothesis that CSF concentrations of the biomarker are significantly associated with and/or predict cognitive decline in living individuals with dementia or mild cognitive impairment, as well as in normal elderly subjects.

Our goals are to determine if 1) copper levels in the CSF remain constant in an individual showing no change in cognitive performance, 2) copper levels in the CSF of a control patient decrease as the individual makes the transition to a diagnosis of MCI, 3) copper levels in the CSF of a patient with MCI decrease as the individual makes the transition to a diagnosis of AD, 4) copper levels in the CSF of a patient with AD decrease as cognitive impairment becomes progressively more severe, and 5) cognitive deterioration is predicted by declining CSF copper concentration.

Brent Vernon, Ph.D.

Arizona State University
Award Amount FY07: $150,000

Improved Materials for Endovascular Embolization

Treatment of several vascular disorders such as Aneurysms and Arteriovenous Malformations (AVM) include embolization (injection of a material through a catheter into the vessel that blocks flow from entering the aneurysm or malformation). However, the current materials used for this application in the clinic have serious drawbacks. Materials such as cyanoacrylates used for this application can 'glue' the delivery catheter in to the vessel resulting in vessel damage upon removal of the catheter. Other materials are delivered in organic solvents such as DMSO and NMP which are known to be toxic. Replacement of these materials with a system that can be delivered in water and then gel within the vessel without adhesion to the tissue will help to overcome these issues. Unfortunately, materials that can be delivered in water will swell giving high water content and lower mechanical properties. To overcome this swelling and low mechanical properties with waterborne materials, temperature-sensitive polymers that undergo a transition from liquid to gel upon injection in the body as the material transitions from being water soluble to water insoluble with the change in temperature have been investigated for this application. However, these gels have the potential to 'creep' (flow of a solid under constant stress/pressure). This creep can potentially cause the material to inappropriately block flow to crucial tissues. The design of a material that will simultaneously gel chemically (with a self-reactive chemistry) and physically (due to temperature) can help to overcome these issues. A collboration between Barrows Neurological Institute (BNI) in Arizona, a premier neurovascular clinical and research institution, and ASU can lead to successful development of an improved material for endovascular embolization. This would further lead to successful commercialization in an Arizona Biomedical Company.

We have developed a new class of simultaneously physically and chemically cross-linking materials in the laboratory. In a simultaneous physical and chemical cross-linking polymer solution, the polymer solution will initially undergo physical gelation when introduced within the blood vessel at 37°C to prevent the material from being washed away by the blood flow. However, concurrent and subsequent chemical cross-linking (curing) will reduce the potential for creep (flow of a viscoelastic solid) by making the material more elastic (versus viscoelastic). Therefore, the immediate short-term goal of this proposed work is to investigate the feasibility of using these simultaneous chemically and physically gelling materials for endovascular embolization in pig models of AVM and aneurysm. Specifically, we will synthesize novel simultaneously physically and chemically cross-linking biomaterials by combining multi-acrylate-functionalized N-isopropylacrylamide copolymers with a multifunctional thiol compound, pentaerythritol tetrakis 3 mercaptopropionate, and characterize the structure-function relationships for these materials. We will then assess the *in vitro* cytotoxicity of simultaneously physically and chemically cross-linking biomaterials based on these materials. Finally, we will then assess *in vivo* compatibility and gelation of these selected simultaneously physically and chemically cross-linking biomaterials in the pig models. In the end, a novel class of simultaneously physically and chemically gelling materials

will be developed as injectable biomaterials with increased creep resistance, and the feasibility of using these materials for endovascular embolization will be established in a porcine rete mirable model. A thorough understanding of the chemical, temperature, and mechanical properties of these new materials will be obtained and can be used to rationally design future generations of these materials. In addition, the information obtained will permit customization of these materials for specific biomedical applications which would include, but not be limited to, endovascular embolization for aneurysms, tumor treatment, and arteriovenous malformation treatment, tissue reconstruction and plastic surgery, bladder bulking for incontinence, and fallopian tube embolization for permanent contraception.

Danzhou Yang, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Novel AP-1 Inhibition of a Highly Potent Anticancer Drug XR5944

The systemic therapies currently available for the treatment of various solid tumors of adult life, including those of lung, colon, breast, prostate and ovary, remain primarily palliative, and there is an urgent need for more effective therapies. Although recently there has been a significant effort in developing molecular targeted therapies, cytotoxic agents remain the major form of therapy for the majority of cancers. Moreover, there is reason to believe that a therapeutic advantage remains to be gained from cytotoxic drugs with novel mechanisms of action, with safety profiles and different spectra of anti-tumor activity. The bis-phenazine XR5944 is a novel cytotoxic agent with exceptional anti-tumor activity against a range of human and murine tumor models both *in vitro* and *in vivo*. It has recently entered Phase I clinical trials. Although initial reports showed that XR5944 can bind strongly to DNA and that it may interfere with the normal function of topoisomerase I and II *in vitro*, recent studies have indicated that the novel biological activity of XR5944 is related to a novel mechanism of action-inhibition of DNA transcription. We have recently determined the molecular structure of the DNA complex of XR5944 and have shown that the *in vitro* DNA binding to the AP-1 site of c-Jun protein, a major member of the AP-1 transcription factors, is significantly inhibited by XR5944 in a dose-dependent manner. Since AP-1 is the key family transducing multiple signals for cell proliferation in response to various pathways, targeting AP-1 transcription factors can be an effective approach to inhibit cancer cell growth. However, no example of small molecule inhibitor of AP-1 transcription factors has been proposed. In this proposal we intend to identify the molecular target of XR5944 and to characterize the novel mechanism of action of the drug. This information will not only be used to understand the novel mechanism of action of XR5944 accounting for its exceptional anticancer activities, structural features that promote AP-1 binding and subsequent AP-1 inhibition can also be utilized to guide further drug development. If successful, this study is going to be quite significant, as we will identify a potential target for cancer treatment, the AP-1 family of transcription factors, and a potent small molecule inhibitor that can be used as a template for further structure-based rational design of

small molecule inhibitors specifically targeting the AP-1 pathway.

In summary, this research is not only important for bringing a new novel anticancer drug for cancer patients in Arizona, but can also provide an important basis for the development of new drugs bearing this novel and unique mechanism of action for the treatment of cancer, a disease that impacts a large number of citizens of the State of Arizona.

The hypothesis to be tested is that the exceptional biological activity of XR5944 is due to its novel DNA binding model leading to a unique mechanism of action, the inhibition of the AP-1 family of transcription factors. We will characterize the AP-1 inhibition by XR5944 both *in vitro* and *in vivo*, as well as identify the cellular targets of XR5944 in solid tumor cell lines and determine the AP-1 pathway involved in the drug action. We will also explore the molecular basis for interactions of XR5944 with AP-1 DNA consensus sequences. Structural features that promote AP-1 binding and subsequent AP-1 inhibition will be utilized to guide further drug improvement. We will use high-field nuclear magnetic resonance (NMR) spectroscopy and molecular computational modeling, microarray and real-time PCR, in combination with the *in vitro* and *in vivo* biochemical and cellular assays for the proposed study.

The objectives of the proposed research are:
1. To understand the mechanism of action of XR5944 and to characterize the *in vitro* and *in vivo* inhibition of the AP-1 family of transcription factors by XR5944; and
2. To determine the three-dimensional structures of XR5944 with AP-1 consensus sequences, and to use this information as a basis for designing better AP-1 inhibitors.

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Award Amount FY07: $49,999

Mechanisms for Local Translational Control in Drosophila Neural and Germ Stem Cells

Fragile X syndrome is the most common form of inherited mental retardation, which according to recent estimates affects 1 in 4,000 males and 1 in 8,000 females throughout the United States. This disease, for which there is no cure but only palliative treatments, is due to loss of function for the FMRI gene which encodes an RNA-binding protein named Fmrp. In the nervous system, Fmrp has been shown to function in early development by contributing to neuronal arborization and growth, as well as morphology of synapses, all of which are critical for the formation of proper neuronal circuits in the brain. At the cellular level, Fmrp is thought to function in various neurons by associating with specific messenger RNAs and regulating their transport, localization and translation into proteins in the vicinity of synapses. Thus, one of the critical problems is elucidating the mechanisms underlying

Fragile X syndrome is to define the mRNAs controlled by Fmrp and how they contribute to various aspects of the disease. Furthermore, an understanding of Fmrp function early in neural development, particularly in stem cells which give rise to all cells comprising the nervous system, has the potential to uncover new therapeutic strategies for Fragile X syndrome. Here, we propose to address these issues by characterizing Fmrp function in stem cells and defining Fmrp specific associated mRNAs. More importantly, we will determine the *in vivo* significance and contribution of these mRNA targets to specific aspects of Fmrp function. Thus, we hope to shed new light on the molecular pathways involved in Fragile X syndrome as well as discover novel therapeutic target for this devastating neurological and cognitive disorder.

Our work hypothesis is that Frmp functions in stem cells by controlling the translation of specific mRNAs and that Fragile X syndrome is a consequence of aberrant translation of such mRNA targets. Here, we propose to use Drosophila as a model system for it is simple and has been successfully used to model other human diseases such as neurodegenerative disorders. Drosophila harbors a Fmrp homolog which is highly conserved in structure and function with its mammalian counterpart. Furthermore, phenotypic analysis of Drosophila mutants lacking Fmrp show amazing similarities in terms of neuronal and cognitive deficits with the mouse model and patient manifestations. In addition, the genetic tools we have available in Drosophila are making this model system a very powerful one for studying the molecular mechanisms underlying Fragile X syndrome. Here, we propose to define the specific roles of Fmrp in Drosophila germ and neural stem cells. We will investigate the consequences due to loss of Fmrp on stem cell number, renewal as well as cell division properties. These experiments will allow us to determine what is the function of Fmrp early in development, before any neurons differentiate and form neuronal circuits. We also propose to identify the translational targets which function as effectors of Fmrp in stem cell function. We will use microarrays to identify mRNAs specifically associated with Fmrp. Using mutants in these predicted targets and Drosophila Fmrl gene, we will test the functional significance of these mRNA targets and will determine what aspects of Fmrp function they mediate in stem cells. These experiments are critical in that they should begin to demonstrate what targets of Fmrp are responsible for specific phenotype. This should ultimately shed light on how Fmrp deficiency leads to developmental and neurological defects.

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