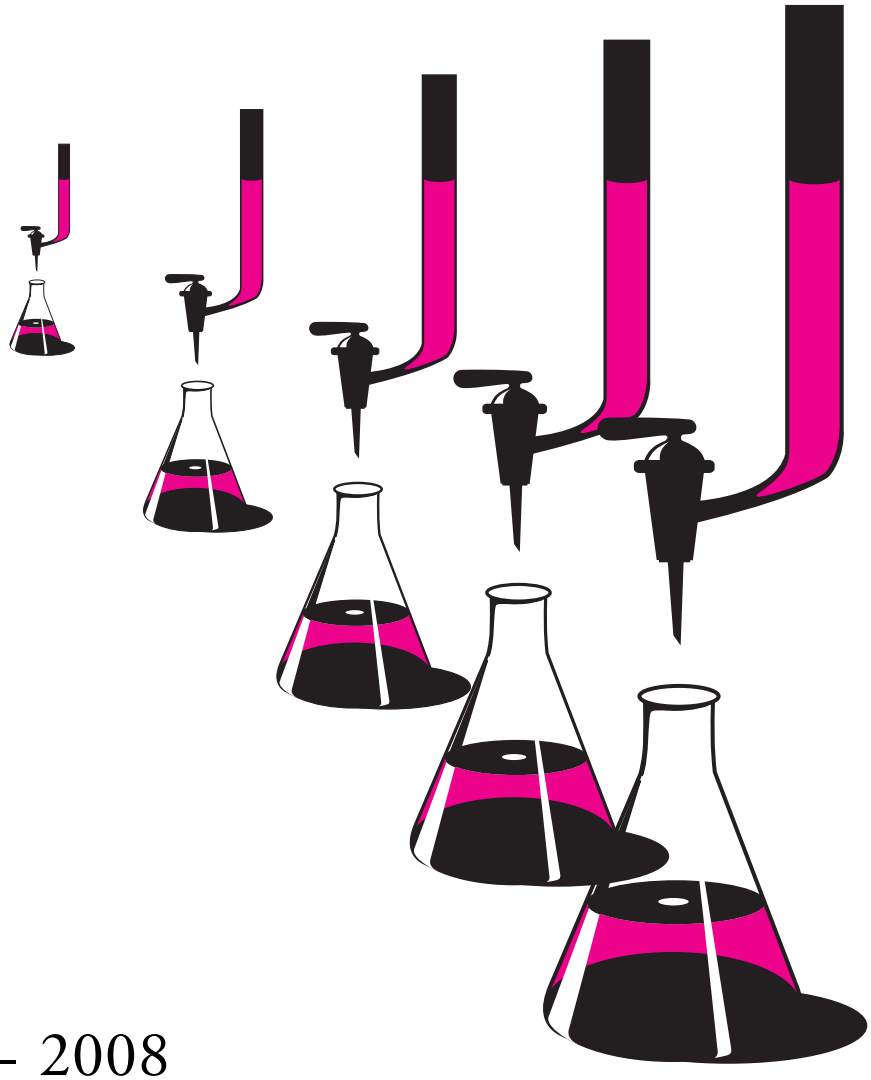
Arizona Biomedical Research Commission



2007 – 2008 Annual Report

January 2009

ARIZONA BIOMEDICAL RESEARCH COMMISSION ANNUAL REPORT 2007–2008

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.

Staff
Executive Director: Dawn C. Schroeder, D.D.S., M.A.
Deputy Director: James Matthews, M.P.A.
Executive Staff Assistant: Damika D. Brock
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David Landrith, M.P.A.

Message from the Chairman

Medicine has changed in the 21ST century. Life expectancy is the longest in the history of humankind.

The challenge of the 21ST century is not to expand the length but to improve the quality of life by preventing and defeating deadly and debilitating diseases. The Commission is supporting the Arizona Parkinson's Disease Center a significant neurological research project. The Commission supported at a critical juncture the Arizona scorpion anti-venom project. The Commission continues to support an innovative approach to decrease the amount of scar tissues after heart attack. The Commission also supports a personalized approach to cancer therapy. These programs as well as efforts designed to support individual researchers are core to the mission of the Commission.

The Commission awarded nineteen new scientific research contracts this year. There will be a total of seventy-four research projects under contract with the Commission beginning in FY2009. 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.

The Commission continued to work in cooperation with the Flinn Foundation to move the recommendations of the Arizona Bioscience Roadmap forward. The Commission has taken the lead in accomplishing the translational research goals. Commission sponsored researchers are pursuing translational research in the neurosciences, cancer, bioengineering, and bioimaging. Of particular note is the progress being made in innovative approaches to research in Native American communities. The Commission created and continues to support the Arizona Translational Resource Network. AzTransNet is focused on removing institutional barriers to research, for example, establishing statewide or community based Institutional Review Boards; developing collaborative associations and clinical research networks; and providing streamlined business practice templates such as uniform intellectual property contract documents and material transfer agreements.

The Commission is undergoing the ten-year sunset audit review process. The Commission thanks the Office of the Auditor General for the time and effort they spent in learning about the work of the Commission. The audit recommendations are timely and useful. The Commission is adopting all of the recommendations.

The Annual Report is prepared and submitted each year to the Governor, the President of the Senate, the Speaker of the House of Representatives. It is the hope of all of the members of the Arizona Biomedical Research Commission that encouraging both new researchers and large scale multi-institutional/multidisciplinary investigation 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. His term expired in 2007, and he was re-appointed by Governor Napolitano in 2008.

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.

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 in 2007.

Summary of 2007–2008 Commission Activities

In the fiscal year 2007-2008, Arizona biomedical researchers received more than $7 million in 74 contracts administered by the Commission. The Commission continued its commitment to individual investigators, investigator collaborations, and further expansion into translational research. 19 new contracts and approximately $1.7 million from the Health Research Fund and the Disease Control Research Fund were directed toward assisting individual investigators in developing proof of their research concepts, collecting preliminary data, and in continued support of translational research.

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 170 scientific publications, abstracts, and presentations arising out of the research are also listed. Section D provides information on new contracts awarded beginning July 1, 2008 (FY2009).

Over 1,000 Requests for Proposals (RFPs) for the 2007-2008 awards were mailed to potential applicants in September 2006. The Commission has seen a decrease in revenue available from the sale of tobacco products which in turn decreased the amount of funding for new biomedical research to approximately $1.2 million. The decline may be attributed to the impact of the Early Childhood Development and Health Initiative adopted by the voters and effective starting in January of 2007. The initiative earmarked tobacco funds for the Childhood Development and Health program and reduced the availability of funds for the ABRC compared to previous years.

While revenue from the tax on tobacco sales fell during the fiscal year, revenue from the proceeds of the State Lottery remained consistent with the previous fiscal year. Lottery revenue in Fiscal Year 2007 was $2.4 million and in Fiscal Year 2008 it was $2.5 million.

In response to the RFP, the Commission received 131 unrestricted medical research proposals. In November and December the medical research proposals were sent to a panel of national and international scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more than 170 out-of-state peer reviewers. Three reviews were sought for each proposal. In the spring and summer of 2007 the Commission selected 19 proposals for funding.

ABRC Projects Submitted/Accepted FY 2008
(Health Research Fund and Disease Control Research Fund)

Institution	Submitted	Accepted	Percent Accepted	Amount $	Percent of Total $
Arizona State University	23	3	12	350,000	20
Northern Arizona University	2	1	50	300,000	18
St. Joseph's Hospital	10	1	10	50,000	3
Sun Health Research Institute	6	0	0	0	0
TGen	7	1	14	150,000	9
University of Arizona	77	13	17	850,000	50
Others	5	0	0	0	0
Total	**131**	**19**	**15**	**1,700,000**	**100**

During 2007-2008 the ABRC managed a total of 74 translational and biomedical research projects representing eight research institutions.

ABRC Total New and Continuing Project Contracts 2008

Institution	Awarded	% of Total Awarded
Arizona State University	7	10
Inter Tribal Council of Arizona	1	1
Mayo Clinic Scottsdale	2	2
Northern Arizona University	3	4
Sun Health Research Institute	5	7
St. Joseph's Hospital	7	10
TGen	4	5
University of Arizona	45	61
Total	**74**	**100**

In June of 2008 the Commission awarded 28 new research contracts for a total of approximately $1.3 from all sources. The contracts were effective on July 1, 2008. Progress on these projects will be reported in the next Commission Annual Report.

ABRC Projects Submitted/Accepted FY 2009

Institution	Submitted	Accepted	Percent Submitted	Percent Awarded	$ Amount Awarded	Percent of Total $
ASU	22	6	18	21	297,705	23
St. Josephs	21	7	17	25	347,572	25
TGen	2	1	2	4	50,000	3
UA	70	14	56	50	648,801	49
All Others	9	0	7	0	0	0
Total	**124**	**28**	**100%**	**23**	**$1,344,078**	**100%**

The Commission remains committed to making the results of scientific discovery more readily available to health care providers and then to patients. The Commission currently has 15 translational 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 these translational projects will result in more rapid deployment of medical therapies to Arizonans.

The Commission's matching fund program has been well received by researchers and their home institutions. Growing from the initial three research programs, the Commission is managing research contracts for seven projects. In each of the programs the Commission investment is matched dollar for dollar by the sponsoring institutions. In order to be eligible for the matching fund program the research project must be multi-disciplinary, inter-institutional, and collaborative. The goals are to promote cooperation among researchers and between institutions, and to tackle significant biomedical research issues. In every project there are more than one principal investigator, more than one institution, and multiple research hypotheses.

Jointly Funded Research Projects

Project Title	Home Inst	3-yr Amt
Arizona Parkinson's Disease Center	Mayo	$ 750,000
Scorpion Treatment and Imaging of Neurotoxicity Group	UA	540,553
Multimeric Ligands for Targeting Cancer for Imaging and Therapy	UA	750,000
Drug Targeting the i-Motif in the c-MYC Promoter	UA	517,500
Biomimetic Scaffolds for Spinal Cord Regeneration	BNI	750,000
Modeling Vulnerability of Cancer	NAU	414,768
Translational Research on AB Metabolism from Synthesis to Clearance	SHRI	675,000

One measure of research success is the publication of research findings in a recognized peer reviewed scientific publication. In 2007-08 ABRC sponsored researchers reported 147 publications, abstracts, and presentations of their research findings. Publication of scientific findings is a critical step in the research enterprise. The data reported provides the foundation upon which the next research project is founded. It is through this iterative process than scientific discovery takes place.

During the Annual Report period fiscal year 2007-2008, 223 full time and part-time jobs were created including principal investigators.

If a scientific discovery has the potential for commercial application, it is important that the rights to that discovery be protected. The Commission on behalf of the citizens of Arizona holds seven patents on biomedical compounds with potential commercial value.

Five Rose, et al patents are focused on technologies that will increase the effectiveness of cancer chemotherapy drugs by reducing the ability of cancer cells to expel the drugs from the host cell.

Two Gervay-Hague patents are for drugs that block the incorporation of tumor cell markers into cancer cells. These drugs may help stop the spread of cancer in the human body.

One Schroeder patent relates to methods of inhibiting, retarding, and reducing metastatic cancer growth.

TGen the Translational Genomics Research Institute

The ABRC through a contract provides funding to support the basic operational infrastructure of TGen, the Translational Genomics Research Institute. TGen is on the cutting edge of translational research where investigators are able to unravel the genetic components of common and complex diseases.

TGen is pursuing research on oncology, diabetes and heart disease, and neurological disease. All of these disease areas are of extreme importance to Arizona citizens.

Some TGen activities include:

- Developing a $200 million program funded by the Luxembourg government to develop a biobanking and biotechnology initiative in Luxembourg.
- A project to address the disproportionate impact of renal cell carcinoma in the Salt River Pima-Maricopa Indian Community with a study conducted in collaboration with the members of the community.
- The Partnership for Personalized Medicine which is a $45 million program designed to make personalized medicine a reality. Funding comes from the Virginia G. Piper Charitable Trust and the Flinn Foundation. The initiative is headed by 2001 Nobel Laureate Dr. Lee Hartwell.
- A team of researchers who are conducting a genomic study of autism, a disease which touches 1 in 150 children in Arizona.

TGen researchers:

- Have published more than 80 scholarly articles in peer-reviewed academic journals.
- Are conducting nearly 30 clinical trials for advanced and/or rare cancers.
- Have submitted 127 grant requests receiving 24 awarded grants in excess of $10.2 million; 39 grant applications totaling over $24.3 are pending peer review.
- The success rate of 24 percent of total grants submitted nearly doubles the national average of 13 percent.

TGen enterprise and collaborative efforts:

- 25 invention disclosures have been filed in FY08. Disclosure is the first step toward protecting intellectual property via the patent process.
- 97 confidential disclosure agreements have been signed in FY08. Confidential disclosure agreements are one measure of collaboration between TGen and the research community.
- 51 material transfer agreements between TGen researchers and other collaborators have been signed in FY08.
- 38 research collaborations have been signed in FY08 including agreements with research institutes and hospitals around the world.

TGen job creation:

- There are 270 full-time TGen employees with 80 percent holding a college degree
- 39 new full-time equivalent positions were created in FY08 with pay and benefits totalling approximately $1,862,000.
- 58 high school, undergraduate, and graduate interns had part-time employment with TGen in FY08.

The energy and focus of TGen leadership and researchers is rapidly advancing collaborative, multi-institutional, interdisciplinary research in Arizona. The financial infrastructure support provided by the Arizona Biomedical Research Commission makes these things possible. The Commission is encouraged by the efforts of TGen, and heartened by the impact that TGen research is making in biomedical research.

Section A

Continuing Contracts

Medical Research

Year Three

FY 2008

Charles H. Adler, M.D., Ph.D. **Mayo Clinic Scottsdale**

Award Amount FY 2008: $250,000

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

The APDC is working on clinical biomarkers and novel treatment strategies for Parkinson's disease and PD with dementia (PDD). There is a clinical core, which prospectively examines PD and control subjects enrolled in the brain and body donation program, and a neuropathology core that performs the autopsies and provides CSF and brain tissue to the laboratory scientists. To date >5,000 clinical evaluations of >1,000 subjects have occurred. In the past year 22 parkinsonism (11 PD) and 16 control subjects were autopsied. Projects 1 and 2 have found changes in BDNF, α-synuclein, and DJ-1 proteins in PD. Project 3 found dysregulation of multiple sets of genes in PD and PDD while Project 4 found differences in cerebrospinal fluid proteins in PDD. Stated goals have been met. In the past year 15 papers were published, 10 are in process, and 5 presentations were made. Funding was received from the Michael J. Fox Foundation and the National Parkinson's Foundation.

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Leslie Boyer, M.D. **University of Arizona**

Award Amount FY08: $188,526

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 for severe scorpion neurotoxicity, and 2) the lack of a validated clinical assessment tool with which to further new drug development and train emergency providers. Since the start of this project, a total of 21 rural and urban hospitals in Arizona have joined the study network and have treated over 548 patients, ranging in age from 25 days to 80 years. Digital video images of patients and of age-matched controls have been collected for development of a medical training tool. This project has resulted in 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.

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Boyer LV. Clinical Trials of Scorpion Antivenom. **Venom Week**. Tucson, AZ. 2007.

Ruha M. The Phoenix Experience. **Venom Week**. Tucson, AZ. 2007.

Fox F. Challenges of Treating Scorpion Envenomation in a Neonate in a Rural Setting. **Venom Week**. Tucson, AZ. 2007.

Thomas Bunch, Ph.D. **University of Arizona**
Award Amount FY08: $49,997

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*. In year one we established procedures for eliminating hundreds of genes, one at a time, and asking if this sensitizes cells to die when drug is added. In year two we tested 3 compounds that target pathways that potentially promote abnormal cell growth or survival in tumor cells. Numerous novel interactions have been uncovered, and in year three we have worked to validate these finding in human tumor cell lines. Additionally, we have completed new screens that involved 5 additional cancer therapeutic drugs as well as ionizing radiation. Interactions have been discovered in all of these screens and are now being validated.

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Johanna DiStefano, Ph.D. **TGEN**

Award Amount FY08: $50,000

Diabetic Kidney Disease in American Indians

Diabetic kidney disease is caused by genetic and environmental factors, and previous microsatellite genotyping has shown that linkage for diabetic nephropathy in Pima Indians is centered on an interval of approximately 40 cM on chromosome 3q. We genotyped 17 additonal markers on 3q and reevaluated the evidence for linkage. We estimated LOD scores of 1.94 using the mean IBD method and 2.63 using the scoring method. To further narrow the region of linkage, we genotyped 359 SNPS between markers *D3S1763* and *D3S3041* in Pima families with diabetic nephropathy and individuals with end-stage renal disease (ESRD) caused by type 2 diabetes. However, results from the additional saturation genotyping on 3q did not significantly narrow the region of linkage, nor did we observe an increase in the evidence for linkage in this area. Analyses defined two peaks, the highest peak having a LOD score of 1.5 and the corresponding SNPs under this peak lie within the inactive N-acetylated-alpha-linked acidic dipeptidase-like protein 2 (*NAALADL2*) gene. In conjunction with this approach, we also genotyped nine markers in the candidate gene, *SUCNR1*, that were prviously shown to be significantly associated with type 2 diabetic ESRD in Pima Indians, in a study sample comprised of Caucasians with ESRD attributed to type 1 diabetes. None of these markers showed evidence for association at levels that reached statistical significance in this study sample suggesting that different variants underlie susceptibility to ESRD in diabetes in these independent study samples. Future studies include further investigation of genes under the linkage peak, including *NAALADL2*, as well as other genes that may have a biological effect on the progression of diabetic kidney disease. Preliminary analyses of the associated *SUCNR1* SNPs in the Pima population indicated that these mutations may influence the binding efficiencies of two transcription factors. We plan to investigate these SNPs further to see they impact the function of this gene.

David Duggan, Ph.D. **TGEN**

Award Amount FY08: $50,000

Genetic Basis of Auricular-Condylar Syndrome in Two AZ Families

Auriculo-condylar syndrome (ACS) is a disorder characterized by congenital ear, jaw, temporomandibular joint and other abnormalities. By studying two Arizona families, our goal is to determine the genetic basis of ACS. In years 1 and 2, a disease-causing region in the human genome had been localized to chromosome 20 and a candidate disease-causing gene re-sequenced. Four novel DNA sequence variants were identified. Unfortunately, further testing of the identified and putative disease-causing changes revealed that they were not the causative changes. In this final year (year 3), we have begun to make use of the latest in state-of-the-art genetic technologies that allows a more thorough examination of the candidate disease-causing gene(s). Using next generation re-sequencing technologies and cost-efficient approaches developed by the TGen scientists, we intend to re-sequence the entire candidate disease-causing gene found on chromosome 20. For cost reasons associated with earlier re-sequencing technologies, only the protein coding regions of the DNA were re-sequenced in year 2 and only in a subset of the family members. This year, the entire gene including protein-coding, protein non-coding, and DNA sequences up- and down-stream of this gene will be sequenced. Such an approach has a high likelihood of identifying the disease-causing genetic change(s) in these two Arizona families.

Katerina Dvorak, Ph.D. **University of Arizona**
Award Amount FY08: $153,922

Barrett's Esophagus Esophageal Adenocarcinoma and Apoptosis

Barrett's esophagus (BE) is an esophageal lesion that arises as a consequence of chronic heartburn. BE is often associated with esophageal adenocarcinoma. More than one hundred patients will die of this cancer in Arizona in 2008 based on death rates in prior years. The mechanism of development of BE is poorly understood. Gastric acids, secreted from the stomach, and bile acids, secreted in response to a high fat diet, are implicated in the development of BE. We found that bile acids and gastric acid induce oxidative damage, abnormal cellular signaling, and mutations, which may lead to cancer. However these processes can be reversed by the replacement of bile acids with a special, protective bile acid, glycoursodeoxycholic acid, that is commonly used to treat liver diseases. An understanding of the development and progression of BE to esophageal carcinoma is crucial for the design of targeted strategies to prevent and eradicate this cancer.

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Mohamed A. Gaballa, Ph.D. **Sun Health Research Institute**
Award Amount FY08: $50,000

Human Umbilical Progenitor Cell Based Therapy for Myocardial Infarction

Intramycocaridal and intravenous delivery of human umbilical cord blood cells (hUCBCs) improves vascularization (the number 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 µl of media containing one million stem cells or media only. Animals were studied three 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 cell delivery. In conclusion, intracoronary delivery of hUCBCs improved heart function after an acute heart attack. The improvement was due to the generation of *de novo* blood vessels. No significant number of newly-formed cardiac myocytes (cardiac muscle cells) was observed. This study provides a strong support for the use of umbilical cord blood as a treatment option for patients with heart attack.

Arthur F. Gmitro, Ph.D **University of Arizona**

Award Amount FY08: $50,000

Ultra-Miniature Endoscopes for Biomedical Imaging

The objectives of this research was to build miniature endoscopes with simultaneous white-light and fluorescence imaging capability and to demonstrate unique applications for such an instrument. We have built 0.5mm diameter catheters exploiting the illumination concepts of numerical aperture sharing and crossed polarizers. Such techniques provide excellent rejection of unwanted signal from the input end of the imaging catheter and enable illuminations and collection down the same fiber optic imaging bundle. Some assembly and testing remains to be done on the multi-modal aspects of the system (white-light and fluorescence). Submillimeter catheters were built with conventional separate illumination and imaging channels for imaging mouse models of Barrett's esophagus. Such endoscopes can image *in vivo* mouse esophagus with little trauma to the animal as well as detect color and large textural variations. Further testing of the ultra-miniature multi-modal endoscope will be conducted with this animal model.

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Leslie Gunatilaka, Ph.D. **University of Arizona**
Award Amount FY08: $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. During the course of the third year of this project, mutasynthesis was used to produce analogs of beauvericin. It was demonstrated that a KIVR knockout *B. bassiana* strain can be used for the efficient mutasynthesis of unnatural beauvericin congeners. Simultaneous feeding of precursor analogs enabled the combinatorial mutasynthesis of scrambled beauvericins, some assembled entirely from unnatural precursors. The effects of the introduced structural changes on the antiproliferative and cell migration inhibitory activities of these analogs were also evaluated. To expedite production of additional analogs of beauvericin, the gene encoding the BbBEAS nonribosomal peptide synthetase was isolated from *B. bassiana* and confirmed to be responsible for beauvericin biosynthesis by targeted disruption. Heterologous expression of the bbBeas gene in *E. coli* to produce the BbBEAS enzyme provided a strain proficient in beauvericin biosynthesis which could be exploited for the production of further analogs of beauvericin. We are hopeful that non-cytotoxic compounds with cell migration inhibitory activity will provide lead compounds that can be developed into natural product-based drugs that can be used to treat metastatic solid tumors.

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Laurence H. Hurley, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

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

The overall goal of this proposal is to study the structure and function of GGA repeats in the *c-myb* promoter and identify small molecules that selectively bind to the *c-myb* promoter and prevent transcription.

During the time period covered by this report, we have made progress on specific aims 1 and 2. These specific aims are 1) to identify the *cis* elements within the GGA repeat region required for *c-myb* promoter activity, and 2) to identify transcription factors that bind to the GGA repeat region.

In addressing specific aim 1, we have employed a differentiation system of leukemia cells to investigate the role of the *c-myb* G-quadruplexes in regulation of the endogenous (originating from within the organism) *c-myb* promoter, and our data verify that the *c-myb* G-quadruplexes are involved in downregulation of *c-myb* expression in the cells. In addressing specific aim 2, we have investigated the role of endogenous MAZ in *c-myb* transcription and demonstrated that the levels of MAZ are inversely related with those of *c-myb* transcription, implicating that the role of endogenous MAZ is downregulation of *c-myb* transcription.

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Laurence H. Hurley, Ph.D. **University of Arizona**
Award Amount FY 2008: $166,269

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 a 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 1–3. These specific aims are 1) to define the structure of the biologically relevant i-motif in the promoter region of c-MYC and its drug complexes by NMR, 2) to define the structure of the biologically relevant i-motif in the promoter region of c-MYC and its drug complexes by calorimetry, and 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.

In the last 12 months we have transferred our efforts to the Bcl-2 promoter but the specific aims have remained the same. The reason for this change is two-fold. First, the i-motif-interactive compounds identified for Bcl-2 are more potent and specific for Bcl-2 than c-MYC. Second, our G-quadruplex drug discovery effort for c-MYC has progressed to a point where prioritizing c-MYC as a drug target fell lower than Bcl-2. Thus, what we had learned from the c-MYC i-motif project was quickly applied to the Bcl-2 with the results outlined in the report. We are now close to having a viable proposal for a drug discovery program using the i-motif as a target for Bcl-2. We could not have done this without the two years prior experience with the i-motif in c-MYC.

Edwin A. Lewis, Ph.D. **Northern Arizona University**
Award Amount FY 2008: $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. Approximately one third of all cancers involve Bcl-2 over expression. Studies of anti-Bcl-2 agents have demonstrated improved antitumor activity.

The objective of this research was to characterize non-helical structures formed in the regulatory region of the Bcl-2 gene. We have characterized the structure and stability of Bcl-2 promoter quadruplexes using DSC, CD, AUC, and Computational methods. We have shown that the Bcl-2 promoter sequence quadruplexes and their small drug interactions are similar to those determined previously for c-MYC. We have shown that the WT Bcl-2 sequence forms fewer folded conformers than might be predicted from its sequence. We found a similar result for K-ras, whose expression is a hallmark for pancreatic cancer.

The hope is that drug recognition or binding to non-helical promoter sequence structures could be used to turn off a number of cancer causing genes like Bcl-2, c-MYC, and K-ras.

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John Lewis, M.D., MPH

Inter Tribal Council
Award Amount FY: $150,000

Promoting Tribal Community Participation in Biomedical Research

This project is designed to promote tribal participation in health research. There are 22 tribes in Arizona, and most have not actively participated in research. The tribes are suffering from poor health generally, and health research holds the possibility of improving health in these communities. The project is being conducted in three phases:

1. Overview of current tribal research review processes–identifying what processes tribes are currently doing to determine whether or not they will participate in research.
2. Tribal research agenda setting–providing assistance to tribes to set their own research agendas and priorities.
3. Development of a Regional Tribal Institutional Review Board (IRB)–the IRB focuses on human subjects protections as well as community protections in the research process.

Phase 1 is complete, and we are in the process of conducting phases 2 and 3 with 18 tribes that have agreed to participate. We will finish the project by June 2009.

Rena Li, Ph.D.

Sun Health Research Institute
Award Amount FY08: $50,000

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

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Postmenopausal women have a higher risk of developing AD than age-matched men, which might be related to the loss of estrogen after menopause. We recently discovered that β-secretase (BACE1) enzymatic activity is significantly increased in AD brains and CSF. While it is not known whether estrogen plays a role in BACE1 activity, we studied the effect of estrogen and its receptors on regulation of the BACE1 in animal model and culture cells. Our data demonstrated that BACE1, the key enzyme for generation of beta amyloid protein, was elevated in AD animals lacking brain estrogen. The elevation of BACE1 in the AD mice is associated with earlier and more severe AD neuropathology observed in the brain. To further study the effect of estrogen on BACE1 gene regulation, we transfected BACE1 promoter into a stable cell line and examined the effect of 17α- and 17β-estradiol on the transcriptional activity of BACE1. Our data showed a down regulation of BACE1 by estrogen *in vitro*. Our ultimate goal is to identify the key mechanisms of estrogen action in AS pathogenesis and provide scientific evidence for developing novel alternative estrogen therapies to treat and even prevent AD.

Lonnie P. Lybarger, Ph.D. **University of Arizona**

Award Amount FY08: $50,000

CD8 Cell Printing Using Engineered 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 eliminated 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 mouse models that mimic human cancers. The preliminary data we have generated during the course of these studies strongly suggest that our molecules are potent vaccine agents.

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Istvan Molnar, Ph.D. **University of Arizona**

Award Amount FY08: $49,623

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 was 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. During the 3-year project, we have investigated 1,079 bacterial strains, conducted solid-state fermentations in 4 culture media with 716 strains, and assayed the resulting 3,268 extracts both in a mechanism-based and a general cytotoxicity assay. Prioritization of positive extracts involved molecular taxonomy (523 strains total);counter-screening against normal human cells (208 extracts total); scale-up of the fermentation process in liquid cultures (31 extracts total); and determination of apparent yield and potency. Ten large scale extracts, produced by10 strains representing 6 bacterial species, were finally selected for further study as future extension of the project. These extracts could contain novel cytotoxic metabolites that might be used for anticancer chemotherapy, improving the health prognosis of cancer patients.

Jitendran Muthuswamy, Ph.D. **Arizona State University**
Award Amount FY08: $150,000

Microactuated Microelectrodes to Assess Cortical Roles in Memory Deficits

During FY08, progress was made in the following four critical areas: 1) we demonstrated that novel doped polysilicon interfaces can suppress noise in electrical recording from single neurons; 2) we assessed the tissue response to microelectrodes that move in the brain after implantation; 3) we designed, developed and tested a novel MEMS microflex interconnect (MMFI) approach for packaging our microchip; and 4) we demonstrated long-term recoding from cortical neurons in the brain.

The doped polysilicon interfaces significantly suppress noise in recording from single neurons in the brain. In addition, the novel MMFI approach to packaging our implantable microdevice now enables dramatic size and weight reduction and improvements in the reliability of the interconnect. Both of the above strategies are novel and will find wide applications in other small-signal sensing contexts as well as packaging of other implantable microdevices. Finally, using our novel microactuated microelectrode arrays, we have successfully demonstrated long-term recording from single neurons in the somatosensory cortex for periods lasting up to three weeks.

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Joseph Rogers, Ph.D. **Sun Health Research Institute**
Award Amount FY08: $225,000

Translational Research on αβ Metabolism from Synthesis to Clearance

This multi-institutional research program seeks to discover new diagnostic and treatment methods for Alzheimer's disease based on synthesis and clearance of amyloid β peptide (Aβ), a molecule that forms millions of toxic deposits in the Alzheimer's brain. Projects 1 and 3 are collaboratively developing methods to lower Aβ production. Project 1 has also elucidated a critical pathway for Aβ synthesis that new drugs can target. Project 2 has shown that Alzheimer's patients have a defect in clearing Aβ from the body, and that this defect, 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. 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.

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Adrienne C. Scheck, Ph.D. **St. Joseph's Hospital**
Award Amount FY08: $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 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. We have performed gene expression analysis of a large group of meningiomas to identify genetic markers of aggressive tumors. Alterations in the expression of a number of genes involved in the Wnt signaling pathway suggests that this pathway may be involved in meningioma progression and/or tumor recurrence. In particular, we have found that the expression of genes encoding secreted frizzled related proteins 1, 2 and 4 is highest in grade 1 tumors and goes down in higher grade and recurrent tumors. Our data suggest that Wnt pathway may be a therapeutic target for the treatment of aggressive meningiomas.

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Jiaqi Shi, M.D, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

Deregulation of Translation Initiation by eIF3f in Melanoma

This project investigated the hypothesis that eIF3f is a negative regulator of translation and decreased eIF3f expression contributes to melanoma tumorigenesis by deregulating translation and apoptosis. In previous reports, we have demonstrated allelic loss of eIF3f gene in melanoma using LOH and gene copy number analysis. Our eIF3f gene mutation analysis suggested that mutation may not be the mechanisms of decreased eIF3f expression in melanoma. Here we further confirmed the decreased eIF3f protein expression in melanoma using immunohistochemistry analysis. To characterize the mechanism of eIF3f-mediated regulation of translation and apoptosis, we further investigated the association between eIF3f and hnRNP K. We have established that hnRNP K to 28S rRNA, which stabilized the rRNA and increased translation. These results support our hypothesis that eIF3f regulates rRNA degradation, translation and apoptosis through interaction with hnRNP K.

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Daekyu Sun, Ph.D. **University of Arizona**

Award Amount FY08: $48,984

Targeting Tumor Angiogenesis Using I-motif Interactive Ligands

As a direct consequence of this work, we have made the important observation that the C-rich strand of the double-stranded polyguanine/polycytosine (pG/pC) sequence of the VEGF promoter can spontaneously convert to i-motif structures in a cell-free system even under physiological pH conditions. Interestingly, we found that hnRNP K, which binds to the C-rich element in the single-stranded DNA form, also interacts with C-rich strand of the (pG/pC) sequence of the VEGF promoters to promote the transcription of the VEGF gene both *in vitro* and *in vivo*. However, hnRNP K binding to the C-rich strand could inhibit conversion of the single-stranded DNA to i-motif structures. On the basis of our accumulated data, we propose that an entirely new approach to anticancer drug design and development could be evolved through the drug targeting of these secondary DNA structures.

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Guo K, Gokhale V, Hurley L. Intramolecularly Folded G-quadruplex and i-motif Structures in the Proximal Promoter of the Vascular Endothelial Growth Factor Gene. **Nucleic Acids Res**. 36(14): 4598-4608. August 2008.

Tom Tsang, Ph.D. **University of Arizona**

Award Amount FY08: $50,000

Cancer Immunotherapy by TCR-Modified HSC Transfer

More than 10,000 people die of cancer each year in Arizona. Improved treatments are urgently needed. Our project aims to combine gene therapy, immunotherapy, and non-embroyonic stem cell therapy into a novel anti-cancer strategy. New genes that can recognize and destroy cancer cells will be created and introduced into non-embryonic stem cells to create a new cancer-fighting, designer immune system. We have successfully constructed a new cancer fighting gene construct and inserted it into a replication-defective lentiviral (HIV) virus. We are now evaluating the transfer of this construct into non-embryonic stem cells and its expression in mature immune cells. We will then test the ability of animals with the anti-cancer, designer immune systems to reject cancer cells.

Jie Wu, M.D., Ph.D. **St. Joseph's Hospital**
Award Amount FY08: $48,885

Nicotine Acetylcholine Receptors in the VTA and Nicotine Dependence

Nicotine 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. Under ABRC support, we have clarified three functional subtypes (ID, IID, and IIID) of nAChRs, which are combined with different subunits. We further found that three nAChR subtypes activation and desensitization are different to smoking relevant-concentrations of nicotine, suggesting they play different roles in nicotine reward and dependence. More interestingly, chronic exposure to nicotine (mimic smoking process) selectively up-regulated ID but down-regulated IID nAChRs, further suggesting that ID type of nAChR likely plays critical roles in nicotine reward and dependence. The finding of "the switch of nAChR subunits" during chronic nicotine exposure provides not only the new insights into an improved understanding of nicotine reward and dependence, but also opens a new window to develop novel drugs for smoking cessation in Arizona.

Section B

Continuing Contracts

Medical Research

Year Two

FY 2008

Craig A. Aspinwall, Ph.D. **University of Arizona**
Award Amount FY08: $48,323

Stabalized Polymer Phospholoid Imaging Probes

For project year 2 we have further developed the proposed nanometer-sized, biomimetic chemical labels, further expanding the utility, range of applications and performance of these novel imaging agents. Our primary efforts have focused on developing second generation fluorescent cores that are brighter, less environmentally sensitive and contain a broader range of dye molecules, enabling higher degrees of multiplexing. Additionally, we have investigated a second generation coating process which allows the utilization of commerically available, more chemically benign phospholipids. Physical and chemical characterization of the probes has been performed and functional characterization in model systems was begun. When fully developed, these probes will provide a novel class of imaging agents for disease diagnosis, prognosis and early detection of disease states.

Kobus Barnard, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

Genetics Diagnostics of Angiogenesis

The overall goal of this project is to identify genes that, when on or off are 1) indicators of small blood vessel (microvessel) health, and 2) potential targets for new therapies. Work in the previous funding period showed that microvessels can exist in three distinct health conditions. Recent work has focused on characterizing and exploiting the highly dynamic gene expression profiles associated with these conditions. For example, by examining gene expression profiles of implanted tissue, we have found that fully 60% of the genes were upregulated in the second week, relative to the first, pointing to great potential for determining reliable assay to diagnose microvessel health. To understand the reliability of such assays, we have also pursued a new approach for evaluating microarray data analysis methods that is based on phenotypic classification performance as this directly links to what is needed to characterize diagnostic tools.

Yongchang Chang, M.D., Ph.D. **St. Joseph's Hospital**
Award Amount FY08: $49,973

Mechanisms of ρ1 $GABA_C$ Receptor Activation and Antagonism

With the support of ABRC last year, we have studied additional regions around the GABA binding pockets and accumulated more data for future publication. With enough preliminary data, we have submitted an NIH R01 grant proposal. We also performed additional experiments for the previous two manuscripts to address peer-reviewers' comments and have recently published one paper entitled "Structural determinants for antagonist pharmacology that distinguish the ρ1 $GABA_C$ receptor from $GABA_A$ receptors" in *Moleuclar Pharmacology*. We have submitted another manuscript entitled "Agonist- and antagonist-induced conformational changes of loop F and their contributions to the ρ1 GABA receptor function" to *Journal of Physiology*. These studies helped us to better understand the structural basis for GABA receptor function. They will help us in the future to design new GABA receptor subtype specific compounds to treat neurological and psychiatric disorders such as sleep disorders, seizures, depression, and schizophrenia, which affect many Arizonans.

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J. Richard Coast, Ph.D.

Northern Arizona University
Award Amount FY08 : $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 muscles and whether those activities could be used to train the respiratory muscles. Initially, we performed studies that showed non-respiratory exercises (sit-ups) caused fatigue of the respiratory muscles, and that other easier, non-respiratory exercises such as toe-touches activated the respiratory muscles. In the second year, we used those exercises to train the respiratory muscles. The first study used sit-up training over 12 weeks to show that both inspiratory and expiratory muscle strength increased. Along with these increases, we saw increases in diaphragm thickness as measured by ultrasound, which indicated an increase in muscle mass in response to the training. This study also showed that respiratory muscle endurance was increased. The second study used some of the easier core exercises (e.g. toe-touches, trunk twists) to determine whether that type of exercise would also increase respiratory muscle strength. While the results were not as clear cut as those of the sit-up training study, they did result in increases in both inspiratory and expiratory muscle strength. This work, particularly the second training study, argue that in people suffering from respiratory muscle weakness (e.g. pulmonary patients), non-respiratory training may result in improvements in respiratory muscles strength and/or endurance. If proven to work, this may provide a useful adjunct therapy to pulmonary rehabilitation. We are currently trying to find clinical collaborators for future studies in pulmonary patients.

Paul Coleman, Ph.D.

Sun Health Research Institute
Award Amount FY08: $50,000

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

We now know that Alzheimer's 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 Alzheimer's disease starts decades before it is diagnosed using current methods argues for a better method of diagnosis. We have evidence that a blood test may provide this better method. Our first goal is to determine whether we can use a blood test to distinguish people already diagnosed with Alzheimer's disease. To that end we have collected blood samples from almost 200 people, half with Alzheimer's disease, the other half without Alzheimer's disease. Our collection of sample material is almost complete at which time we will analyze our data to determine how well our blood test works.

John K. DiBaise, M.D. **Mayo Clinic Scottsdale**
Award Amount FY08: $136,322

Tranmucosal 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 and diabetes control. The 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 study's second year, we have successfully a) augmented our *in vitro* release data showing erythromycin release from Carbopol across cellular monolayers, b) displayed the pharmacokinetics and tissue sequestration of the drug in our animal models, and c) compared this performance to human subjects. Remaining work will refine our formulation and document its effect on gastric emptying.

Kathleen Dixon, Ph.D. **University of Arizona**
Award Amount FY08: $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 NAU College of Engineering and Natural Sciences. 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.

Shona T. Dougherty, M.B. Ch.B., Ph.D. **University of Arizona**

Award Amount FY08: $49,535

Molecular Therapy of Bladder Cancer

Over 1000 cases of bladder cancer are diagnosed each year in the state of Arizona. The objective of this study is to explore the therapeutic potential of a novel approach to the treatment of this disease that exploits the differential production by the tumor cell population of a particular soluble molecule known as VEGF. Specifically, through the use of various genetic engineering techniques we have generated an artificial receptor that can bind VEGF and, upon doing so, induce cells to die. This receptor has been introduced and expressed in bladder cells using a specially modified non-infectious virus as a vehicle and its functional activity confirmed. Ongoing studies are concerned with further exploring the therapeutic potential of this exciting approach. It is essential that such work be carried out in order to ensure that the proposed treatment is both safe and effective prior to the initiation of clinical studies.

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Dougherty ST. Exploiting the Tumor Microenvironment in the Development of Targeted Cancer Gene Therapy. **The 11th International Tumor Microenvironment Workshop**. Miami, FL. 2008.

Lars Ewell, PhD **University of Arizona**
Award Amount FY08: $50,000

Diffusion Weighted MRI and Magnetic Resonance Spectroscopy to Differentiate Radiation Necrosis and Recurrent Disease in Gliomas

We have continued to analyze the substantial amount of image data we have acquired from the imaging protocol that was initiated in conjunction with the study this grant is supporting. The funds have allowed us to hire additional researchers, Tim McDaniels (PhD) and Amarjeet Bhullar (PhD). They have taken over from Naren Vijayakumar, who graduated this (fall) semester with his master's degree in electrical/computer engineering. His thesis title was "Multimodality Image Registration Using Gradient Information and Clustering." In his thesis, he discussed how best to align (register) two different sets of MRI images. This alignment is important when comparing MRI scans taken at different times, such as when attempting to assess disease progression. The analysis of imaging data has progressed, as indicated below.

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Bhullar A, Ewell L, McDaniels T, Stea B. Metabolic Concentrations and Ratios of Brain Tissue. **Frontiers in Medical Research**. Tucson, AZ. 2008

Robert J. Gillies, Ph.D. **University of Arizona**

Award Amount FY08: $250,000

Multimeric Ligands for Targeting Cancer for Imaging and Therapy

Our goal for treatment of pancreatic cancer is to develop multimeric therapeutic ligands that can target cancer cells from normal cells in humans. A ligand is a molecule that contains more than one ligand binding motif attached to a backbone linker, a nanoparticle. Cell surface receptors are targets since they express highly selective binding for ligands and are accessible from outside the cell for ease of interaction. These particles can contain copies of the same ligand or copies of different ligands allowing cross-linking of two or more different receptors for higher specificity.

This project is important as it is focused on pancreatic adenocarcinoma, a devastating disease with high mortality. The technology was invented at the University of Arizona and our research group remains a leader in the discovery and design of multi ligands complexes. Two emerging strengths in Arizona are both therapeutics and imaging for which these ligands are used.

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John G. Hildebrand, Ph.D. **University of Arizona**
Award Amount FY08: $49,999

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

We continued our studies on the species distribution of kissing bugs in the vicinity of Tucson. The most abundant species continue to be *Triatoma rubida*. We launched an extensive collection campaign with public participation that has yielded about 800 bugs since March 2008, all of which will be individually analyzed for the presence of the parasite *Trypanosoma cruzi*, the causative agent of Chagas' Disease. This parasite is transmitted through the insects' feces. We are finalizing a comprehensive study that demonstrates that only adult-female *T. Rubida* could be efficient transmitters of the parasite. We have successfully accomplished one of the main goals in the grant, which is to increase public awareness for the risks attributable to these insects. During the last year of the project we will devote our efforts to testing a protoytpic trap in the field that could divert kissing bugs from entering human houses and thus contribute to reducing the incidence of human contacts with them in southern Arizona and the allergic reactions that often result from their bites.

Richard D. Lane, M.D., Ph.D. **University of Arizona**

Award Amount FY08: $150,000

Neural Basis of Vagal Tone Dysregulation 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 reduction in the prevalence of depression in Arizona.

Nine depressed patients and five controls have given consent thus far (four subjects with MDD and two controls have completed the protocol, and one depressed subject is currently active). Thirty-two fMRI imaging sessions have been completed, and HRV and clinical data has 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 FY08: $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. For the first year of the study, patients from the mammography center received their results as per usual care and patients from the surgical clinic received their results via video conference by a teleoncologist and also completed a satisfaction survey. The second year of the study, recruitment continued only at the mammography center. During this second year, patients had the option to receive results per usual care or via video- or teleconference by a teleoncologist. 100% of patients opted to receive results via teleconference by a teleoncologist where they also completed a satisfaction survey.

Year 1: There were 153 mammography center and 38 surgical clinic participants. On average, tissue processing and telepatholgy interpretation was completed in < 5 hour (19% (37/191) presented difficult diagnoses and took longer (+24 hours) to diagnose). The 38 participants from the clinic reported overall satisfaction with EBC again, indicated they would seek EBC again, and expressed relief at receiving prompt results.

Year 2: There were 49 mammography center participants. Tissue processing and telepathology interpretation times are currently being evaluated. The participants from the clinic 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 FY08: $50,000

Blood Flow Measurements 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 cases, 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.

Lawrence J. Manderino, Ph.D. **Arizona State University**
Award Amount FY08: $150,000

Metabolic Syndrome and Inflammation

Insulin resistance is reduced response to 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 lkB, a protein involved in inflammation. Our studies show that obese individuals have lower lkB protein than lean individuals, indicating muscle inflammation. After completing these preliminary studies, we currently are completing the *in vivo* portion of the clinical research (fat infusions).

Jesse D. Martinez, Ph.D. **University of Arizona**

Award Amount FY08: $49,999

Nutritional Modulation of Colorectal Cancer Risks

Bile acids have been implicated as gastrointestinal factors that can modify risk for developing colon cancer. Here we examine the molecular mechanism by which bile acids act at the molecular level in order to gain insight into how they function in modifying colon cancer risk. We found that the tumor promoting bile acid, deoxycholic acid (DCS), can modify the composition of the cell's membrane and that this results in simulation of growth signaling pathways. In contrast we found that a bile acid which was shown to suppress tumor development also suppresses growth signaling pathways by also acting at the cell's membrane. To date our results confirm that bile acids act at the membrane, but that the effect that they have on the membrane determines their effect on tumor development. This has important implications for colon cancer chemoprevention because it suggests that manipulating the composition of the plasma membrane may be a useful strategy for the prevention of colon cancer.

Raymond B. Nagle, M.D., Ph.D. **University of Arizona**

Award Amount FY08: $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. We have previously demonstrated that LM-332 loss in prostate cancer is not due to alterations in translational regulation, nor transcriptional regulation. This finding has led us to investigate other possible mechanisms of LM-332 loss, such as targeted protein degradation. We have shown evidence for this mechanism of loss in this current report.

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Alyssa Panitch, Ph.D. **St. Joseph's Hospital**
Award Amount FY08: $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 regeneration 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 regeneration 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 and improves functional recovery. *In vitro*, coupling the gels with regeneration signals improves neurite extension and is anticipated to further increase functional recovery after spinal cord injury in our *in vivo* model.

Mark C. Preul, M.D. **St. Joseph's Hospital**
Award Amount FY08: $150,000

A Novel Peptide Mimetic for the Immunotherapy of Brain Tumors

Direct activation of phagocytic cells therefore the final effectors of the immune response against tumors, is a promising approach to cancer treatment. Ednogenous human serum macrophage activating 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 previous findings reported in 2007 continue. In addition, Cytokine assays are confirming the effect of the L4MAF radiation sensitization for tumor destruction. These promising results are anticipated to lead to further animal studies and future clinical trials.

Naomi E. Rance, M.D., Ph.D. **University of Arizona**
Award Amount FY08: $49,504

Effects of Estrogen Withdrawal on Hypothalamic Thermoregulation

Our goal is to provide basic information on how estrogen affects the regulation of body temperature and thus provide insights into the cause of menopausal flushes. In the past year, we examined the effects of estrogen and changes in environmental temperature on the activity of neurons in the hypothalamus, the area of the brain that controls body temperature. We found that the activity of cells in the median preoptic nucleus (MnPO) of the hypothalamus was increased in rats exposed to warm temperatures, providing evidence that this area controls heat loss from the body. Furthermore, the activity of MnPO neurons was reduced by estrogen replacement. These data implicate MnPO neurons as a site of integration between the reproductive and the thermoregulation control centers in the brain. Thus, this site could be involved in the generation of menopausal flushes.

Seth Rose, Ph.D. **Arizona State University**
Award Amount FY08: $50,000

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

Cancer cells grow and spread because they have overcome normal cellular processes that cause abnormal cells to self-destruct. Drugs that restore the normal behavior suffer from cancer cells becoming resistant to them by expelling the drugs from the cell. To combat this, we devised, made, and tested thirteen new compounds, as well as five previously prepared ones, against cancer cells. The compounds were designed to chemically bind to their target in a novel way so they would interfere with cancer cell growth without being subject to the effects of expulsion of the drug. We treated an enzyme with some of these compounds and found proof of concept of the strategy. We examined how some of these compounds may chemically achieve their cell-killing effects and used computer methods to evaluate a possible additional, cellular target enzyme. These studies further the development of anticancer drugs for the benefit of Arizona cancer patients.

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Samuel Schluter, Ph.D. **University of Arizona**
Award Amount FY08: $150,000

Modulation of Autoimmune Disease by Natural Autoantibodies and Immunopeptides

Our focus is on natural autoantibodies that bind to the T-cell Receptor (TCR) present on T-cells, the central players in the immune system cells. We hypothesize that such antibodies may act to suppress pathogenic autoimmune reactions. We derived a natural anti-TCR auto monoclonal antibody (mAAb) using B cells from an arthritis (RA) patient. This mAAb can induce the secretion of low levels of IL-10 *in vitro*. However, in the presence of specific antigen, the mAAb induces very large amounts of IL-10. This is very significant since IL-10 is one of the main cytokines used by the immune system to inhibit and suppress immune responses. In particular, T-regulatory cells, a chief cell involved in suppressing autoimmune reactions, secrete IL-10. The results are extremely encouraging supporting our proposal that anti-TCR mAAbs could, in the future, be used to treat autoimmune diseases such as RA by inducing specific T-regulatory cells.

Adelman MK, Schluter SF, Robey IF, Marchalonis JJ. Natural and Autoantibodies to Human T-cell Receptor Vbeta Segments: Potential Roles in Immunomodulation. **Crit Rev Immunol**. 27(30): 221-32. 2007.

Ranganathan P, Adelman MK, Schluter SF. Attempted Immunocorrection by Autoantibodies in Inflammatory Autoimmune Disease. **Immunotherapy 2008: Implementing Combinations**. Havana, Cuba. 2008.

Philip M. Service, Ph.D. **Northern Arizona University**
Award Amount FY08: $47,535

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

During this year, we made progress in two areas. First, we successfully completed screening genetic markers that we need in order to map genes that influence life span in our fly populations. In all, we have screened more than 370 candidate markers and have obtained 34 - 38 informative markers for each of our three mapping crosses. During the past year we genotyped almost 1,700 individual flies for each marker. This data will be analyzed in the coming year. Second, we substantially completed a large-scale simulation study. These simulations are necessary to determine the best strategy for analysis of our actual data. As a result of these simulations, we have developed a strategy that will improve our power to detect life span genes while at the same time reducing the likelihood that we will "identify" non-existent genes. This strategy for analysis of genetic data and gene discovery should be broadly applicable.

Edward B. Skibo, Ph.D. **Arizona State University**
Award Amount FY08: $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 (type II) 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 active analogues based on QSAR studies carried out last year
- Work on isolation of recombinant human Type II IMP dehydrogenase
- Investigation of new purine mimics.

Hargreaves RHJ, David CL, Whitesell LJ, LaBarbera DV, Jamil A, Chapuis JC, Skibo EB. Discovery of Quinolinediones Exhibiting a Heat Shock Response and Angiogenesis Inhibition. **Journal of Medical Chemistry**. 51(8): 2492-501. 2008.

Hoany H, Huang X, Skibo EB. Synthesis and *in vitro* Evaluation of Imidazole-based Wakayin Analogues. **Organic and Biomolecular Chemistry**. 6(17): 3059-64. 2008.

Lucy J. Treiman, Ph.D. **St. Joseph's Hospital**
Award Amount FY08: $50,000

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

Febrile seizures in neurologically normal young children appear to be an important risk factor for developing epilepsy later in development. In an animal model of febrile seizures, infant rat pups exposed to hyperthermia-induced seizures have been reported to have a lowered threshold to experimental seizures as adults. The mechanisms of this increased neuronal excitability are not understood. Differences in neuronal gene expression in specific areas of the brain after hyperthermia-induced seizures will be compared with control rats 48 hours after seizures and in adult rats. The hypothesis of this project is that acute and/or long-term changes in gene expression after hyperthermia-induced seizures compared with control rats may help explain the increased neuronal excitability and lowered seizure threshold seen in adult rats exposed to hyperthermia-induced seizures as pups. Identification of differences in gene expression may lead to the development of neuroprotective interventions for children with febrile seizures to prevent the subsequent development of chronic epilepsy.

Tsu-Shuen Tsao, Ph.D. **University of Arizona**

Award Amount FY08: $49,999

Biological Function and Chemistry of Adiponectin Oligomerization

The overall objective of this project is to understand the role of impaired adiponectin oligomerization in the pathogenesis of Type 2 diabetes mellitus, a major and rapidly increasing health threat in Arizona. The data generated during the course of completing the goals set forth in Aim 1 have allowed us to successfully apply for a research grant from the American Diabetes Association. This grant will bring to Arizona a total of $414,000 over a period of three years from a nationally recognized foundation. This represents a 176% return on ABRC's investment in the form of this contract. We continue to make significant progress in further characterizing the oligomerization mechanisms of adiponectin as part of Aim 2. We have also confirmed our initial observation that adiponectin oligomers can interconvert outside of circulating blood as part of Aim 3. These results will allow us to compete for additional funding from national sources.

Thompson ACS, Davidson R, Nunez M, Tsao TS. Enhanced Adiponectin Production in Larger 3T3-L1 Adipocytes Suggests Altered Regulation of Adipocyte Size in Obesity. **Keystone Symposia in Pathogenesis of Diabetes and Obesity**. Keystone, CO. 2008.

Choi JE, Gorman G, Gilbert D, Tsao TS, McDonagh P. Adiponectin Treatment Attenuates Myocardial Infarction in Zucker Diabetic Fatty Rats and Is Associated with Reduced Neutrophil Accumulation. **American Diabetes Association 68th Scientific Sessions**. San Francisco, CA. 2008.

Briggs DB, Mashalidis EH, Tsao TS. Distinct Assembly Pathways for Adiponectin Oligomers Suggests Improper Intra-Trimer Disulfide Formation as Cause of Decreased HMW Adiponectin in Insulin Resistance. **American Diabetes Association 68th Scientific Sessions**. San Francisco, CA. 2008.

Marlys H. Witte, M.D. **University of Arizona**
Award Amount FY08: $49,676

Massage Therapy in Childhood Lymphedema

Swollen or unequal limbs of children are most commonly due to birth defects of lymphatic drainage ("congenital lymphedema [LE]") or complications of cancer treatment ("acquired LE"), which slows down removal of excess tissue fluid (lymph). These children often suffer severe physical and psychological disabilities. At least several hundred Arizona children, occasionally with other family members, suffer from childhood LE (CLE). We are testing simplified CLE management, namely a specialized massage therapy (manual lymph drainage-MLD) without compressive bandaging in growing children to determine responsiveness to a short and maintenance MLD regimen, compliance, and long-term outcomes. During the second year, we trained personnel, individualized protocols, screened and enrolled subjects ranging from toddler to adolescents with congenital and acquired LE of arms, legs and face. Promising preliminary results showed substantial volume reduction in the swollen limbs during the short intensive MLD monotherapy, which is being followed during home maintenance. To accrue more subjects, we are networking with Arizona and national pediatric and cancer centers, physicians and therapist, CLE families, and through Telemedicine links with international CLE centers of excellence for multi-institutional collaboration.

George T. Wondrak, Ph.D. **University of Arizona**
Award Amount FY08: $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 particularly aggressive tumor that originates from pigment producing cells in human skin. In this ABRC sponsored research period, my laboratory has examined a novel molecular mechanism that regulates melanoma cell survival and progression. This molecular mechanism involves reactive intermediates from cellular metabolism and their protein targets. Based on our understanding of this mechanism, novel prototype drugs that effectively kill melanoma cells without harming normal cells have been identified. A compound similar to reactive intermediates from metabolism, cinnamic aldehyde derived from cinnamon, has been identified as a promising anti-melanoma agent. Further research will define the role of this compound and close chemical derivatives as potential drugs for chemoprevention and treatment of melanoma skin cancer.

Wondrak GT, Cabello CM, Villeneuve NF, Zhang S, Ley S, Sun Z, Zhang DD. Cinnamoyl-based Nrf2-activators Targeting Human Skin Cell Photo-oxidative Stress. **Free Radic Biol Med**. 45(4): 385-95. 2008.

Wondrak GT. Rectivity-based Drug Discovery Using Vitamin B(6)-derived Pharmacophores. **Mini Rev Med Chem.** 8(5): 519-28. 2008.

Cabello CM, Bair WB3rd, Wondrak GT. Experimental Therapeutics: Targeting the Redox Achilles Heel of Cancer. **Curr Opin Investig Ddrugs**. 8(12): 1022-37. 2007.

Bause SA, Lamore SD, Wondrak GT. More Than Skin Deep: The Human Skin Tissue Equivalent as an Advanced Drug Discovery Tool. **Frontiers Drug Disc Dev**. 2009. In press.

Cabello CM, Wondrak GT. Targeting the NQO1-redox Achilles Heel of Cancer Using Small Molecule Prooxidant Redox Cyclers. **Proceedings of the 99th Annual Meeting of the American Association for Cancer Research**. San Diego, CA. 2007.

Yitshak Zohar, Ph.D. **University of Arizona**

Award Amount FY08: $ 50,000

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

The goal of this project is to use bio-technology to create microdevices for capturing specific populations of cells from complex suspensions, such as blood, for further analysis. So far we assembled and tested a system, incorporating fabricated microfluidic devices with bio-functional surfaces, designed to capture cancer cells from a complex mixture. After developing the assay for functionalizing surfaces with antibodies, we test the specific interaction between functionalized surfaces and target cells. The results show that an anti-N-cadherin coated is highly specific in binding N-cadherin expressing prostate cancer cells while suppressing non-specific binding of other cell types. Furthermore, although possible, the capture of a moving cell on a functionalized surface is difficult. However, under no-flow condition, about 5 minutes of incubation time is sufficient to capture almost all the target cells present in the microchannel. We also obtained preliminary results regarding cell deformation and detachment due to applied flow field.

Cheung LSL, Zheng XJ, Stopa A, Schroeder JA, Heimark RL, Baygents JC, Guzman R, Zohar Y. Attachment and Detachment of Prostate Cancer Cells in a Microfluidic System. **12th International Conference on Miniaturized Systems for Chemistry and Life Sciences**. San Diego, CA. 2008.

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Guidpaty T, Cheung LSL, Jiang L, Zohar Y. Cluster Formation in Partical-laden Microchannel Flow. **First Sensor, Signal, and Information Workshop**. Sedona, AZ. 2008.

Guidpaty T, Cheung LSL, Jiang L, Zohar Y. Cluster Formation and Growth in Flow of Dilute Particle Suspension in Microchannels. **9th Annual ASME Conference on Engineering Systems Design and Analysis**. [Keynote lecture]. Haifa, Israel. 2008.

Section C

Continuing Contracts

Medical Research

Year One

FY 2008

Emmanule Akporiaye, Ph.D. **University of Arizona**

Award Amount FY08: $50,000

Targeting TGF-β Signaling to Enhance Dendritic Cell Based Therapy of Melanoma

A major obstacle to the success of cancer immunotherapy is the diminished immune responsiveness of patients with progressing disease. This is in part due to the suppressive effect of tumor-derived factors such as transforming growth factor-β (TGF-β). Our goal was to block TGF-β-mediated signal transduction to improve the effectiveness of a specialized immune cell known as dendritic cell (DC) in treating established TGF-β-secreting malignant melanoma. We demonstrated in the test tube that the TGF-β signaling inhibitor SM16 was able to block TGF-β-mediated signaling in DC and melanoma tumor cells. We also successfully generated gene-modified mice with DC that are unresponsive to TGF-β and showed that such DC are better able to initiate an immune response. Furthermore, we tested the combination of DC plus SM16 to treat melanoma and demonstrated that although DC vaccination was effective in suppressing tumor growth the addition of SM16 did not improve the outcome.

Klaus Beyer, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

Membrane Interactions and Neurodegeneration in Parkinson's Disease

This project is focused on an investigation of biomembrane associated structural transitions in the Parkinson related protein α-synuclein. It has been shown previously that α-synuclein undergoes a coil-helix transition upon interaction with raft forming membranes. Membranes that are prone to forming domains are prevalent in the brain and are believed to be involved in vesicular nerve transmission, a key process that seems to be impaired in Parkinson's disease. This causal relationship prompted us to initiate a thorough investigation of structural properties and of the mixing dynamics of raft forming model membranes using solid state Nuclear Magnetic Resonance (NMR) techniques. The particular role of cholesterol on the non-ideal mixing behavior and on structural parameters such as hydrocarbon thickness and thermal expansion coefficients in sphingomyelin containing membranes could thus be demonstrated for the first time.

In this study we used a number of peptides selected from the primary sequence so as to represent the structural elements of the membrane bound protein as determined earlier by NMR spectroscopy. Our results provide strong evidence that the N-terminal protein segment, comprising residues 1-2, is crucial for proper folding of the full length protein.

Bartels T, Lankalapalli RS, Bittman R, Beyer K, Brown MF. Raft Like Mixtures of Sphingomyelin and Cholesterol Investigated by Solid-State 2H NMR Spectroscopy. **Journal of American Chemical Society**. 130: 14521-32. 2008.

Bartels T, Zhang H, Beyer K, Saavedra SS, Brown MF. Plasmon Waveguide Resonance Shows Preferential Binding Oligomeric α-Synuclein to Raft-Like Lipid Mixtures. **Biophysical Society Annual Meeting. Boston**, MA. 2009.

Bartels T, Kamp F, Nuscher B, Odoy S, Haass C, Beyer K. Lipid Interaction of α-Synuclein: The N-Terminus Triggers Vesicle Binding and Helix Folding. **The Journal of Biological Chemistry**. In press.

Bartels T, Lankalapalli RS, Bittman R, Brown MF, Beyer K. Solid-State 2H NMR Spectroscopy Reveals Micromechanics of Raft-Like Ternary Lipid Membranes Containing Sphingomyelin and Cholesterol. **Biophysical Society Annual Meeting**. Boston, MA. 2009.

Christopher Buneo, Ph.D. **Arizona State University**
Award Amount FY08: $50,000

Contribution of Parietal Areas to State Estimation

This research is aimed at understanding how the brain combines sensory information from various sources and uses it to guide our limb movements. Such basic sensorimotor functions can be altered by 'strokes' and traumatic brain injuries, as well as by the normal aging process and developmental abnormalities. We have been studying these functions by monitoring the activity of cells in a brain region known to be involved in sensorimotor control, the posterior parietal cortex (PPC). We have found that in one part of the PPC cells appear to signal the position of the arm solely by using information obtained from the muscles, i.e. they do not appear to use visual information when it is available, suggesting visual and muscular information are integrated in another area. It is hoped that a more complete understanding of this important function will benefit the citizens of Arizona through improved rehabilitation protocols and assistive technologies.

Apker GA, Darling T, Bueno CA. Sensorimotor Integration During Online Movement Corrections. **Society for Neuroscience Annual Meeting**. Washington, DC. 2008.

Shi Y, Bueno CA. Patterns of Directinal Errors Resulting from Endpoint Estimates of Varying Precision. **Society for Neuroscience Annual Meeting**. Washington, DC. 2008.

Setsuko K. Chambers, M.D. **University of Arizona**
Award Amount FY08: $50,000

Regulation of c-fms Proto Oncogene Related Breast Cancer Risk

We have shown that the HuR protein, by binding to the tail end sequences of the RNA for the c-fms oncogene, elevates the levels of this oncogene, leading to enhanced invasiveness of breast cancer cells. This year, we studied whether a novel binding protein we have identified, vigilin, can compete for binding of the HuR protein to the same c-fms RNA sequences. If this competition exists, then this could be a mechanism which could be exploited to decrease c-fms oncogene levels and turn off invasiveness and spread of breast cancer cells. We have shown that vigilin is expressed in breast cancer cells, that it binds to c-fms RNA both in the laboratory and in breast cancer cells, and that it binds to the same regions of the tail end of c-fms RNA as does HuR. Most importantly, we have successfully shown that vigilin competes for HuR binding to c-fms RNA.

Harinder Garewal, Ph.D. **University of Arizona**
Award Amount FY08: $150,000

Hypothesis-Driven Biomarkers of Colon Cancer Risk

Arizona has 6.5 million residents. Lifetime risk of colorectal cancer is 5-6%, so 360,000 residents will develop a colorectal cancer. Current tests to indicate need for removal of pre-cancerous growths (polyps) or cancers in the colon are only 20-50% accurate. We have obtained 6 colon biopsies from each of 121 female and 116 male patients undergoing colonscopies at University Medical Center and evaluated most of these biopsies for the enzyme Cytochrome c Oxidase I (CcOI). If one or more patient biopsies has 20% or more areas in the biopsy deficient for CcOI, this corresponds fairly closely with the expected risk for the patient, determined from polyps found, of colon cancer. Also 17 of 21 colon cancers had deficiencies for CcOI, indicating the cancers may have arisen from a CcOI defective tissue area. These results may allow us to devise a reliable test, performed in a physicians office, that predicts need for removal of pre-cancerous growths.

Dvorak K, Bernstein H, Payne C, Bernstein C, Garewal H. Bile Acid Induction of Apoptosis in Relation to Gastrointestinal Cancer. [Eds Jenkins G, Hardie L.] **Bile Acids: Toxicity and Bioactivity**. Chapter 3. Royal Society of Chemistry. Cambridge, UK. 2008.

Bernstein C, Bernstein H, Payne C, Dvorak K, Garewal H. Field Defects in Progression to Gastrointestinal Tract Cancers. **Cancer Letters**. 260:1-10. 2008.

Bernstein H, Bernstein C, Payne C, Garewal H, Dvorak K. Adverse Effects of the Steroidal Bile Acids in the Gastrointestinal Tract. [Ed Columbus F.] **Adverse Effects of Steroids**. Nova Science Publishers, Inc. 2008.

Bernstein H, Payne C, Bernstein C, Garewal H, Dvorak K. Cancer and aging as Consequences of un-Repaired DNA Damage. **Cancer Research Journal**. 2:2-3. 2008.

Leslie Gunatilaka, Ph.D. **University of Arizona**

Award Among FY08: $150,000

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

Among all cancers, pancreatic cancer has the worst survival rate and is the fourth leading cause of cancer deaths in the United States. Unfortunately, there are no good chemotherapies available, and there is thus an urgent need for the discovery and development of new and effective anticancer drugs to treat pancreatic cancer. Our preliminary studies with the natural product withaferin A (WA), isolated from the desert medicinal plant *Withania somnifera* (winter cherry), has led to the discovery of the protein Annexin II as the target. With the aim of exploiting the unique potential of Annexin II as a novel target for pancreatic cancer, we have assembled a multidisciplinary team for *in vitro* and *in vivo* evaluation of naturally-occurring and semi-synthetic analogs of WA.

In our studies to produce WA by soil-less aeroponic cultivation of *Withania somnifera*, three new and eight known analogs of WA were isolate and characterized. WA produced by this technique was used for the semi-synthesis of seventeen of its analogs by chemical conversions and microbial bio-transformations. All twenty-eight natural and semi-synthetic analogs together with WA were subjected to anticancer bioassays involving inhibition of cancer cell proliferation/survival, heat shock induction, disruption of cytoskeleton in normal human diploid cells, and inhibition of angiogenesis in HUVEC-2 cells. Based on the results obtained in these assays, ten WA analogs were selected for evaluation in progressively aggressive pancreatic cancer cell lines, BxPC-2, Miapaca-2, and Panc-1. Outcome of these assays will be used to select promising compounds for gene expression analysis and *in vivo* evaluation against pancreatic cancer.

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

Melissa D. Halpern, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

Enterohepatic Circulation of Bile Acids in Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is a life-threatening gastrointestinal disease of premature infants 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 nationwide has steadily increased and the number of babies with NEC has also increased. Statewide, it is responsible for up to 5% of all neonatal intensive care 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. 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 intestinal tissue cultured with inflammatory components of the NEC intestine, including bile acids. In this year of study, we have determined that newborn intestine reacts differently to bile acids than do intestines for older animals. The newborn intestine appears to be less able to protect itself from potential damage from bile acids, and these data suggest a mechanism by which premature infants may be at risk for development of this devastating disease.

Ronald P. Hammer, Jr., Ph.D. **University of Arizona**
Award Amount FY08: $49,995

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

The broad, long-term objective of the project is to determine the brain mechanisms underlying symptoms of schizophrenia using an experimental animal model to better define cellular and molecular alterations. The specific aims are 1) to utilize molecular approaches to determine the nerve cells and circuits affected by drugs that produce schizophrenia symptoms, 2) to compare the pattern of functional activity produced by these drugs to that produced by auditory stimulation, and 3) to determine the precise connections of the affected nerve cells. The results will allow us to better understand the final common pathway involved in producing symptoms of schizophrenia, possibly resulting in better therapeutic interventions targeting this pathway to help treat or cure patients with this devastating disorder.

Paul S. Keim, Ph.D. **TGEN**

Award Amount FY08: $150,000

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

The goal of this project is to develop novel genotyping techniques for *Coccidioides*, the cause of Valley fever. We have made significant progress in accomplishing the stated objective. We completed the bioinformatics analysis of all available *Coccidioides* genome sequences, identifying 1500 targets that will be used in the genotyping assays. We also established a comprehensive repository of over 450 individuals strains of *Coccidioides*. Additionally, we developed two separate genotyping strategies, an initial strategy to target multiple (~50) foci and a more comprehensive analysis system using all 1500 identified targets. We began development of the probes for the first system and plan to have 200 isolates typed by the first quarter of year 2. We also began design of the second system which we expect to complete in the first quarter and move to genotyping in the second quarter. We will conduct final analysis of data in the final quarters.

Pawel R. Kiela, Ph.D. **University of Arizona**

Award Amount FY08: $50,000

Modulation of Neutroophil Function by Curcumin in Inflammatory Bowel Disease

Neutrophilis play a key role in the immune response by eliminating pathogens. However, Inflammatory Bowel Disease (IBF), a disproportionate and persistent inflammatory process mediated by transepithelial neutrophil migration and also a reduction of epithelial barrier function, causes perpetuation of the inflammatory processes and tissue destruction via oxidative damage and the release of proteases. Our central hypothesis is that curcumin affects the recruitment and function of neutrophils at multiple levels to reduce the extent of mucosal damage in colitis. To date, we have determined that a) curcumin affects production of epithelial cells and macrophage-derived key chemokines to reduce recruitment of neutrophils to the site of inflammation, b) curcumin inhibits migration of neutrophils in a chemoattractant gradient, c) curcumin inhibits neutrophil chemotaxis and chemokinesis by interfering with signaling cascade leading to actin polymerization and formation of the leading edge, d) this is partially due to inhibition of chemokine-induced AKT (protein kinase B) activation in neutrophils.

Susana Martinez-Conde, Ph.D. **St. Joseph's Hospital**
Award Amount FY 2008:$ 50,000

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

Our eyes move continually even while we fixate our gaze on an object. If fixational eye movements are counteracted, our visual perception of stationary objects fades completely due to neural adaptation. When we explore a visual scene, we spend approximately 80% of the time fixating our gaze on the various points of interest of the image. Therefore much, possibly most, of our visual experience occurs during fixation, and fixational eye movements drive much of our perception. Thus pathological fixational eye movements are expected to lead to vision loss, yet this has not been previously addressed in any clinical therapy regime. We have begun to establish the contribution of fixational microsaccades in normal and pathological vision. Our central hypothesis that microsaccades are critical to normal visual perception during fixation, and that deficiencies in microsaccades contribute to pathological vision in progressive supranuclear palsy (PSP). Our initial data shows that PSP patients make a pathological type of eye movement, called a square-wave jerk, with higher prevalence than normal healthy subjects. We have also developed a new (and first) objective algorithm that objectively characterizes square-wave jerks from eye position data collected with an ocular monitor.

Chen Y, Martinez-Conde S, Macknik SL, Bereshpolova Y, Swadlow HA, Alonso J-M. Detection Difficulty Modulates Neuronal Activity in Primary Visual Cortex. **Nature Neuroscience**. 11(8): 974-982. 2008.

Macknik SL, King M, Randi J, Robbins A, Teller R, Thompson J, Martinez-Conde S. Attention and Awareness in Stage Magic: Turning Tricks Into Research. **Nature Reviews Neuroscience**. July 2008. Also featured in **The New York Times**, **The Boston Globe** and National Public Radio.

Troncoso XG, Macknik SL, Martinez-Conde S. Microsaccades Counteract Perceptual Filling-in. **Journal of Vision**. 8(14): 1-9. 2008.

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Martinez-Conde S, Macknik SL. Fixational Eye Movements Across Vertebrates: Comparative Dynamics, Physiology, and Perception. **Journal of Vision**. 8(14): 1-16. 2008.

Macknik SL, Martinez-Conde S. Consciousness: Neurophysiology and Visual Awareness In. [Ed. Squire L.] **New Encyclopedia of Neuroscience**. 3: 105-16. Oxford Press. 2009.

Otero-Millan J, Leigh RJ, Serra A, Troncoso XG, Macknik SL, Martinez-Conde S. Objective

Characterization of Square-wave Jerks Allows to Differentiate Between Progressive Supranuclear Palsy Patients and Healthy Volunteers Populations. **Society for Neuroscience**. 38th Annual Meeting. Washington, DC. 2008.

Otero-Millan J, Leigh RJ, Serra A, Troncoso XG, Mackink SL, Martinez-Conde S. Objective Characterization of Square-wave Jerks Differentiates Progressive Supranuclear Palsy Patients from Healthy Volunteers. **Vision Sciences Society**. Naples, FL. 2009.

Otero-Millan J, Leigt RJ, Serra A, Troncoso XG, Mackink SL, Martinez-Conde S. Objective Characterization of Square-wave Jerks in Progressive Supranuclear Palsy Patients and Healthy Volunteers. **Vision Sciences Society**. Naples, FL. 2008.

Otero-Millan J, Leigh RJ, Serra A, Troncoso XG, Mackink SL, Martinez-Conde S. Objective Characterization of Square-wave Jerks in Progressive Supranuclear Palsy Patients and Healthy Volunteers. **Society for Neuroscience**. 37th Annual Meeting. San Diego, CA. 2007.

George R. Pettit, Ph.D. **Arizona State University**
Award Amount FY08: $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 to 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 proven 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 are extending this very successful research focus to making improvements in the treatment of human prostate cancer.

Richard D. Posner, Ph.D. **Northern Arizona University**
Award Amount FY08: $133,111

Modeling Vulnerability of Cancer

Signal-transduction systems comprise the decision-making apparatus of cells. Many serious health problems, including cancer, can develop when signal transduction pathways go awry. In the past decade there has been a major effort to identify the chemical species that populate specific signaling pathways and, for each of these species, to determine how it is regulated, with what molecules it interacts, and its role in the signaling process. This has led to the development of a new generation of "targeted" pharmaceuticals. As the number of identified signaling molecules has grown and new regulatory mechanisms have been discovered, networks to describe these systems have grown exponentially in complexity. It is no longer possible to use intuition alone to guide targeted drug discovery. This project develops and experimentally verifies computational models of signaling networks to guide and streamline target identification and selection, as well as affords testing of multi-agent treatment regimens for cancer.

Donato F. Romagnolo, Ph.D. **University of Arizona**
Award Amount FY08: $49,445

Epigenetics of Breast Cancer and Chromatin Remodeling

The long-range goal of this project is to identify the factors that increase the risk of sporadic breast cancer through epigenetic regulation, and develop dietary strategies that prevent this event. The central hypothesis of this project is that the activation of the aryl hydrocarbon receptor (AhR) by dietary and environmental ligands leads to epigenetic modulation of tumor suppressor and promoter genes, whereas these effects can be prevented by dietary compounds. In year 1 of this project, we investigated the protective effects of dietary selective AhR modulators against changes in transcription induced by AhR-ligands. To begin answering this question, we have investigated the molecular interactions of the AhR and a number of transcription factors at the BRCA-1 and cyclooxygenase-2 (COX-2) promoters and found that the regulation of BRCA-1 by estrogen required the participation of members of the transcription factor Sp, in detail Sp1 and Sp4. This observation is important to the overall scope of the project because the AhR physically binds to the estrogen receptor (ER) and Sp factors. This physical interaction represents a mechanism for transcriptional repression of BRCA-1 by the AhR. Conversely, the recruitment of the AhR to the COX-2 promoter led to activation of transcription.

We also have investigated the time-course recruitment of the AhR to xenobiotic response elements (XRE), which are binding targets for the activated AhR. Results of DNA binding and chromatin immunoprecipitation (ChIP) studies documented that TCDD-induced the association of AhR to XRE found in promoter oligonucleotides from the CYP1A1 promoter selected as a control model. In addition, we observed that the TCDD-induced binding of the AhR was reduced by small-interfering RNA (siRNA) for th AhR. The recruitment of AhR was also reduced by cotreatment with the compound 3-methoxy-4 naphthoflavone. These data sugest that the recruitment of the AhR to target XRE is time dependent and can be prevented by intervention with siRNA for the AhR or antagonists for the AhR. Previous studies have shown that, unlike BRCA-1, the COX-2 gene is induced by the AhR ligand TCDD. Results of binding studies indicated that the treatment with TCDD induced the binding of the AhR, but this effect was reduced by cotreatment with the dietary factor 3, 3'-Diindolylmethane (DIM), a condensation product of indol-3-carbinol (I3C) found in Brassica (cruciferous) vegetables. Results of these studies have been accepted for publication.

Taken together, these data suggest that naturally occurring modulators of th AhR such as SIM may be effective agents in dietary strategies targeted to modulate the recruitment of AhR to target sequences in the BRCA-1 and COX-2 promoters. Current experiments are investigating the chromatin remodeling events that accompany these changes.

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Hockings JK, Degner SC, Morgan SS, Kemp MQ, Romagnolo DF. Involvement of a Specificity Proteins-binding Element in Regulation of Basal and Estrogen-induced Transcription Activity of the BRCA-1 Gene. **Breast Cancer Research**. 10(2):R29. 2008.

Degner SC, Papoutsis AJ, Selmin O, Romagnolo DF. Dietary Targeting of Aryl Hydrocarbon Receptor-Mediated COX-2 Gene Expression in Breast Cancer Cells. **The Annual AICR Research Conference on Food, Nutrition, Physical Activity, and Cancer**. Washington, DC. 2008.

Degner SC, Papoutsis AJ, Selmin O, Romagnolo DF. Targeting of Aryl Hydrocarbon Receptor-Mediated Activation of COX-2 Expression by the Indole-3-Carbinol Metabolite 3,3'-Diindolylmethane in Breast Cancer Cells. **Journal of Nutrition**. In press.

Romagnolo DF. Modifying Cancer Risk with AhR Based Phytochemicals: Epidemiological and Mechanistic Evidence. Presentation Department of Nutritional Science University of Arizona. Tucson, AZ. 2008.

Joyce A. Schroeder, Ph.D. **University of Arizona**

Award Amount FY08: $49,999

Mechanism of CD44-HA Mediated Inhibition Breast Cancer Metastasis

We have evaluated the effects of altering the main ligand for CD44, HA, in the ECM. Using HA either embedded in the ECM (eHA), or presented in the media (sHA), we have determined that HA differentially alters proliferation depending on its presentation in the cellular microenvironment. In the presence of sHA, proliferation is inhibited. Conversely, when interacting with eHA, cells decreased spreading onto collagen gels while increasing proliferation. When HA is absent from the cellular microenvironment, cells adhere and proliferate at rates in between those seen with soluble or matrix associated HA. The differential effects seen in the presence of sHA and eHA are mediated by ERK and PAK, as demonstrated through the use of their inhibitors in proliferation assays, an analysis of protein expression and activation. Overall, these results demonstrate that the manner in which HA is presented to the cells alters adhesion, migration signaling, and proliferation. These data will be used to design future experiments for peptide development and treatment to determine if a CD44-directed therapy can be developed.

Ornella Selmin, Ph.D. **University of Arizona**
Award Amount FY08: $49,954

Folate as a Nutrient Competitor Against Environmental Exposure to Trichloroethylene

During the first year of the study we have bred four groups of rats using two types of folic acid supplemented diet (either 2 or 8mg/kg), in the presence or absence (control groups) of 10ppb TCE in the maternal drinking water. Embryos from each group were harvested at day 11, and RNA was isolated for gene expression analysis. Preliminary analysis of the data indicates that exposure to TCE in the presence of maternal high folate diet (8mg/kg) may reduce the number of reabsorbed embryos when compared to the control non-exposed group animals. Future molecular analysis will reveal whether or not this effect may lead to an increased number of neonates carrying heart defects. These results are significant in light of the low dose of TCE used in this study and the fact that large groups of Arizonians may be at risk from exposure to similar doses of TCE.

D. Larry Sparks, Ph.D. **Sun Health Research Institute**
Award Amount FY08: $150,000

CSF Copper and Cognitive Performance

Alzheimer's disease (AD) affects nearly 5 million Americans and 125,000 Arizonans, as a progressive dementing disorder gradually ending in the total loss of self. The financial and emotional burden on the caregiver (normally the spouse) of an AD patient can be staggering. Identifying a method of predicting which individuals might develop AD could reveal a method of treating and hopefully delaying progression or onset of the disorder. In an autopsy study we found that there is a gradual reduction of copper levels in the cerebro-spinal fluid (CSF) as an individual coursed from normal cognitive performance to dementia of AD. We have shown that we can quantify copper levels in human saliva, but have yet to show that they co-vary with CSF levels. We are testing the hypothesis that reduced copper levels in CSF/saliva are predictive of future cognitive impairment by yearly assessment in this three-year longitudinal investigation.

Sparks DL, Ziolkowski C, Connor D, Beach T, Adler C, Sabbagh M. Copper and Cognition in Alzheimer's Disease and Parkinson's Disease. **Cell Biology and Toxicology**. 24:426-30. 2008.

Brent L. Vernon, Ph.D. **Arizona State University**
Award Amount FY08: $150,000

Improved Materials for Endovascular Embolization

For treatment of aneurysms and arteriovenous malformations (AVM), an efficient material is needed to block blood flow to the site. Due to its temperature-sensitive properties and wide use in the biomedical field, NIPAAm is suitable for such applications. The structural, thermal and mechanical properties of the NIPAAm-based copolymers were analyzed using various techniques and demonstrated promising characteristics for embolization of AVMs and aneurysms. Due to the simultaneous chemical crosslinking which occurs through the Michael-type addition reaction and the physical gelling undertaken at higher temperatures as the polymer undergoes phase transition, the polymer has shown its ability to endure stress and strain experienced in the endovascular system. Cytotoxicity studies have also demonstrated its non-toxic effect on cells. Once tested *in vivo*, these materials can then be used to treat patients suffering from endovascular malfunctions such as aneurysms and AVMs.

Danzhou Yang, Ph.D. **University of Arizona**
Award Amount FY08: $50,000

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

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*. The objective of this project is to characterize the novel AP-1 inhibition of XR5944. We have shown previously that the *in vitro* DNA binding of the c-June AP-1 protein is inhibited by XR5944. Now, our electrophoretic mobility shift assay (EMSA) results demonstrated that XR5944 remarkably inhibits the DNA binding of AP-1 proteins from nuclear extracts. Using mouse keratinicytes, we have shown that XR5944 is capable of inhibiting UVB induced transcriptional activity of AP-1 in cells, whereas the level of AP-1 proteins is not changed. AP-1 is the key family for transducing multiple signals for cell proliferation, thus targeting AP-1 by a small molecule drug can be an effective approach to inhibit cancer cell growth. Our research will be useful to identify a potential anticancer drug target.

Daniela C. Zarnescu, Ph.D. **University of Arizona**
Award Amount FY08: $49,999

Mechanisms for Local Translational Control in *Drosphila* Neural and Germ Stem Cells Gene

Fragile X syndrome is the most common form of inherited mental retardation and affects one in 4,000 males and one in 8,000 females in the state of Arizona. The disease is due to deficits in Fragile X protein (FMRp) function. Here we hypothesize that FMRP functions in stem cells by controlling specific target mRNAs. To test this, we investigated whether FMRP loss affects the development of germ and neural stem cells. We are using *Drosophila* as a model system due to its powerful genetic and molecular tools. During the first year of the project we made significant progress toward our goals. We discovered that germ and neural stem cells lacking FMRp overproliferate and produce more cells. Our data suggest that FMRP may control proliferation via a ubiquitin ligase enzyme, Cbl. These results identify new pathways affected by FMRP and have the potential to be used for new therapeutic approaches in the future.

Zarnescu D. Fragile X Protein Controls the Proliferation and Differentiation of Neural Stem Cells. **Annual *Drosophila* Research Conference**. San Diego, CA. 2008.

Section D

New Contract Awards

Beginning in FY 2009

Mohamad Azhar, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Role of TGF-beta Ligands in Aortic Aneurysm

An aneurysm is a localized, blood-filled dilation of a blood vessel caused by disease or weakening of the vessel wall. Aneurysms most commonly occur in the aorta (the main artery coming out of the heart). Aortic aneurysms can occur in the abdomen, chest and aortic root of the heart. The bulge in a blood vessel can burst and lead to death at any time. Aneurysms are a common finding in patients of Marfan syndrome (MFS, MFS2), familial thoracic aortic aneurysms and dissections (TADD) and Loeys-Dietz syndrome (LDS), and they are associated with tobacco smoking and with gene mutations in TGF-β receptors (i.e., TGFBR2 or TGFBR1). Based on the positive benefits of Losartan therapy (which lowers TGF-β activity) in Fibrillin-1 mutant mice (mice which model MFS), a phase III clinical trial is being currently conducted by the Pediatric Heart Network to determine whether blocking TGF-β activity by Losartan can prevent aneurysm. One of the ways Losartan blocks TGF-β activity is to reduce expression of TGF-β ligands. Therefore, it is important to delineate the function of individual TGF-β ligands in the pathogenesis of aneurysm.

The major goal of this proposal is to identify the specific function of TGF-β ligands in aortic aneurysm disorders. Extensive experimentation by others in Fibrillin-1 mutant mice has found that these mice exhibit enhanced TGF-β activity in the vessel wall due to the breakdown of wall architecture, and that this increased TGF-β activity causes aneurysm in these mice. It is not known which TGF-β ligands contribute to this increased TGF-β activity and aneurysm in these mice. Our preliminary studies suggest that the TGF-β2/ TGF-β3 doubly-deficient (DKO) embryos develop aortic aneurysm and die at around 14.5 day of gestation. The proposed hypothesis is that TGF-β2 and TGF-β3 are both required for protection against aneurysm, whereas excess TGF-β1 causes aortic aneurysm. Combinations of TGF-β deficiencies in the mouse and pharmacological approaches will be used to exacerbate or rescue aortic aneurysm by systematically altering dosage of TGF-β activity in order to determine the effects of the individual TGF-βs.

Objective 1) Determine the molecular, cellular and developmental aspects of aortic aneurysm in TGF-β2/ TGF-β3 DKO mice to assess the underlying mechanisms by which these ligands protect against aortic aneurysm.

Objective 2) Determine whether enhanced TGF-β activity mediates aneurysm in DKO mice by pharmacologic treatment of female pregnant mice carrying DKO embryos with inhibitors of TGF-β activity (i.e., Losartan, TGF-βR1 inhibitor and TGF-β neutralizing antibodies).

Objective 3) Generate TGF-β1/Fibrillin-1 DKO mice to determine whether a reduction in TGF-β1 rescues aortic aneurysm in Fibrillin-1 mutant mice.

These studies will determine the underlying molecular pathogenesis of aneurysm disorders and have implications in developing safe therapies for aneurysm disorders. These studies are highly significant to the biomedical issues facing Arizona because tobacco-caused cardiovascular diseases remain the leading cause of death of Arizonans. Also, aneurysms are more common in the elderly, and Arizona has a high percentage of population 65 and older.

Ross Bremner, M.D., Ph.D. **St. Joseph's Hospital**
Award Amount FY09: $50,000

Predictors of Therapy Response in Adenocarcinoma of the Lung

Non-small cell lung cancer remains the leading cause of cancer death in industrialized countries and yet lags behind in preclinical models and genomics investigations. In Arizona, there are 2,760 new cases of lung cancer each year of which 2,550 men and women will die of lung cancer. The last decade has seen a shift in the most common histologic sub-type of lung cancer from squamous cell to adenocarcinoma. For unknown reasons, the incidence of adenocarcinoma of the lung in non-smoking women is escalating. Unfortunately, lung adenocarcinoma is often a metastatic process at discovery, even if the primary cancer is small. Surgical cure rates for early lung cancer are only in the range of 60-70% and chemotherapy has historically been disappointing, with only about 25-30% of cancers showing any meaningful response. Recently new small molecule biologic therapies have demonstrated promising activity against cancer, suggesting a break through in cancer treatment by matching a targeting agent to a specific aberrant molecular pathway. As more targeting agents are developed and as tumor-specific targets are identified, systems to align these agents to proper application will greatly accelerate and refine personalized medicine.

Our overall hypothesis is that a molecular signature in patient tumors may direct optimal or effective therapy selection, thereby enabling personalized treatment planning. In accordance to Arizona's Bioscience Roadmap, this collaborative interface between St. Joseph's Hospital and the Translational Genomics Research Institute will advance the science and understanding of lung cancer and build a strong collaboration for translating laboratory discoveries into clinical care, treatment, and commercialization

of technology in the state of Arizona.

The long-term goal of this project is to develop more effective therapies against lung adenocarcinoma based on molecular features in patient specimens. The objective of this project, and an important step toward accomplishing our long-term goal, is to implement a collaborative clinical and laboratory network for lung tumor tissue collection, which will foster the development of xenograft models for biological and therapeutic testing coupled to proficiency in expression profiling for guidance of individualized therapy of lung adenocarcinoma. The strategy for this research is to develop, test, and implement genomic profiling of lung adenocarcinoma and develop biological models for personalized medicine.

To accomplish the objectives of this project, we will pursue the following specific aims:

1) Establish a pre-clinical xenograft model for lung adenocarcinoma and determine whether molecular profiling can identify predictors of tumor response or non-response to conventional chemotherapeutic agents. We will operate a successful collection, preservation, and serial propagation model of lung adenocarcinoma suitable for follow-on tumor biology, molecular genetic, and therapy testing experiments. We propose to establish 3-4 primary lung adenocarcinoma xenografts per year suitable for serial propagation as subcutaneous tumors in mice. We will next use whole genome expression profiling of xenografts to discern whether molecular profiling can predict / guide therapy selection.

2) Validate candidate genes discovered in Aim #1 and determine the cellular and biochemical mechanisms of action of these genes in relation to the responses to chemotherapeutic treatment. Clinical associations between therapy responses and gene candidates identified in Aim #1 will be evaluated by comparing the differential expression of these genes in the therapeutic responders and non-responders xenografts. Quantitative RT-PCR will be used to either eliminate candidate genes or to advance them to mechanistic experiments. Specific inhibition or activation strategies (antibodies, siRNA, or transfection of identified genes, respectively) will be used to determine the function of the genes in therapeutic responses. Additionally, we will develop a tissue microarray using archival human lung adenocarcinoma specimens to survey patterns of markers for tumor progression and therapeutic selections.

Michael R. Caplan, Ph.D. **Arizona State University**
Award Amount FY09: $50,000

Reverse Engineering the Basement Membrane

Many medical devices designed to address cardiovascular problems fail due to material-host interactions, in particular inflammation. Examples include artificial vascular grafts, stents, pacemaker leads, heart valves, and almost all other blood-contacting systems. These are examples of pathological cellular response to materials, but there is a material in our bodies that for the most part works well for decades. The material is the basement membrane which is a material made mostly of proteins to which vascular endothelial cells adhere in normal blood vessels. These endothelial cells receive cues from the basement membrane and their other surroundings that cause them to respond by not recruiting inflammatory cells or platelets except in pathological situations. What is it about this material that elicits desirable responses from endothelial cells whereas synthetic materials elicit undesirable responses? Can doctors and engineers use that knowledge to trick cells into responding to synthetic materials in a desirable way? To answer these questions, it is essential to understand how these cells sense their surroundings and convert those sensations into gene regulation.

Several aspects of this project are of great importance to the state of Arizona and Arizona residents. First, improved materials for medical devices, particularly cardiovascular devices, would directly help many Arizona residents due to the rising rate of cardiovascular disease. Secondly, as design principles for creating better materials are discovered, it is likely that graduates trained in this work will work for Arizona-based companies such as Bard Peripheral Vasculature and W.L. Gore, thus building Arizona's biomedical industry. Finally, this research will cement collaborations among ASU, University of Arizona, and the Translational Genomics Institute forged to develop preliminary data for this proposal. This collaboration has potential to provide Arizona with a center of expertise in systems biology applied to biomaterial design which does not currently exist anywhere else in the world. In these ways, the research can enhance the health, economy, and prestige of the state of Arizona and Arizona residents.

In this study we propose to systematically control the stiffness and protein composition of a material and then study the responses of human umbilical vein endothelial cells that are brought into contract with those materials. We will measure the activity of six or more molecules that transmit sensation from the cell's surface to its nucleus (each representative of a distinct signaling pathway) for cells in contact with materials of which we have varied stiffness and protein composition. To aid in interpretation of the data, we will use principal component analysis and partial least squares regression which are well-established techniques for making sense of highly complex, multivariate systems. Using these techniques we will correlate material properties with the signals that they activate. We hypothesize that unique material properties cause a unique pattern of cell signaling which in turn controls cellular production of molecules necessary for recruitment of inflammation. This hypothesis will be tested by completing three aims: 1) quantify signaling cascade activation when cells contact materials; 2) identify

cell behaviors that result from these activated signaling cascades; and 3) study the role of individual signaling pathways via inhibitors of signaling molecules.

Qin M. Chen, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Oxidant Induced c-Fos Phosphorylation

Tobacco smoking causes an increased risk for coronary heart diseases. Arizona is among the states that have the highest population of tobacco smokers. Smoking produces oxidants. In addition, oxidants are produced as byproducts of aerobic metabolism and during ischemia-reperfusion. Oxidants can damage macromolecules or cells inside our body. But recent studies with antioxidant administration have generated controversial results. It is not known at mechanistic levels how oxidants may cause heart diseases and whether antioxidants can prevent heart disease due to smoking. Cardiomyocytes are the major type of cells in the heart and are responsible for contractile function of the heart. Enlargement of cardiomyocytes is often observed in failing hearts. It is known that hypertension can cause cardiomyocytes to enlarge. This is usually resulting from the action of small peptide endocrine factors, such as angiotensin II and endothelin-1, on the heart cells. We find that low to mild concentrations of oxidants cause cardiomyocytes to enlarge. We are trying to find whether there is a central switch, such as c-Fos transcription factor, within cardiomyocytes that controls cell enlargement.

While studying the mechanism of oxidant effect on cardiomyocytes, we found that oxidant causes changes in a certain transcription factor. One component of AP-1 transcription factor, c-Fos, undergoes phosphorylation when cells encounter oxidants. We are trying to understand the upstream molecules regulating c-Fos phosphorylation and downstream consequence of c-Fos phosphorylation. By focusing one molecule, e.g. c-Fos, at a time, we can trace back to the question whether or not this molecule serves as a central switch that controls cardiomyocyte enlargement and whether this molecule can serve as a target for curing certain types of heart disease associated with oxidant overexposure, such as in the cause of tobacco smoking. We have come up with three specific aims to test the hypothesis that H_2O_2 induces c-Fos phosphorylation at specific amino acid residues and phosphorylation of c-Fos at these residues increases the stability of c-Fos protein and, therefore, the activity of AP-1 transcription factor.

Cheryl D. Conrad, Ph.D. **Arizona State University**
Award Amount FY09: $49,940

Stress and Estrogen Actions in the Female Hippocampus

Many psychiatric conditions such as Alzheimer's disease (AD), schizophrenia, and major depressive disorder (MDD) show changes in the brain that are most prominent in the hippocampus, a region important in learning and memory. Projections for the year 2020 indicate that MDD will be the leading cause of disability and disease burden throughout the world, and this should apply to Arizona as its population and retirement communities continue to grow.

MDD and chronic stress show changes in similar brain regions, circuitries and mediators, especially within the hippocampus, suggesting that MDD and chronic stress may share common mechanisms. Moreover, many symptoms in AD, schizophrenia and MDD are triggered or exacerbated by stress. Work from our lab and others show that chronic stress in males causes hippocampal dendritic retraction which is characterized by reduced dendritic complexity of hippocampal neurons. This chronic stress-induced hippocampal dendritic retraction is associated with impaired hippocampal function, such as poor memory, and increases the susceptibility of the hippocampus to metabolic challenges. These metabolic challenges may include ischemia/stroke (blood flow obstruction), hypoxia (lack of oxygen), and hypoglycemia/hyperglycemia (abnormal sugar levels). While all of these metabolic challenges can be damaging by themselves, these events become even more crippling under conditions that the hippocampus is already compromised from AD, schizophrenia MDD and chronic stress. Therefore, delineating the mechanisms by which the hippocampus can be protected under compromised conditions will be essential for protecting it against further damage.

The problem is that the majority of research on MDD and stress focus on male subjects, but females are more than twice as likely as men to develop MDD. In our research, chronic stress exacerbates hippocampal damage caused by a neurotoxic insult in males, but does not exacerbate hippocampal damage in females. Why females are protected from further damage following chronic stress has not been studied and is the purpose of these studies. However, we hypothesize that estrogen may be neuroprotective against chronic stress actions for two main reasons. 1) Pilot data show that estrogen protects against chronic stress-induced hippocampal dendritic retraction in females. 2) The presence of hippocampal dendritic retraction has been a marker for hippocampal susceptibility to a neurotoxic challenge. Therefore, conditions that prevent hippocampal dendritic retraction may also prevent the detrimental effects of chronic stress on the structure of the hippocampus.

The goal of these studies is to determine whether estrogen and the estrogen receptor protect females against chronic stress-induced exacerbation of hippocampal damage caused by a neurotoxin challenge using ibotenic acid (IBO).

We hypothesize that estradiol protects against chronic stress-induced hippocampal vulnerability to

neurotoxin exposure. Objective 1 is to examine whether estrogen mediates the neuroprotective effect within the hippocampus that is observed in chronically stressed females in response to an Ibotenic acid (IBO) challenge. Objective 2 is to determine the involvement of estradiol receptors in the neuroprotective effects against chronic stress-induced exacerbation of hippocampal damage following an IBO challenge in females.

Keith D. Coon, Ph.D. **St. Joseph's Hospital**
Award Amount FY09: $47,749

Perioperative Strategies to Improve Outcomes in Patients with NSCLC

Non-small cell lung cancer (NSCLC) is the most common cause of cancer death in the western world. Most treatments have not been very effective as demonstrated by the overall survival rate of 15%. There are 2,760 new cases of lung cancer in Arizona annually, of which 2,550 men and women will die. If detected early, surgical removal of the tumor can increase a patient's long-term survival rate to 60%-70%, but many will still recur with distant metastases. This rate of recurrence implies the presence of lingering cancer cells at the time of surgery, and the severity of the surgical procedure has been shown to impact the incidence and magnitude of recurrence. Historically, there has been no therapy given around the time of surgery for fear of interfering with a successful surgical outcome; however, it has been suggested that perioperative treatment with an anti-cancer agent might reduce the risk of recurrence. One promising group of anti-cancer agents that could potentially be used in this fashion is cyclooxygenase-2 (COX-2) inhibitors. The COX-2 inhibitor celecoxib has been shown to have anti-cancer effects and does not demonstrate significant side effects, especially when used for limited periods of time. The use of microarrays for studies of gene expression has increased the accuracy of disease characterization in many cancers including NSCLC. However, this valuable tool has not yet been applied to characterizing the effects of surgery on the potential to develop distant metastases or assessing the response to therapeutics given around the time of surgery.

We hypothesize that surgical resection of NSCLC tumors produces discernible changes in gene expression that create a local and/or systemic microenvironment that is conducive to both the recurrence of the

primary tumor and the ability of the primary tumor to metastasize to a distant locale, thus promoting further tumorigenesis. We further hypothesize that perioperative administration of celecoxib around the time of the introduction of surgical stress will demonstrate a significant inhibition of these tumorigenic effects. Our goal is to determine the effect that surgery has on gene expression, whether these effects are tumorigenic, and whether perioperative administration of celecoxib can counteract these changes.

It is our objective to make Phoenix, Arizona a nationally-known clinical and research hub for thoracic disease and we are uniquely positioned to accomplish this. St Joseph's hospital and the recently developed Heart and Lung Institute has the only dedicated thoracic surgical oncology division in Phoenix and has the only CyberKnife in Arizona. The thoracic surgeons routinely perform minimally invasive (VATS) lobectomies, which is usual for this city, and St Joseph's has one of the largest CyberKnife lung cancer programs in the country. Consequently, in combination with our existing genomics expertise, we have an exclusive opportunity to study the effects of these innovative procedures at the molecular level. Further, our background in the study of inflammation and the cyclooxygenase enzyme enables us to perform a translational study in our patient population that as the potential to provide unique insights into not only the tumorgenic effects of surgery, but the potential to overcome these effects with a non-toxic therapeutic agent, in this case celecoxib. We expect the results from this study to provide a fertile foundation to further study the "perioperative period" and to improve the outcomes for our patients undergoing surgery for cancer. Though celecoxib has been tested extensively as an anti-cancer therapeutic *in vitro* and in animals, this study represents the first attempt to utilize a perioperative treatment regimen of celecoxib in a randomized, prospective study of human subjects to directly minimize the reported effects of surgery-induced promoters of metastases and interrogate the changes induced by both surgery and celecoxib treatment at the molecular level via microarray-based gene expression analyses. This study will provide important information as to the molecular mechanism that underlies the high incidence of recurrence/metastasis observed in lung cancer patients. It also has the potential to move rapidly into clinical trials, resulting in a potential cancer treatment that could be implemented in the clinic in a relatively short amount of time. This treatment has the added benefit of being inexpensive with potentially little to no side effects. Though the hypotheses being tested in this investigation will need to be further validated in a larger cohort of patients, validation of this novel therapeutic avenue could have tremendous impact on not only the surgical treatment of lung tumors, but on surgical treatment for other cancers, and as such, represents an exciting potential advance in our approach to cancer therapy. In summary, utilizing well-established genomic strategies, this project will address novel questions relating to patient outcome following surgical removal of cancerous lesions via three standard procedures and has distinct potential to have significant impact on the health of cancer patients as well as surgical patients in general.

Torsten Falk, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Transplantation of Adult RPE Cells as a Treatment for Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting the senior population in Arizona (2% of the population over 65 years of age). At the present time, treatment of this movement disorder is inadequate. Developing new treatments for PD has been identified as a major focus area defined by the Arizona Bioscience Roadmap report commissioned by the Flinn Foundation. This project will test a novel cell-based therapy approach to the treatment of PD. PD occurs due to the loss of a small but important population of brain cells that produce the chemical messenger know as dopamine. These cells are critical for the normal control of movement. Patients with PD can be adequately treated with oral dopamine replacement therapy for about 5 years, but after this time the response to oral medications becomes inadequate and this has no effect on the ongoing process of cell loss and degeneration. This is in part due to the fact that oral medications cannot penetrate into the brain where they are needed and in part due to the ongoing loss of the normal cells that produce dopamine. In this proposal, we will explore the possibility of transplanting dopamine producing cells into the brain that will act as a direct source of dopamine. In addition, the cell type which we are studying, known as "rentinal pigment epithelium" or RPE cells, may have the ability to slow or prevent the ongoing degeneration of the normal dopamine-producing cells in the brain. The RPE cells are obtained from eyes donated to the eye bank from deceased donors. In the future, we could also obtain RPE cells by taking a sample from a patient suffering from PD and later transplant the patient's own cells into the brain. RPE cells are not stem cells and can be obtained from adult sources.

We hypothesize that RPE cells can produce both dopamine and a neuroprotective factor called PEDF that can prevent the loss of dopamine-producing neurons in Parkinson's disease. Early clinical trials have already shown that RPE cells can provide dopamine and help with the symptoms of PD. In this study our goal is to test whether the cells can also prevent the ongoing loss of dopamine producing cells. When successfully complete, this proposal will answer several important scientific question regarding RPE cells. These are:

1) What chemical factors do RPE cells produce that protect dopamine-producing cells from Parkinson's disease?
2) Do RPE cell lines derived from different donors produce different levels of these protective factors?
3) Can the protective factor known as PEDF (which is produced from RPE cells) protect dopamine cells from experimental Parkinson's disease? If so, what dose of PEDF is necessary?
4) Can RPE cells provide the necessary dose of PEDF to protect dopamine cells?

Once this information has been obtained, we will be in a position to further develop this project for use in a clinical trial.

Hanna F. Fares, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Deciphering Endocytosis in Multicellular Eukaryotes

Endocytosis is used for the uptake and processing of nutrients. Endocytosis is also used to regulate other essential cellular functions. At least 70 inherited human diseases result from defects in endocytosis. Deregulation of endocytosis also plays a causative role in cancer and can contribute to different aspects of aging and to the pathogenesis of some age-related disease. Finally, many bacterial and viral pathogens utilize the endocytic machinery to invade cells. Treatment strategies for various diseases have focused on endocytosis as a vehicle to deliver therapeutic substances, especially for the treatment of neurological defects where the substances have to cross the blood-brain barrier.

Traditionally, studies on endocytosis have been done either in mammalian tissue-culture cells or using genetic approaches in the unicellular yeast. We decided to use the worm *Caenorhabditis elegans* as a model system to study this process because *C. elegans* allows a level of sophistication in genetic analyses not available with vertebrates. Studies in *C. elegans* have been instrumental for identifying and analyzing the molecular pathways that regulate central conserved processes like aging, cell death, and neurobiology and behavior.

We have established a system to study endocytosis in *C. elegans* and have shown its effectiveness by identifying and characterizing novel proteins that regulate this process specifically in multicellular eukaryotes, worms and humans. This has allowed us to propose novel mechanisms that regulate endocytosis and to provide a cell biological explanation for the symptoms seen in various human diseases, including Myotubular Myopathy, Charcot-Marie Tooth syndrome, and the lysosomal storage disorder Mucolipidosis type IV. We have also used our system to identify potential leads for the treatment of Mucolipidosis type IV. Continuing studies on endocytosis will yield a more detailed understanding of its functions and will suggest novel treatments and applications through modulations of its pathways.

It is estimated that 2% of all cellular proteins regulate endocytosis in a given cell. This translates to 400-600 proteins in worms and humans. Yet only a small fraction of these proteins have been identified and are being studied. The long-term goal of this work is to fully understand how endocytosis is regulated in multicellular eukaryotes. This requires that we identify all of the proteins that are involved in this process. We will use the system we have established in *C. elegans* to identify additional proteins that are used to regulate endocytosis in multicellular eukaryotes. We will also use our assays to characterize the functions of the identified proteins, which will allow us to construct more elaborate models of this complex process. These studies will provide more avenues for the treatment of human diseases and infections through modulations of the activities of newly identified regulators of endocytosis. We will also use our system to understand the cell biological basis for endocytosis-related diseases in humans and to identify possible therapies.

Sourav Ghosh, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Cell Polarity Regulation During Differentiation of Embryonic Stem Cells

More than 600 disorders afflict the nervous system. Well known are disorders such as stroke, epilepsy, Parkinson's disease, autism, etc. Motor Neuron Diseases such as ALS are a group of progressive neurological disorders that destroy cells that control essential muscle activity such as speaking, walking and swallowing. Eventually, the ability to control voluntary movement can be lost. In adults, symptoms often appear after age 55 and are thus of particular concern for states such as Arizona. In children, particularly in inherited or familial forms of the disease, symptoms can be present at birth or appear before the child learns to walk. Many other neurological disorders are rare, known only to the patients and families affected. Neurological disorders strike an estimated 0.8 million Arizonans each year, exacting an incalculable personal toll and an annual economic cost of millions of dollars in medical expenses and lost productivity. Is there a treatment? There is no cure or standard treatment. Prognosis varies depending on the type of disease and the age of onset; however, many cases are typically fatal. There are many prospective applications for using embryonic stem (ES) cells to treat neurological diseases and injuries; however, a critical barrier to progress is the inability to efficiently and reliably enhance neuron generation from these cells while simultaneously minimizing the risks of abnormal proliferation and cancer. Understanding the molecular processes will eventually lead to improved techniques for deriving neurons from ES cells for regenerative medicine and improve the quality of life for Arizonans. The training of scientists who will serve as future educators and researchers, and potentially novel intellectual property essential to creating high quality jobs in biotechnology and pharmaceutical industries will be added benefits.

aPKCζ gene codes for an enzyme are essential for the three-dimensional structural organization and architecture or 'polarity' of cells. The main goal of this project is to define this enzyme-directed, essential regulatory step that may operate to control the acquisition of neuronal fate from ES cells. Because so little is known about how ES cells differentiate, this project will focus on understanding how aPKCζ function and polarity changes as a single neuronal subtype–motor neuron–is generated. Our hypothesis is that manipulation of aPKCζ gene will enhance motor neuron generation. Thus, our study will be directly applicable to ES cell use for treating motor neuron diseases. Nevertheless, the mechanisms that operate to control motor neuron formation are also likely to be relevant to many other neurons; and thereby, our study will serve as a platform for treating a wide range of neurological diseases. The flip side of the coin is that stem cells, in many ways, resemble cancer seed cells and alterations of aPKCζ activity may cause cancer. We will investigate this possibility, study the molecular mechanism and consequently reduce this risk. Over the long term we plan to identify small compounds (drugs) targeting this enzyme. Drugs that enhance neuron formation are likely to improve scientists' ability to experiment with ES cells and create *in vitro* models for studying neurological diseases. These drugs may also be used to stimulate endogenous stem cells within adults to self-repair damaged areas of the brain, while at the same time reducing the risk of inducing cancer.

Steven Goldman, M.D. **Southern Arizona VA Healthcare System**
Award Amount FY09: $50,000

Seeding Fibroblast Patch for Chronic Heart Failure

This project is designed to help treat patients with heart failure after a heart attack. When patients have a heart attack, the blood vessel that supplies the heart, the coronary artery, is blocked or occluded and part of the heart muscle dies. If we cannot open the artery or there is a lot of other damage to the heart, the patients goes on to develop heart failure. Although there are good medical treatments for heart failure, these treatments can not increase blood flow or add new heart cells in a damaged heart. There is currently a lot of research interest in developing ways to put new cells in a damaged heart, but to-date this work has been disappointing in large part because when new cells are injected into a damaged heart, most of the injected cells do not survive. Our plan is to create a new blood supply in the damaged heart and then transplant new heart cells. We will use a 3-dimensional fibroblast construct (3DFC) matrix graft or patch that was, in part, developed at the University of Arizona. With the new blood supply, we can then put new heart cells into the damaged heart and these cells will survive. We have preliminary data in a model of acute myocardial infarction or heart attack with this patch that show new blood vessel growth, improved blood flow and better heart function. The purpose of this project is to explore the use of this 3DFC patch in a rat model of chronic heart failure. We will use this 3DFC patch as a delivery system, and we will seed the patch with cardiac stem cells or new heart cells. Thus we will be able to insure that the new heart cells will survive because these new cells will have a blood supply and a matrix support system.

The newest treatment for acute myocardial infarction (MI) and heart failure is direct injection of stem cells into the damaged heart. Although the pre-clinical data are very encouraging, results from clinical trials have been disappointing. The explanation for the lack of success in clinical trials is not clear. Most investigators believe the cells themselves do not improve left ventricular (LV) function because majority of transplanted cells do not survive after injection. Injecting cells directly into an injured heart does not work because this damaged tissue will not support new cell growth; the blood supply is inadequate and there is no support matrix for cell attachment and growth. We have a new approach to cell-based therapy for heart failure using a viable 3-dimensional fibroblast construct (3DFC) matrix patch implanted on the infarcted heart. Our data show that this 3DFC matrix patch improves LV function and myocardial blood flow while decreasing remodeling in acute MI. Although these data are promising, the real need is to develop a new treatment for chronic heart failure, not acute MI. This project develops a new treatment for chronic heart failure using the 3DFC seeded with cardiac stem cells.

Hypothesis: Use of a viable 3-dimensional fibroblast construct seeded with cardiac stem cells will improve myocardial perfusion, reverse maladaptive LV remodeling, and improve LV function in chronic heart failure after myocardial infarction.

Objective 1) Define changes in myocardial blood flow, LV remodeling and LV function after implanting

the 3DFC patch onto the infarcted myocardium in a rat model of chronic heart failure.

Objective 2) Determine if seeding the 3DFC with cardiac stem cells improves LV function and decreases LV remodeling in chronic heart failure.

Objective 3) Isolate and characterize the properties of the newly formed myocytes by measuring myocyte shortening the Ca+ transients in the transplanted cardiac stem cells.

Stephen Helms Tillery, Ph.D. **Arizona State University**
Award Amount FY09: $50,000

Role of The Basal Ganglia In Learning To Control Neuroprosthetics

The basal ganglia are a set of forebrain structures that are known to be involved in both the control of movement and in reinforcement driven learning. While elegant work has shown signals in basal ganglia that convey information about reward, most of the neuronal activity in basal ganglia that is observed during movement is largely similar to neuronal activity that can be observed in motor cortical areas. This has made it difficult to discern the role of basal ganglia in motor learning. We have embarked on a series of studies in our laboratory on how the brain learns to control neuroprosthetic systems, with a special emphasis on probing the limit of the brain's ability to adapt to novel control algorithms. Literature on basal ganglia and motor learning suggest that the basal ganglia likely will play a significant role in modifying signal processing within the central nervous system enabling control of these systems. In the research proposed here, we will record the activity of neurons in three nuclei of the basal ganglia, the putamen, globus pallidus pars externa, and globus pallidus pars interna, as an animal learns to control a neuroprosthetic system from cortical signals. We expect that signals within these structures will parallel learning curves, with activity in striatum deviating from the movement related signals typically seen. This is particularly the case since our neuroprosthetic tasks are performed with no overt movement.

Our overall goal in this research is to gain a more thorough understanding of how the basal ganglia participate in reward-driven learning. We have postulated that the basal ganglia acts as a system to a)

identify, and b) amplify patterns of cortical activity that have become associated with rewards. This hypothesis is difficult to evaluate in standard movement tasks because movement related signals dominate within those parts of the basal ganglia which interface to motor cortical areas. Our specific goal here is to take advantage of our experience in brain machine interface to require learning in motor cortex that is independent of movement. Our hypothesis is that signals in the putamen early in the process of learning will parallel the learning rather than the actual output of cortex that is controlling the prosthetic system. This contrasts with later in learning, where the neuronal activity in putamen particularly will come to more closely resemble activity the cortical signals which are driving the prosthetic system. Thus, our objective is to use this entirely novel learning environment as a probe for neural processes which underlie reinforcement driven learning.

Jui-Cheng Hsieh, Ph.D. **University of Arizona**
Award Amount FY09: $49,999

Functional Analyses of the Mammalian Hairless Protein

Since the hairless gene (Hr) was cloned in 1996, the functions of hairless protein have not and cannot be predicted on the basis of homology. Its amino acid sequence is not related to any known protein with the exception of six cysteine residues in central region that have been proposed to form a DNA-binding zinc finger. The autosomal recessive gene Hr is responsible for the complete hairlessness in mice homozygous for this gene. In addition to the skin disorders, the Hr mutant mice also displayed some defects in neuronal morphology and inner ear. Despite the importance of hairless function in the skin and brain, very little is known about its biomedical properties such as DNA-binding capacity, heterodimeric partners, phosphorylation sites by target kniases, the target ligands, etc. One of our major difficulties in the field of Hr studies is the limited availability of sufficient quantities of this large, labile protein. Therefore, how to obtain the enough quantities of purified hairless protein becomes an urgent issue. Previous reported repressors such as N-CoR and SMART inhibit their target proteins, but we failed to observe their transrepression for VDR. Recently, we found that the 1,25(OH) 2D3-VDR-mediated transactivation is strikingly inhibited by coexpression of rat Hr corepressor to inhibit VDR. It is noted that Hr becomes the only found repressor of VDR action. Vitamin D has been

reported to be involved in the development of many diseases including osteoporosis, colon and breast cancers. It raises the questions about how Hr is involved in this 1,25(OH)2D3 signaling transduction. If successful, the project will create a new critical mass of information of how Hr transpresses its target genes and target proteins.

We have previously shown that Hr functions as a corepressor for vitamin D receptor (VDR) transactivation in COS-7 cells and keratinocytes (Hsieh et al., JBC 278: 38665-74, 2003). Hr interacts directly and selectively with VDR as demonstrated at GST pull down assays. Further, like other nuclear transcription factors, Hr contains a putative DNA-binding zinc-finger motif via six conserved cysteine residues. Based on these observations, an attractive hypothesis is that Hr binds directly to DNA and displaces VDR from its vitamin D responsive element (VDRE). However, there is as yet no evidence that the Hr protein can bind to DNA. The first objective in this project is to determine whether Hr binds to DNA including the well-characterized VDREs, thyroid hormone responsive elements (TREs) and unknown DNA motifs. We have been interested in studying the negative gene regulation mechanism of VDR. This Hr-mediated transrepression on VDR mechanism is important because of the wide prevalence of 1,25(OH)2D3-VDR action in many tissues and organs such as skin, intestine, kidney, brain, heart, bone, etc.

Many proteins involved in gene regulation have all been found to be phosphoproteins, including thyroid hormone receptor, VDR, estrogen receptor. In the case of VDR, protein kinase C (PKC) phosphorylates VDR at serine-51 and significantly reduced DNA-binding activity of VDR, while serine-208 is the target site for casein kinase II (CK-II) and boosts VDR transactivation. Therefore, considering the important influence of phosphorylation in Hr, the second aim of the project is to determine the phosphorylation sites of Hr. Our immediate plan includes the purification of large quantities of homogeneous Hr protein for molecular biological characterization and the identification of Hr phosphorylation sites. This project is designed to provide insight into the architecture of Hr-binding DNA and the functional relevance of Hr phosphorylation sites. The goals of this proposal are to 1) study Hr DNA binding capacity and 2) identify Hr phosphorylation sites and examine their potential functional roles.

Leland S. Hu, M.D. **St. Joseph's Hospital**
Award Amount FY09: $49,500

Distinguishing Post-Treatment Radiation Effects From Glioma Recurrence Using Dynamic Susceptibility Contrast (DSC) MRI

A number of new and existing therapies can help maximize patient survival following initial treatment of high grade gliomas. Applying the appropriate treatment plan relies on accurate distinction between tumor regrowth and post-treatment changes. Accurate diagnosis requires surgery since current imaging tests are not sufficient. However, development of specialized imaging tests can vastly improve accuracy and may help forgo the need for invasive procedures. Dynamic Susceptibility Contrast (DSC)-MRI is a specialized imaging test which provides information about tissue blood flow and can improve non-invasive detection of tumor growth. The preliminary results are very promising but further work is needed. The overarching goal of this pilot study is to establish DSC-MRI as a non-invasive diagnostic tool which provides accurate treatment planning for tumor patients.

Present evidence suggests DSC-MRI can exploit the inherent differences between high blood flow in tumor growth and low blood flow in post-treatment tissue to provide measurements which distinguish the two entities; however, the DSC-MRI measurements must first be validated by directly correlating with surgical tissue specimen histopathology, which is the diagnostic gold standard. This pilot study will first compare DSC-MRI measurements with tissue specimen histopathologic diagnosis of tumor growth or post-treatment change to establish a set of threshold DSC-MRI values to distinguish the two diagnoses. In addition, the DSC-MRI measurements will be compared with the amount of blood vessels histopathologically identified from the same tissue specimens to provide further validation of this technique.

Mrinalini Kala, Ph.D. **St. Joseph's Hospital**
Award Amount FY09: $50,000

Role of B Cells In Glatiramer Acetate Mediated Suppression of Multiple Sclerosis

Multiple Sclerosis (MS) is an autoimmune disease that affects the central nervous system (CNS). The CNS consists of the brain, spinal cord, and the optic nerves. Surrounding and protecting the nerve fibers of the CNS is a fatty tissue called myelin, which helps nerve fibers conduct electrical impulses. Myelin not only protects nerve fibers, but makes their job possible. Myelin is the target in an MS attack. When myelin or the nerve fiber is destroyed or damaged, the ability of the nerves to conduct electrical impulses to and from the brain is disrupted, and this produces the various symptoms of MS. In MS, myelin can be lost in multiple areas, leaving scar tissue called sclerosis in the brain or spinal cord. These damaged areas are also known as plaques or lesions. Sometimes the nerve fiber itself is damaged or broken.

It has been estimated that MS is a leading cause of disability among young adults in North America and Europe. Approximately 400,000 Americans have MS, and every week about 200 people are newly diagnosed. World-wide, MS affects about 2.5 million people. Drugs recommended for its treatment were designed to reduce autoimmune responses. Some of these therapies such as mitoxantrone, corticosteroids and drugs in development like Fingolimod are based on generalized immunosuppression. Other therapies including cytokine based strategies and monoclonal antibodies to various immune targets are more selective in their effects. These treatments have improved risk to benefit ratios over generalized immunosuppressant, but they are only partially effective and still have significant risks. All of these therapeutic strategies are incapable of permanently stopping the immune attack on the CNS that characterizes MS. The only immunotherapy for MS that is not based on immunosuppression is Glatiramer Acetate (GA). GA (copolymer-1 or Copaxone) is a randomized copolymer that consists of 4 amino acids: L- alanine, L-lysine, L-glutamic acid and L-tyrosine. GA slows the progression of disability as well as the relapse rate with few side effects. This makes GA a good candidate for our studies. Our lab has shown that B lymphocytes have a role in suppression of MS by GA. In this proposal we will examine how GA can modulate B lymphocytes and thereby affect the immune system. Our studies will be carried out initially in the mouse model of MS and if they are successfu,l we will extend them to humans. Understanding the mechanism of successful drug therapy will be a major advance towards developing an effective therapy for Multiple Sclerosis.

Annual economic cost of MS in the United States is approximately $28 billion. In Arizona alone there are at least 6000 people suffering from MS, and developing or improving a successful therapy will be an immediate benefit for them and their families.

Lei Lei, Ph.D. **Arizona State University**
Award Amount FY09: $50,000

Function of Sox11 In Mammalian Cortical Development

The human brain has over 100 billion neurons and these neurons form specific connections. It is now widely recognized that abnormal brain development during the critical periods such as prenatal, neonatal and adolescent stages, underlies the etiologies of many mental disorders. According to the National Institute of Mental Health, each year as many as 44 million Americans meet criteria for some mental disorder, with roughly 12 million reporting symptoms so severe as to cause significant disability and interference with everyday living. The economic cost of mental disorders are estimated at over $150 billion a year. Furthermore, mental disorders can also be fatal. Each year more Americans die from suicide than from homicide. Like the rest of the nation, Arizona faces the ever-increasing challenge to develop new diagnostics and therapeutics to treat patients with various mental disorders. However, in order to accomplish this goal, it is imperative to understand the biological pathways and mechanisms that control normal brain development and identify gene mutations and environmental factors that cause mental disorders. In the past twenty years, although research using genetically engineered mice has significantly improved our knowledge of development and behavioral neuroscience, we are just beginning to have a comprehensive understanding of brain development and function. In order to reduce the burden of mental and behavioral disorders, our government needs to continue to invest in fundamental research in neuroscience.

Our long-term goal is to understand the molecular pathways and mechanisms that control brain development. During fetal development, all of our neural cells are derived from a special type of cells called neural progenitor cells. Neural progenitor cells divide and generate neurons, which then migrate to occupy appropriate positions in the brain and form the correct connections with other neurons. Abnormal generation or migration of neurons leads to dysfunction of the brain. In adult brains new neurons are continuously generated in specific brain regions by neural progenitor cells albeit at a much reduced level. Like other organs in our body, the development of brain is largely controlled by transcription factors, which are proteins that serve as "switches" to tell cells what to do or what to become. Our central hypothesis is that the transcription factor Sox11 controls neural progenitor cell proliferation and neuronal migration during fetal development and in adult brains. Our objectives are 1) to determine the function of Sox11 in regulating neural progenitor proliferation and 2) to determine the function of Sox11 in regulating neuronal migration. In order to demonstrate the function of Sox11 in brain development, we will use genetically engineered mice in which Sox11 is removed from specific nerve cells. Because mice and humans share similar biological processes and developmental pathways, our studies using mice will translate into a better understanding of human brain development. Moreover, our genetically engineered mice may serve as animal models for understanding the causes of mental disorders. We anticipate that the experiments proposed here will not only provide significant new insights into the specific mechanisms of brain development, but may also lead to the identification of novel therapeutic targets for combating developmental and neurological disorders including Schizophrenia, depression, and mood disorders.

Kathryn Lemery-Chalfant, Ph.D. **Arizona State University**
Award Amount FY09: $47,438

Molecular and Quantitative Genetic Approaches to Understanding Child Psychopathology

The Arizona Biosciences Roadmap emphasizes the need to strengthen the medical research base and build a critical mass of firms and jobs with increased collaboration among higher education, nonprofits, and industry. This project outlines an innovative interdisciplinary collaboration between Arizona State University and the Translational Genomics Research Institute. Specifically, we aim to elucidate the role of specific functional genes in child psychopathology (i.e., anxiety, depression, conduct disorder, and attention-deficit hyperactivity disorder). The need to understand genetic influences on mental health is underscored by findings that 10-15% of children exhibit clinical or subclinical disorders, and most adult disorders have roots in childhood.

Mental disorders are the leading cause of disability in the US and Canada (World Health Organization, 2004), costing the US economy an estimated $105 billion dollars each year in lost productivity (National Mental Health Association, 2001). Arizona currently ranks 18th in the country for employee poor mental health days and 33rd in the country for overall health (United Health Foundation, 2007), so psychopathology is a central issue to Arizona citizens and the Arizona economy. Investing in children is an excellent way to ensure that overall health increases and the drain on the economy decreases in the future. Lastly, effective pharmacological and behavioral prevention and intervention efforts require an understanding of the role of genes and gene-environment interplay in child psychopathology.

We propose a molecular genetic study of child psychopathology. The participants are twin children (many of whom have diagnoses of anxiety, depression, conduct disorder or attention-deficit hyperactivity disorder), other biological siblings and biological mothers and fathers. These families have already been extensively assessed with clinical interviews, hormonal measures, and observational assessment of behavior and the home environment. In general, we believe that the identified candidate genes will convey general risk for greater that one disorder and be associated with intermediate biological and behavioral factors. For example, we hypothesize that genes that influence levels of the neurotransmitter serotonin in the brain will increase risk for anxiety and depression through their influence on child temperament, auditory and tactile sensitivities, and stress hormone levels. Further, we hypothesize that the role of these genes on child psychopathology will depend on the family environment such as low social class, negative parenting practices, and stressful life events. Genes are turned on and off depending on the context, so models that allow for gene-environment interplay best represent reality.

Lonnie Lybarger, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Regulation of Immune Activation By Ubiquitination

The immune system is critical for the control of both infectious agents and cancers. However, this powerful system also holds the potential for unwanted reactions against normal, "self" targets (tissues). Such untoward immune reactions are referred to as autoimmune reactions. Unfortunately, as a whole, the frequency of autoimmune diseases has increased sharply in recent years. This includes diseases such as asthma, arthritis, and food allergies, to name a few. In Arizona, the incidence of asthma exceeds the national average and is on the increase. Further, with our aging population, diseases like arthritis are particularly relevant. Clearly, this trend is alarming and represents a serious concern. The number of individuals that are affected by autoimmune disorders is expected to continue to increase in the coming decades and represent and ever-larger health system burden. Therefore, it is imperative to understand the mechanisms that contribute to the generation of autoimmunity.

A general feature of autoimmune disease is uncontrolled activation (or priming) of the immune system against self tissues. Normally, several "checks-and-balances" exist to prevent such aberrant activation, many of which have been characterized in detail. Nonetheless, there is much that remains to be learned about the cellular pathways that regulate activation. Indeed, a protein called MARCH1 was recently identified that appears to play an unexpected role in controlling the initiation of immune responses. MARCH1 is expressed in a subset of immune cells called antigen presenting cells (APC). APC are critical for immune priming; they have the unique capability to activate T-cells. In APC, MARCH1 seems to diminish the ability of APC to activate T-cells by causing the selective destruction of other proteins within the cell that are necessary for T-cell activation. Thus, MARCH1 is positioned to play a substantial role in the control of immune priming. In spite of this, little is known regarding the molecular details of MARCH1 function. We will explore this critical area and characterize the cellular pathways utilized by MARCH1 to regulate the immune response. In turn, this knowledge will likely prove useful for the future development of strategies designed to treat immune disorders such as autoimmunity.

Our lab has a longstanding interest in quality control pathways within cells. Such pathways are essential to all cells and can remove unwanted or defective proteins. MARCH1 employs such pathways to cause the destruction of other cellular proteins in APC that are required for immune activation, though the details of the mechanisms employed by MARCH1 to accomplish this feat are poorly understood. We have applied many of the techniques and reagents already available in the lab to the study of MARCH1 and have generated exciting preliminary data regarding the pathways involved in MARCH1 function. We will extend our finding and test the hypothesis that MARCH1 invokes ubiquitin-dependent cellular pathways to negatively regulate APC function. We will focus our analysis on a known target molecule of MARCH1 called CD86, an essential protein for T-cell activation. Using a variety of cell biological techniques, we will define the pathways employed by MARCH1 to target CD86 for destruction. In addition, we will determine the role of these pathways in immune priming by APC. This project

represents an essential step toward understanding the means by which MARCH1 is able to affect immune priming, and it is fully anticipated that this knowledge will ultimately inform future studies designed to alter priming for therapeutic benefit.

Anjan Misra, Ph.D. **St. Joseph's Hospital**
Award Amount FY09: $50,000

Exploiting Receptor Tyrosine Phosphatases for Diagnosis and Treatment of Malignant Astrocytoma

Malignant astrocytoma are lethal brain tumors accounting for more than 50% of all brain tumors. Survival has not changed in 3 decades despite most aggressive therapy. These tumors come in two grades, Anaplastic Astrocytoma (AA, grade 3) and Glioblastoma Multiforme (GBM, grade 4). GBM has a median survival of about one year and AA, about 3.5 years. Protein Tyrosine Phosphatase Receptor D (PTPRD) is lost in AA and GBM (41-80% and 65-88%, respectively) and is associated with poor survival in GBM (n=195). This suggests that loss of this protein helps malignant astrocytoma tumors. Other groups reported that PTPRD is lost infrequently in other cancers (e.g., neuroblastoma, esophageal, stomach and lung cancer). This makes PTPRD an ideal target for diagnostic and therapeutic use in many cancer types, but specially in malignant brain tumors with protest outcome.

We hypothesize that PTPRD is a candidate tumor suppressor gene and loss of it helps tumor initiation and/or progression maintenance of malignant astrocytic tumors.

The goals and objectives of this project are to investigate a) how frequently PRPRD is lost in malignant brain tumors at protein level (as expected from preliminary data), b) whether higher grade malignant brain tumors have a higher frequency loss than the lower grade tumors, c) how loss of this protein affect tumor initiation, progression and behavior, and response to therapy, d) what are the normal mechanisms of PTPRD loss in human brain tumors, and e) what are the pathways that are modulated by this loss so that an intervention can be directed either directly to PTPRD or to pathway(s) it influences. This information will help us strategize diagnostic and therapeutic applications of PTPRD loss.

Jong M. Rho, M.D. **St. Joseph's Hospital**
Award Amount FY09: $49,819

Epilepsy - Induced Impairment of Circadian Rhythms

Epilepsy is common neurological disease and afflicts 1-2% of the general population. The highest incidence of epilepsy occurs in very young children and elderly adults. It is well known that epileptic seizures can occur more commonly during sleep, and there is growing evidence indicating that many, if not most, epileptic patients experience significant sleep problems. Since virtually all epileptic patients are treated with anticonvulsant medications, it is unclear whether such sleep disturbances result from the condition itself or as a consequence of chronic treatment with drugs. Sleep problems are often linked to abnormal circadian rhythms (i.e., dysfunctional day-night cycling), and it is well known that the brain's internal master clock responsible for regulating these rhythms resides in the suprachiasmatic nucleus (SCN) which is part of the hypothalamus. The major goal of this project is to evaluate circadian rhythm disturbances in an animal model of early-onset epilepsy (the epileptic Kcna1-null mutant mouse) and to determine the cellular and molecular basis for this dysfunction by focusing on associated changes in the SCN. There is growing evidence that SCN integrity is paramount to general health, and its importance is underscored by the SCN's role in regulating seizure activity, contributing to sleep disorders, obesity and overall neuropsychological functioning. Given the epidemic of sleep problems and obesity currently faced by the general population in Arizona and throughout the United States, the results of the proposed studies may help expand our knowledge regarding SCN function to a wide range of physiological and neuroendocrine functions.

The principal goal of the project is to determine whether there are intrinsic functional abnormalities of the suprachiasmatic nucleus (SCN) of epileptic knockout mice and to identify the gene(s) responsible for abnormal circadian function. This research study comprises three specific aims. In the first aim, we will determine whether impairment of normal circadian rhythms in our mouse model is associated with the post-natal development of epilepsy. We hypothesize that circadian rhythm expression and phase shifts are altered after onset of spontaneous seizures in Kcna1-null epileptic mutant mice, and that prior to the onset of spontaneous seizures, circadian rhythm expression does not differ between Kcna1-null mutant and wild-type mice. In the second specific aim, we will examine the circadian firing patterns of SCN neurons in organotypic slice cultures prepared from Kcna1-null mutant mice using multi-electrode array recordings. We hypothesize that circadian firing patterns of SCN neurons form Kcna1-null mutant mice are either phase-shifted, or are irregular or shortened, compared to those of wild-type mice.We further predict that prior to the age of seizure onset, circadian firing patterns of SCN neurons from Kcna1-null mutant mice will resemble those of wild-type mice. Finally, in the third specific aim, we will determine whether circadian rhythm abnormalities in epileptic Kcna1-null mice are associated with differential expression of clock gene protein products. We hypothesize that SCN neurons will express abnormal circadian expression patterns of clock genes (e.g., Per1, Per2, and BMAL) in Kcna1-null mutant mice, and that prior to the onset of spontaneous recurrent seizures, circadian expression patterns of these clock genes in Kcna1-null mutant mice will resemble those of wild-type control mice.

Overall, we hope to demonstrate that there are intrinsic abnormalities of clock gene expression, SCN firing patterns and circadian phase shifts in epileptic mice, and that these finding occur after the onset of the epileptic state.

Leslie S. Ritter, Ph.D. **University of Arizona**
Award Amount FY09: $47,608

Novel Use of a Natural Product for Acute Stroke Therapy

Stroke is one of the leading causes of death and disability in the United States and in Arizona. However, there is currently only one approved treatment, a clot-busting drug, to protect brain cells from death during the time of a stroke. Other drugs have all failed for several reasons. First, the experimental drugs that were tested were not well-characterized, or treated just one cause or pathway of brain cell death. Second, the experimental models that were used did not fully represent the human stroke condition. Our study is designed to overcome these problems. Natural products isolated from medicinal plants have not previously been tested for stroke treatment but are attractive candidates for testing because they are made up of multiple compounds that can act at multiple levels to prevent brain injury. Turmeric, the natural product to be tested in this study, is well-characterized and is known to affect multiple inflammatory pathways that injure the brain after stoke. Also, the experimental model of stoke that will be used in this study is one that is recommended by national advisory panels. The inflammatory pathways that injure the brain after stoke that we will study involve the white blood cells (neutrophils) and the cells that line the blood vessels (endothelial cells). Dr. Ritter, the Principal Investigator, was the first to discover that a tremendous amount of white blood cells stick to the blood vessels of the brain after a stroke. When this occurs, the white blood cells release an overabundance of toxic substances which damage the blood vessels and the brain tissue. Dr. Funk, the Co-Investigator, was the first to discover that a well-characterized turmeric dramatically reduced severe joint damage in rheumatoid arthritis by blocking the damaging effects of the white blood cells. These collaborating investigators asked the question, "Would this natural product also block the damaging effects of the white blood cell after stroke?" Our project has been designed to answer this important question.

The major hypothesis to be tested is that a well-characterized natural product, turmeric, will reduce brain damage after stroke, and it will do so by affecting multiple inflammatory pathways that involve white blood cells (neutrophils) and blood vessels (endothelial cells).The specific objectives of our study are 1) to determine the ability of a well-characterized turmeric extract to prevent brain injury in a animal

model of stroke that is relevant to the human condition, 2) to assess the ability of turmeric to block neutrophil adhesion to the blood vessel endothelial cells, an event that is a major contributor to brain injury after stroke, and 3) to identify inflammatory signaling pathways at the neutrophil/endothelial interface that are targeted and blocked by turmeric.

As strokes are the third leading cause of death and major cause of disability, identification of a safe and beneficial effect of turmeric that could act in a novel way to limit brain injury in stroke could have a major impact on the health and health care costs of the state of Arizona. This is particularly true at a time when baby boomers approach retirement and the percentage of our population over the age of 65, i.e. those individuals at greatest risk of stroke, is projected to double. Strong public interest in botanical supplement use is clearly supported by recent national health surveys (CDC). This project will also provide opportunities for the Arizona Board of Regents to be at the forefront of a new business model that will allow for the development and commercialization of complex natural products for stroke using a pharmaceutical approach.

Marek Romanowski, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

Contrast Agent for Colonoscopy

Despite the steady decline of its incidence rate, colorectal cancer remains the third most common cancer and the third leading cause of cancer death, killing one in seventeen men and one in nineteen women in the United States. Most carcinomas of the colon develop from preexisting polyps. Medical practice clearly demonstrates the importance of screening followed by resection of polyps, or polypectomy, and among currently recommended screening methods, colonoscopy is considered the most sensitive. Following polypectomy the incidence of colorectal cancers has been shown to fall by half or even more. However, small or difficult to identify polyps could have been missed only to progress to invasive cancer in the remaining half of patients.

Approximately 2750 Arizonans will be diagnosed with cancer of the colon or rectum in 2007 and 970 will die of this disease. The incidence rate of colorectal cancer dramatically increases with age. The U.S. Census Bureau projections indicate that between the years 2000 and 2030 the elderly population of Arizona will more than triple, an exceptional growth rate projected in only two other, less populous,

states. This evolving demographic will require accelerated development of highly effective and accessible methods of preventive screening.

The overall goal of this project is to develop a contrast agent that would enhance existing diagnostic techniques by marking areas of precancerous changes, or adenomas, with highly visible luminescent dyes. The proposed contrast agent will employ upconverting nanoparticles. This class of optical materials has only recently been contemplated for biomedical use, and its diagnostic application discussed here is new and unique. The safety, chemical stability, and high contrast associated with upconverting nanoparticles are superior to many standard molecular probes or quantum dots. These silica-encapsulated particles have no discernable color under ambient light. However, when exposed to invisible infrared illumination, they emit bright colors. Targeting precancerous changes will be possible by including in this design highly selective and robust molecules capable of recognizing early markers of disease. When fully developed, the proposed contrast agent will provide functional enhancement of colonoscopy and other endoscopic techniques. One of the more exciting possibilities is its use in conjunction with the wireless endoscopic capsule technology recently approved by FDA for examination of esophagus and small intestine and currently in development for imaging of the entire gastrointestinal track. At the successful completion of this project we will seek funding from federal and commercial sources, which would allow for continuing research as part of the colon cancer prevention programs at the Arizona Cancer Center.

Jiong Shi, M.D.,Ph.D.

St. Joseph's Hospital
Award Amount FY09: $50,000

APOE Mimic Peptide as a Novel Therapy on Cognition in a Transgenic Mouse Model

Multiple sclerosis (MS) is a devastating disease that causes not only motor deficits but cognitive ones as well. MS is a leading cause of disability in young adults, with most people experiencing their first symptom between the ages of 20 and 40. Cognitive symptoms of MS are considered the most debilitating and have the heaviest impact economically and socially. Studies indicate and our research confirms that 40-70% of patients with multiple sclerosis (MS) exhibit significant cognitive impairment. Our studies found a strong association between the development of cognitive problems and a gene for apolipoprotein E4 (APOE4). Our studies further indicated that a markedly disproportionate percentage of the young patients with cognitive deficits have the APOE4 gene. Apolipoproteins are lipid-binding proteins which are necessary to promote repair of injured neurons, cells of the brain. APOE 4, however,

is less efficient and more likely to be cleaved by enzymes than the other types. Understanding the role of APOE in MS and how the different types of APOE interact with the disease to affect cognition is our goal. Animal models provide the best methods of determining the mechanisms. Experimental autoimmune encephalomyelitis (EAE) is the best studies animal model for MS. Mouse models allow us to use genetically altered (transgenic) mice which have or do not have the gene of interest.

We will use transgenic mice which have no APOE (APOE knockout) and to have APOE 4 (APOE 4 knock-in) and compare their cognitive function to mice with normal APOE after inducing them with EAE, the animal version of MS. We will test them early in the course of the disease, as our MS studies indicate that cognitive symptoms precede motor and may be the first indication of disease.

We will test their cognitive function using a water maze and examine their brain using special stains for chemical and cellular changes seen in the hippocampus, the memory region of the brain. We will also look for electrophysiologic changes using a wll0-studied synaptic model for memory.

Finally, we will use APOE peptide mimetic, a peptide–small protein fragment–which mimics the therapeutic effect of APOE to determine if it can overcome the detrimental effects of the absent (knockout) and less efficient APOE 4.

Understanding the role of APOE on the MS mouse model will provide us with valuable insight regarding the mechanisms of the cognitive decline in the susceptible MS population. Identification of cognitive symptoms prior to the onset of motor symptoms in the animal model of MS and determination of the role of APOE at this early stage should result in clinical trials designed to identify the disease in the earliest stages enlarging the therapeutic window in which the disease can be addressed and potentially reversed. Determining the mechanism and efficacy of competitive inhibition using APOE mimetic peptides will provide further insight, promote the rapid translation to human trials, and promote further investigations into competitive inhibitory therapies for this devastating disease.

Catharine L. Smith, Ph.D. **University of Arizona**
Award Amount FY09: $49,999

Identification of the Acetylated Transcriptional Proteome in Leukemia

Cancer is the leading cause of death in Arizona children under the age of 15 and leukemia and lymphoma are the first and third most prevalent childhood cancers in the state (American Cancer Society, Arizona Cancer Facts and Figures, 2004-2005). In addition, the incidence of these cancers increases with age; in adults, these cancers are most prevalent after the age of 60 (Leukemia and Lymphoma Society, http://www.leukemia-lymphoma.org/). Given its sizable community of retired adults, its is not surprising that leukemia and lymphoma rank among the top 10 causes of cancer death in Arizona as of 2004. A new class of anticancer drugs, histone deacetylase (HDAC) inhibitors, has shown promise in treating these cancers. These drugs inhibit enzymes that remove acetyl groups from proteins. The presence of these groups can modulate the activity of proteins thus protein acetylation is considered an important regulator of cell function. HDAC inhibitors can induce either a halt to growth of the death of cancer cells by mechanisms thought to involve changes in gene expression. Although they are sometimes effective in leukemia/lymphoma patients, it is difficult to predict who will benefit from these drugs. This is partly due to the lack of identified acetylated proteins known to be important for regulation of leukemia/lymphoma cell growth. Detection of such proteins in cancer cells could serve as an important predictive tool. In addition, cancers are most often treated with multiple drugs and it is unclear which drugs would work best in combination with HDCA inhibitors to increase both the number of patients who might benefit from these drugs and the rate of patient survival.

The hypothesis of this project is that genes rapidly regulated by the inhibition of HDAC activity will be bound and coregulated by proteins which are acetylated and thus targeted by HDACs. Proteins bind to genes by recognition of specific DNA sequences. We propose that computer-aided analysis of such sequences in the genes regulated by HDAC inhibitors will reveal the enrichment of binding sites for proteins potentially regulated by acetylation. The acetylation status of such proteins will be assessed using new and powerful mass spectrometry methods. The project has three aims. First, we will identify the genes commonly regulated by HDAC inhibitors in five leukemia/lymphoma cell lines that are likely to contribute to regulation of cell growth and survival. Second, we will analyze these genes for the presence of common binding sites for gene regulatory proteins. Third, we will determine which gene regulatory proteins are acetylated in response to drug treatment in leukemia and lymphoma cells. The overall objective of the proposed study is to identify acetylated proteins critical for mediating the death of leukemia and lymphoma cells through regulation of gene expression.

This study will address a major gap in our understanding of acetylated gene regulatory proteins important for controlling growth and survival in leukemia and lymphoma. These proteins are likely to be the mediators of drug action and the resulting halt of cancer cell growth. The acetylation status of these proteins could serve as potential diagnostic tools to determine whether individual patients would

benefit from treatment with HDAC inhibitors. The identity of these proteins could also provide insight into which other drugs might be used in combination with HDAC inhibitors to increase their efficacy and boost the rate of disease-free survival. The unique integrated approach we propose could also be applied to other cancers in which HDAC inhibitors show effectiveness and to other anti-cancer drugs which work through changes in protein modification.

Peter N. Steinmetz, Ph.D., M.D. **Arizona State University**
Award Amount FY09: $48,744

Representation of Memory for Spoken Words and Voice Detail by Single Neurons in the Human Hippocampus

The problems posed by illnesses affecting memory, such as Alzheimer's disease, assume increased importance as people live longer on average, creating a growing population that is susceptible. An inability to remember names, statements and instructions can be particularly debilitating. Understanding how such linguistic memories are stored in the brain is a key step toward developing better therapies, but this is difficult to study in non-human animal models of disease because spoken abstract language is not present. Although non-invasive measurements, like brain imaging and scalp electrical activity monitoring, provide information about average activity in the human brain, these techniques lack the ability to describe exactly which cells in different brain areas are involved on the time-scale of single cell activity, which is the fundamental unit of information representation in the brain.

This project overcomes these limitations by measuring the activity of single neurons in the human brain under extraordinary circumstances - when epilepsy patients require implantation of electrodes for clinical monitoring. While the patients are implanted with electrodes, they can volunteer to perform experiments where they are asked to remember spoken words and later recall them. By measuring the activity of single neurons near the tip of the electrodes and correlating this activity with the patient's recollections, we can determine which brain areas and connections, or circuits, between brain cells are critical to correct recollection.

Since memory of spoken language has never been studied in two brain areas where the electrodes are placed (hippocampus and amygdala), our initial goals are to determine how memory for spoken words in a simple task is represented in these brain areas. The task performed by the subjects is simply to remember whether they have heard the word before or during the experiment. The spoken words are played through headphones with different amounts of delay between their first and second presentation, and also spoken half the time in a different voice. Our hypothesis is that both the amount of delay between presentations and whether the same or different voice is used will change the responses of single neurons in the human hippocampus and amygdala. This is based on the observation that both of these variables, delay and voice, affect the ability of normal subjects to accurately recall whether they have heard the word before, as well as many other observations showing that the hippocampus is involved in the recollection of events and facts.

To test this hypothesis, we record the activity of single brain cells near the tips of the implanted electrodes as the patients perform this task. The average activity, or rate of firing, will then be correlated with both the delay and voice, as well as how well the patients recall hearing the words.

The results of these experiments will provide a basic groundwork for understanding how memory for spoken language is represented in these brain areas, facilitating further work examining memory for more complex and abstract linguistic structures such as sentences. These results will also expand our ability to target new therapies for memory disorders to particular cell types and brain areas. The demographics of the state of Arizona make improving these therapies a particularly important issue, since failures of memory (dementias) occur more frequently in the elderly.

Raoul Tibes, MD, Ph.D. **TGEN**

Award Amount FY09: $50,000

RNAi Kinome Screening to Identify Rational Combinations with Cytarabine in Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is the most common adult acute leukemia and one of the deadliest cancers. In fact, leukemias are the 5th and 6th leading cause of cancer deaths in men and women, respectively, and AML is responsible for the majority of these deaths. Overall only 5-10% of AML patients are alive at 2 years. Unlike other leukemias and cancers, AML therapy has not much improved over the last decades. Therefore, new therapies are urgently needed to treat patients with AML. Cytarabine (a "chemotherapy") is the most active drug used in AML and can cure a small fraction of AML patients. However, many patients are resistant to Cytarabine based therapies, leading to inadequate treatment of their AML. It is not yet understood which are the genes that lead to Cytarabine resistance. Identifications of these genes would increase our understanding and guide the development of new and better therapies to treat AML with Cytarabine based therapies.

Many of the genes that are involved in leukemia development and resistance are kinases, a group of enzymes that drive growth of leukemia cells. In the past researchers were only able to analyze one or a few single genes/kinases that they thought might have a role in Cytarabine resistance. This is because the technologies have not been available to test hundreds of kinases at the same time to see if they can slow down growth of leukemia cells. A new discovery that was awarded the Nobel Prize for Medicine now makes it possible to use short stretches of RNA (base pairs similar to the DNA in cells) that can be brought into cells and very selectively reduce the activity of individual genes. Now hundreds of genes and kinases can be studied at the same time to identify which individual kinase plays a role in conferring resistance to Cytarabine in AML. Discovery of these genes can be utilized to develop new therapies to improve response to Cytarabine based therapy and ultimately increase survival for patients with AML.

We hypothesize that certain, yet unknown kinase genes have a function to make AML cells resistant to Cytarabine. This research proposes to inhibit the function of several hundred kinases to test if inhibiting the function of one of those genes can reverse the resistance to Cytarabine. We will take Cytarabine resistant leukemia cells and incubate them with Cytarabine and short RNA pieces that inhibit the function of a single gene (knock down). Consequently, if a gene produces Cytarabine resistance, inhibiting its function would increase leukemia cell death. This implies that cells have regained their sensitivity to Cytarabine. We will do this for ~600 genes, the so-called kinases. The genes that help make cells the most sensitive to Cytarabine will be tested further. This validation step is important to increase our confidence that those kinases are indeed the most important ones. In additional experiments, we will then look at the mechanisms of what happens to the proteins of those genes and assess the activity of those proteins.

The approach in this project will help to find the important kinase genes that confer resistance to

Cytarabine in AML. Once those genes have been identified, new drugs and therapies can be developed against those genes/kinases and be combined with Cytrarabine to improve treatments for patients with AML.

This project is important for Arizona because it contributes to a new program that has started to improve leukemia treatment, care and research in Arizona. This project leverages the clinical expertise of the applicant with the knowledge and resources at the institution where the research will be conducted. The facility for the short RNA stretches to reduce the function of genes is one of the few high-throughput in a not-for profit research institute in the nation. There is a unique opportunity for this project to be successful and to attract attention to Arizona as a new developing biomedical center. Furthermore, the results generated from this project will help develop new projects and allow investigators to apply for national grant funding increasing the competitiveness of Arizona in research and biotechnology. With promising new findings, new drugs can be developed here in Arizona involving new and start-up biotech companies.

Theodore Trouard, Ph.D. **University of Arizona**
Award Amount FY09: $50,000

MRI, MRS and Molecular Modeling of Three Dimensional Cell Cultures

Diffusion-weighted MRI(DWMRI) is a technique that allows non-invasive measurements of the microscopic motion of water in living tissue. Because the motion of water is sensitive to the local cellular environment, DWMRI is being used to study a variety of diseases including stroke and cancer. Within minutes after a stroke has occurred, the area of brain tissue affected by the stroke shows up very bright on a DWMRI image compared to normal brain tissue, which allows physicians to unambiguously identify the tissue involved in the stroke. More recently, DWMRI has also been used to monitor the early effects of chemotherapies in cancer. It has been shown that the motion of water in tumors changes in response to successful therapy and that this change occurs prior to any measurable change in the size of the tumor. Although these observations in stroke and cancer are clinically useful, the underlying reason for the changes in water diffusion is still unknown. The more we know about how water moves within tissue, the better we will be able to interpret results from the numerous DWMRI exams being carried out in stoke and cancer and the better we will be able to treat these diseases.

The goal of this project is to better understand why the motion of water, as measured by DWMRI, changes after stroke and successful cancer therapy. To begin to reach this goal, we will carry out MRI experiments on unique cell culture systems developed at the University of Arizona to study the motion of water in well defined and easily controlled environments. These experiments will allow us to make detailed measurements of water movement in normal cells, swelled cells, shrunken cells and ischemic (stroked) cells. We will also develop realistic mathematical models with which to describe water motion in simple cell systems and predict the results of DWMRI experiments. The mathematical models will allow us to determine which biophysical properties are important to water diffusion and which are responsible for the changes observed in stroke and cancer therapy.

Pak Kin Wong, Ph.D. **University of Arizona**
Award Amount FY09: $49,999

Molecular Probe Biosensors for Rapid Screening of Photoprotective Compounds

Currently, Arizona has among the highest incidence rate of skin cancer in the world, so we are interested in agents that can suppress solar damage relevant to skin cancer. Our team, in collaboration with the Department of Pharmacology and Toxicology, are currently investigating natural and synthetic compounds to serve as prototype photprotective agents. In order to perform a large-scale screening of photoprotective compounds, we plan to develop an automated platform for high-throughput screening of chemopreventive agents. The technological core of the rapid screening system is two molecular biosensors for rapid detection of the cellular antioxidant response. In addition, a microfluidic platform will be developed to facilitate large-scale screening as the microfluidic system automates a large number of measurements simultaneously and the amount of reagents required is dramatically reduced.

The overall goal of the project is to develop a rapid screening system for identifying chemopreventive compounds. Chemopreventive compounds often exert their effects by inducing the expression of cytoprotective enzymes to defend cells from oxidant stress and reactive carcinogenic intermediates. The expression of many cytoprotective enzymes is regulated by the NF-E2-related factor 2 via antioxidant-responsive elements. Here, we plan to develop two molecular probe designs and a microfluidic platform for rapid detection of the ARE-bound Nrf2 protein and Nrf2-mediated gene expression for identifying potential chemopreventive compounds. The molecular biosensors will form the technological core of a high-throughput screening system. To evaluate the assays, human skin cells are chosen as cell-based model. The development will lead to a rapid, quantitative, sensitive, and specific screening system for rapid identification of chemopreventive compounds. The system will provide an alternative for reducing animal usage in predicting the effects of chemopreventive compounds and elucidating their mechanisms of action. The assays should also be generally applicable to a wide range of toxicology studies, as they are compatible with various *in vitro* models.

Section E

Indices

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