Arizona Biomedical Research Commission

2005 – 2006
Annual Report

January 2007

Arizona Biomedical Research Commission
Annual Report
2005 -2006

Janet Napolitano, Governor

C. Eileen Bond, J.D., Chairman

COMMISSION MEMBERS

General Public
C. Eileen Bond, J.D.
David Landrith, M.P.A., Co-chairman
David Jerman, M.B.A.

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

Scientific Community
Gary Krahenbuhl, Ed.D.
Manuel Modiano, M.D.
Walter H. Williams, Ph.D., M.D.

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

15 South 15th Street, Suite 103A
Phoenix, Arizona 85007
Telephone: 602.542.1028
Fax: 602.542.6380

January 2006

Message from the Chairman

Fiscal Year 2006 saw a tremendous increase in activities and public awareness of biomedical research. The Arizona Biomedical Research Commission continued to play a significant role in the advancement of the biosciences in Arizona.

The Commission awarded twenty-eight new scientific research contracts this year. There will be a total of seventy-four research projects under contract with the Commission beginning in FY 2007. The Annual Report contains abstracts of all the projects along with information on funding levels and institutional involvement. The abstracts demonstrate the wide breadth of inquiry being undertaken by Arizona investigators. Commission contract awards enabled many Arizona researchers to prove their investigative concepts and go on to obtain additional funding at the national level. The Commission through its statutory authority continues its technology transfer efforts.

Translational research projects addressing Parkinson's disease, Alzheimer's disease, cancer, bioengineering, and bioimaging have shown great progress and promise. Commission funding supports the streamlining of Institutional Review Board processes, harmonizing business process such as intellectual property agreements, and innovative approaches to research in Arizona's Hispanic and Native American communities.

The Commission has worked in close harmony with The Flinn Foundation sponsoring symposia, convening meetings, and advancing the promise of the Arizona Bioscience Roadmap. The Commission looks forward to playing a pivotal role in Arizona's efforts in becoming a key biomedical research leader and the translation of research findings to Arizona's citizens.

The Annual Report is prepared and submitted in January of each year to the Governor, the President of the Senate, and the Speaker of the House of Representatives. It is the hope of all the members of the Arizona Biomedical Research Commission that encouraging both new researchers and large scale multi-institutional/multi-disciplinary investigations will advance scientific discovery in the search for better health and lives 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.

C. Eileen Bond, J.D. Prescott

Private Practice
Specializing in Child Welfare Law

Commissioner Bond received her B.A. in History (Far Eastern Studies) and Master of Library Science from UCLA. She received her J.D. from Arizona State University in 1971. Commissioner Bond retired from the Arizona Attorney General's Office in 1996 and is in private practice in Prescott, Arizona, where she specializes in the area of child welfare law. Commissioner Bond was recently appointed as a Judge Pro Tem in Yavapai County. Commissioner Bond serves as a Disciplinary Hearing Officer for the Arizona State Bar Association and as a due process hearing officer for the Arizona Department of Education. Commissioner Bond was appointed by Governor Hull in May, 2000 and reappointed by Governor Napolitano in May, 2003. Her term expired in May 2006.

General Public

David Jerman. M.B.A. Phoenix

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 had extensive experience in the pharmaceutical industry and in technology transfer issues. The Arizona Alzheimer's Disease Institute is located within Banner Health. 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. His term expires in 2008.

David Landrith. M.P.A. Mesa

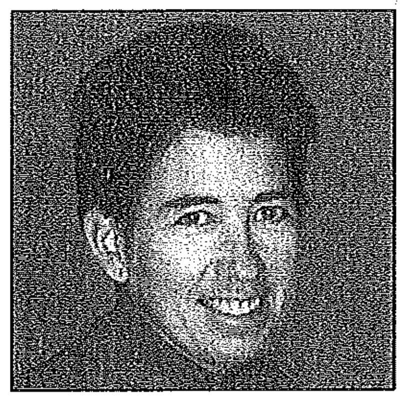
Vice President for Policy and Political Affairs
Arizona Medical Association

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

Medical Community

Colleen M. Brophy, M.D. — Tempe

Chief of Vascular Surgery
Carl T. Hayden VAMC



Commissioner Brophy received her undergraduate and medical degrees at the University of Utah. She completed her surgical residency at Yale University followed by a fellowship in vascular surgery at Harvard University. She is a Research Professor of Bioengineering at Arizona State University, a Clinical Professor of Surgery at the University of Arizona and the director of the Proteins and Peptides As Pharmaceuticals Center, Arizona Biodesign Institute at ASU. She is a founder and president of a biotechnology start-up company developing proteomic based therapeutics, Arizona Engineered Therapeutics. Dr. Brophy is an editor for the Journal of Surgical Research, sits on the Executive Committee of the Surgical Research Committee of the American College of Surgeons, Chairs the Committee on Women's Issues for the Society for Vascular Surgery, and is a member of the NIH Surgery and Bio-engineering Study Section. She was appointed in 2002 and 2006 by Governor Napolitano. Her term expires in May, 2009.

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

Director of Clinical Education
Medicis Pharmaceutical 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 Medicis, she served as a 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 Governor Napolitano in 2006. Her term expires in May, 2007.

Scientific Research Community

Gary S. Krahenbuhl, Ed.D. Tempe

Senior Vice President and Deputy Provost - Retired

Arizona State University

Commissioner Krahenbuhl received his B.S.Ed. and M.S.Ed. from Northern Illinois University. He received his Ed.D. from the University of Northern Colorado in 1969. He came to Arizona State University in 1973 and served as the Director of the Human Performance Laboratory, Associate Dean and Dean of the College of Liberal Arts and Sciences, Senior Vice President and Deputy Provost of the University, retiring from the University in 2003. Commissioner Krahenbuhl's major fields of interest include biogenic amines and acute stress and the physiology of distance running. He has published extensively on human stress response, the effects of catechololamine excretion, and the physiological effects of training regimes in adults and children. Commissioner Krahenbuhl was appointed to the Commission by Governor Napolitano in 2004. Dr. Krahenbuhl resigned from the Commission in May, 2006.

Manuel Modiano, M.D. Tucson

Arizona Oncology Associates

Commissioner Modiano obtained his Bachelor of Science degree from Colegio Collumbia in Mexico City. He received his M.D. from the Universidad Nacioinal 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 conducted community research. Dr. Modiano is director of research and president of Arizona Oncology Associates, medical director and chairman of the board of the Arizona Clinical Research Center, and president of the Arizona Clinical Oncology Society. He was appointed by Governor Napolitano in 2006. His term expires in May, 2008.

Scientific Research Community

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

Professor, Department of Nuclear Medicine and Radiology, University of Arizona

Commissioner Williams received his B.S. with majors in Chemistry and Physics from the University of Missouri in 1963, his Ph.D. in Physical Chemistry from Purdue University in 1968, and his M.D. from Yale University in 1980. Dr. Williams was a member of the Science Team for the Voyager Spacecraft missions to Jupiter and Saturn and was a Senior Scientist at the Jet Propulsion Laboratory, California Institute of Technology. He left to return to school to study medicine. From 1985 to1987, he was a clinical instructor in the Joint Program for Radiology and Nuclear Medicine at Harvard. He has authored numerous publications in the areas of physics and medicine. Commissioner Williams was appointed by Governor Symington in 1994 and reappointed in 1997. Commissioner Williams was reappointed by Governor Hull in May, 2000 and by Governor Napolitano in May, 2003. His term expired in May, 2006.

Summary of 2005 – 2006 Commission Activities

The Commission administered 64 contracts worth over $6 million with medical researchers in Arizona. The Commission continues its commitment to individual investigators as well as expanding into translational research. 28 new contracts and $2.2 million were directed toward assisting individual investigators in developing proof of their research concepts, collecting preliminary data, and in continued support of translational research.

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

Section headings in this report list each program and whether the project is in its first, second, or third year of funding. Research abstracts outlining the progress made during the year are contained in Sections A-D. Citations for scientific publications and abstracts arising out of the research are also listed.

Nearly 1,000 Requests for Proposals (RFPs) for 2006-2007 awards were mailed to potential applicants in September, 2005. The amount of funding available for new unrestricted medical research was approximately $2.2 million. In response to the RFP, the Commission received 113 unrestricted medical research proposals.

ABRC Projects Submitted/Accepted FY 2006

Institution	Submitted	Accepted	Percent Accepted	Amount in $'s	Percent of Total
Arizona State University	12	3	25	$ 250,000	12
Northern Arizona University	5	2	40	$ 88,606	4
University of Arizona	72	17	24	$ 1,446,321	64
Sun Health Research Institute	3	1	33	$ 50,000	2
Barrow's Neurological Institute	12	4	33	$ 299,973	13
Others	9	1	11	$ 116,279	5
Total	**113**	**28**	**25%**	**$ 2,245,179**	**100%**

In November and December the medical research proposals received were sent to a panel of national and international scientific and medical experts for peer review and evaluation. The Commission received the proposal evaluations prepared by more that 170 out-of-state peer reviewers. Three reviews were sought for each proposal. All proposals and evaluations were distributed to Commission members. In the spring a of 2006 the Commission selected 28 proposals for funding. During 2006-2007 the ABRC will be managing a total of 74 contracts.

ABRC Total New and Continuing Project Contracts 2006-2007

Institution	Award	% of Total Awarded	Amount in $'s	% of Total Amount
Arizona State University	9	13	$ 827,041	12
Northern Arizona University	3	4	$ 238,502	3
University of Arizona	43	58	$ 3,967,801	56
Sun Health Research Institute	5	7	$ 540,125	8
Barrow's Neurological/St. Joseph's	7	9	$ 649,244	9
TGen	2	3	$ 100,000	1
Mayo Clinic	2	3	$ 360,279	5
5AM Solutions	1	1	$ 100,000	1
Inter-Tribal Council	1	1	$ 150,000	2
Molecular Profiling Institute	1	1	$ 244,565	3
Total	**74**	**100**	**$ 7,177,557**	**100**

SECTION A

CONTINUING CONTRACTS

Medical Research

Year Three

FY 2006

Paul McDonagh, Ph.D.
University of Arizona
Award Amount FY06: $163,939

Mechanisms by Which Air Pollution Increases the Severity of Heart Attacks

In major metropolitan areas, such as Phoenix and Tucson, the mortality associated with cardiovascular disease, particularly acute ischemic heart disease (heart attacks) increase significantly on days with elevated airborne particulate matter (PM). Stated in another way, inhalation of fine fraction PM somehow causes an excess mortality by increasing the severity of injury associated with a heart attack. This research project aims to ***uncover the mechanisms by which inhalation of airborne particulate matter increases the severity of myocardial infarctions.*** We propose that inhalation of PM induces a multi-stepped scenario that ultimately causes a systemic inflammatory response which, in turn, enhances the severity of heart attacks. In Year 3, we compared the abilities of PM from the combustion of coal to PM from the combustion of diesel fuel to induce a systemic inflammatory response . We believe that PM from diesel fuel is a particularly relevant source of PM for the people of Arizona. In the present study we hypothesized that 18 hours following intratracheal instillation of PM, a pulmonary inflammatory response occurs which then immediately induces a systemic inflammatory response by activating circulating polymorphonuclear leukocytes (PMNs). C57BL/6J mice were instilled with either saline (control), 200ug of fine coal PM, 200ug of diesel exhaust PM, 400ug of diesel exhaust PM or lipopolysaccharide (positive control), and measurements were taken to assess pulmonary and systemic inflammation. Pulmonary inflammation was measured by histological analysis bronchoalveolar lavage cell differential counts and by tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocytechemoattractant protein-1 (MCP-1) concentrations within the bronchoalveolar lavage fluid and pulmonary homogenate. Systemic inflammation was measured by differential white blood cell counts and CD11b expression on activated PMNs in whole blood. The number of PMNs in pulmonary histology and bronchoalveolar lavage fluid, which is indicative of pulmonary inflammation, was greatest in the 400ug diesel dose compared to the 200ug diesel and 200ug fine coal PM doses. Circulating PMNs and CD11b expression were significantly increased in whole blood for both 200ug and 400ug diesel PM treatments compared to control. The consistent trend in the data suggests that the 400ug diesel PM dose elicits a greater pulmonary and systemic inflammatory response compared to the other treatments and, therefore, may be a useful murine model of acute pulmonary and systemic inflammation induced by PM instillation. These results may be particularly relevant to the people of Arizona who are commonly exposed to diesel exhaust.

Andrej A. Romanovsky, Ph.D.

St. Joseph's Hospital
Award Amount FY06: $166,166

Vagal Anti-Inflammatory System

We hypothesized that stimulation of nicotine receptors is protective in systemic inflammation. Mice were made nicotine-dependent (modeling such a dependence in smokers), subjected to non-microbial systemic inflammation (by administration of bacterial lipopolysaccharide, LPS) or microbial sepsis (by intestinal puncture), and then either received no nicotine (mimicking what happens to ICU patients) or continued receiving nicotine. The outcome of non-microbial systemic inflammation was improved by nicotine stimulation, whereas the outcome of experimental sepsis was slightly worsened. These findings suggest that nicotinic stimulation limits both harmful, bad, effects and the anti-microbial, good, effect of systemic inflammation. An important application of these findings is that an abrupt cessation of smoking in patients with aseptic systemic inflammation (e.g., after massive blunt trauma) may be harmful. To test this hypothesis, St. Joseph's Hospital has initiated a retrospective clinical study. During the past year we also continued characterizing the nicotinic system of the gastrointestinal tract and the thermoregulatory responses to systemic inflammation.

Publications:

Almeida MC, Steiner AA, Branco LGS, Romanovsky AA. What Is the "Natural" Thermoregulatory Response of Rats to Bacterial Lipopolysaccharide, Fever or Hypothermia? Eur J Neurosci. 23:3359-67, 2006.

Almeida MC, Steiner AA, Branco LGS, Romanovsky AA. Neural Substrate of Cold-Seeking Behavior in Endotoxin Shock. PLoS ONE. 1: 2006.

Romanovsky AA. Thermoregulatory System. Am J Physiol Regul Inter Comp Physiol. 2007 (in press).

Steiner AA, Romanovsky AA. Leptin: At the Crossroads of Energy Balance and Sytemic Inflammation. Prog Lipid Res. 2007 (in press).

Indraneel Ghosh, Ph.D.

University of Arizona
Award Amount FY06: $49,500

Inhibiting Protein-Protein Interactions Involved in Cancer

The American Cancer Society estimates that in the year 2006, there will be 1,399,790 new cancer cases diagnosed in the United States, of which at least 25,450 will be in Arizona. The prevention of cancer is the long-term goal of our proposal. The unregulated growth of new cells is the hallmark of cancer. The growth of new cells is controlled at the level of proteins that interact with one another. However, the development of new and potent cancer therapeutics that specifically target protein-protein interactions is still in its infancy. In this project we outlined a general strategy for developing leads for potent cancer therapeutics utilizing novel technological advances that target the disruption of protein-protein interactions. We believe that our technological platform for drug design is simple and powerful and will not only aid in designing cancer therapeutics but also impact other human diseases such as Alzheimer's, stroke, and AIDS.

Publications:

Rajagopal S, Meyer SC, Goldman A, Zhou M, Ghosh I. A Minimalist Approach for Protein Recognition by Epitope Transfer from Functionally Evolved Beta-Sheet Surfaces. J Am Chem Soc. 128, 14356-63, 2006.

Smith TJ, Stains CA, Meyer S, Ghosh I. Inhibition of ß-Sheet Presenting Miniature Protein. J Am Chem Soc. Published on-line October 2006.

Haiyong Han, Ph.D.

Translational Genomics Research Institute
Award Amount FY06: $49,822

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

The VHL gene is a tumor suppressor gene that has been found to be inactivated in over 75percent of all Renal Cell Carcinoma (RCC) cases. We previously reported the establishment of a screening approach for identifying new agents that specifically kill RCC cells with deficient VHL. We generated a pair of isogenic cell lines and used them to screen chemical libraries to identify lead compounds. During the past year, we continued to screen library compounds and identified 3 additional leads that showed >2-fold selectively against the RCC cell line with inactivated VHL gene, compared to the cell line with intact VHL. The IC50s of the compounds in the VHL deficient cell line range from low nanomolar to low micromolar. The selectively of the compounds was further confirmed in natural RCC cell lines. Further development of these leads can potentially lead to new therapies for the treatment of patients with RCC.

Publications:

Han H, Zhao Y, Mosley AE, Von Hoff DD. Identification of Anti-Renal Cell Carcinoma Agents Using Pharmacological Synthetic Lethal Screening. Proc Amer Assoc Cancer Res. 47:4699. 2006.

Nancy Horton, Ph.D.

University of Arizona
Award Amount FY06: $49,005

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

Repair of DNA damage is critical in order to avoid changes in the genetic material that could result in cellular malfunction, cell death, and even the formation of cancer. We sought to determine a high resolution crystal structure of the initial DNA damage recognition factor, XPC. Such a structure could be used as the basis for drug design, which would be useful in treating cancers which use the over-expression of XPC to escape the effects of chemotherapeutics. Since proteins crystallize more readily when truncated to smaller, but still functional, domains, and crystallization requires large quantities of the purified protein, recombinant methods using the bacteria *E. coli* are preferred. During the funding period, we have prepared eight different expression clones in *E. coli* and have purified large quantities of a small version of XPC from both human and yeast cells. We found both to be functional and have begun to screen conditions for crystallization.

Natalia A. Ignatenko, Ph.D. University of Arizona
Award Amount FY06: $55,000

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

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

Animal models have provided valuable systems for studying tumor initiation and progression target tissues of carcinogenic agents and preclinical evaluation of potential chemopreventive and therapeutic drugs. In this study we used C57BL/6J-*Apc* min/J mouse strain (Apc min/+) which has the APC gene mutation similarly to the FAP patients.

The non-steroidal anti-inflammatory drug (NSAID) sulindac is a potent inhibitor of intestinal carcinogenesis in the Apc Min/+ mouse model and is used to treat humans with FAP. We evaluated the effect of sulindac on the polyamine pathway and the role of dietary polyamines in colon tumorigenesis. We found that dietary putrescine reduces the ability of sulindac to suppress intestinal tumorigenesis in the mouse model. Dietary pitrescine significantly increased the grade of intestinal adenoma dysplasia in Apc Min/+ mice. These data suggest that reducing polyamine metabolism and dietary polyamine levels may enhance strategies for colon cancer chemoprevention.

To study the role of c-Myc in the mutant Apc-medicated colon tumorigenesis, we developed a transgenic mouse with the mosaic loss of c-MYC expression in the intestinal tract. These mice developed less tumors than control mice. This observation validates the inhibition of c-MYC as a possible therapeutic strategy for suppression of colorectal cancer.

Publications:

Ignatenko NA, Besselsen DG, Basuroy U, Stringer DE, Blohm-Mangone KA, Padilla-Tores JL, Cui H, Gerner EW. Dietary Putrescine Reduces the Anti-Carcinogenic Intestinal Activity of Sulindac in a Murine Model of Familial Adenomatous Polyposis. Nutrition and Cancer. 5:2 (in press).

Emmanuel Katsanis, M.D. | University of Arizona
Award Amount FY06: $108,367

Chaperone Rich Cell Lysate (CRCL) Vaccine for Ovarian Cancer

Purpose: Tumor-derived chaperone rich cell lysate (CRCL) has successfully been used to generate tumor specific T-cell responses and protective immunity against a wide range of mouse tumors. We have investigated the potency of human ovarian cancer-derived CRCL to activate dendritic cells (DCs) and to generate tumor specific T-cells.

Experimental Design: CRCL was generated from primary ovarian cancers and a human ovarian tumor cell line. Peripheral blood mononuclear cells from healthy donors and ovarian cancer patients were stimulated weekly with autologous DC's loaded with ovarian tumor derived CRCL.

Results and Conclusion: CRCL is effective in stimulating T-cell responses against ovarian cancer. The results of our study further substantiate the concept that CRCL may prove to be a potent adjuvant for women suffering from ovarian cancer and that this personalized vaccine may be a promising approach for active immunotherapy against ovarian cancer.

George Pettit, Ph.D. | Arizona State University
Award Amount FY06: $166,250

Anticancer Drug Preclinical Development

This year, nearly 600,000 people in the United States will die of cancer. The tragic death toll, in the US and internationally, will not be reduced until more generally effective and curative anti-cancer drugs are discovered and developed. These continuing death rates for the overall 200+ types of human cancer make it clear that an acceleration in the discovery and development of new anti-cancer drugs is vital and urgent. Three years of ABRC funding have focused on that objective, making progress to the benefit of future cancer victims in Arizona and elsewhere. We have progressed in developing synthetic methods to increase the preclinical supply of four anticancer drug candidates previously discovered based on leads we uncovered among terrestrial plants and marine organisms namely, phenpanstatin, pancratistatin 3,4-0-cyclic phosphate prodrug, iodocomstatin phosphate prosphate prodrug, and one of the auristatins. The current increase in preclinical supplies will now allow further preclinical development.

Publications:

Pettit GR, Eastham A, Melody N, Orr B, Herald DL, McGregor J, Knight JC, Doubek DL, Pettit GR, Garner LC, Bell JA. Isolation and Structural Modification of 7-Deoxynarciclasine and 7-Deoxy-*trans*-Dihydronarciclasine. Nat Prod. 69:7-13. 2006.

Snyder SD, Cooper PA, Millinton NJ, Gill JH, Pettit GR, Bibby MC. Investigation of the Efficacy and Mechanism of Action of Pancratistatin 3,4-cyclic Phosphate. Clin Cancer Res. 11:8971S. 2005.

Pettit GR, Minardi MD, Rosenberg HJ, Hamel E, Bibby MC, Martin SW, Jung MK, Pettit RK, Cuthberson TJ, Chapuis JC. Antieoplastic Agents 509. Synthesis of Sodium Fluorcombstatin Phosphate and Related 3-Halo-Stilbenes. J Nat Prod. 68:1450-58. 2005.

Pettit RK, Pon S, Cichacz ZA, Herald CL, Pettit GR, Woyke T. *In Vitro* and *In Vivo* Antifungal Activities of the Marine Sponge Constituent Spongistatin 1. Medical Mycology. 43:453-63. 2005.

Ali MA, Bates RB, Crane ZD, Dicus CW, Gramme MR, Hamel E, Marcischak J, Martinez DS, McClure KJ, Makkiew P, Pettit GR, Stessman CC, Sufi BA, Yarick GV. Dolastatin 11 Conformations, Analogues and Pharmacophore. Bioorg Med Chem. 13:4138-52. 2005.

Muller IM, Dirsch VM, Rudy A, Lopez-Anton N, Pettit GR, Vollmar AM. Cephalostatin 1 Inactivates Bcl-2 by Hyperphosphorylation Independent of M-phase Arrest and DNA Damage. Mol Pharmacol. 67:1684-89. 2005.

Pettit GR, Zhang Q, Pinilla V, Hoffman H, Knight JC, Doubek DL, Chapuis JC, Pettit RK, Schmidt JM. Antineoplastic Agents 534. Isolation and Structure of Sansevistatins 1 and 2 from the African *sansevieria ehrenbergii*. J Nat Prod. 68: 729-33. 2005.

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

Pettit GR, Melody DL. Antineoplastic Agents 527. Synthesis of 7-Deoxynarcistatin 7-Deoxy-*trans*-dihydronarcistatin, and *Trans*-dihydronarcistatin 1. J Nat Prod. 68:207-11. 2005.

Rinner U, Hudlicky T, Gordon H, Pettit GR. A beta-Carboline 1-one Mimic of the Anticancer Amaryllidaceae Constituent Pancratistatin. Synthesis and Biological Evaluation. Angew Chem. 116:5456-60. 2004.

Pettit, GR, Xu JP, Doubek DL, Chapuis JC, Schmidt JM. Antineoplastic Agents 529. Isolation and Structure of Nootkastatins 1 and 2 from the Alaskan Yellow Cedar *Chamaecyparis nootkatensis*. J Nat Prod. 67:1476-82. 2004.

Pettit GR, Xu DL, Chapuis JC, Schmidt JM. Antineoplastic Agents 510. Isolation and Structure of Dolastatin 19 from the Gulf of California Sea Hare *Dolabella auricularia*. J Nat Prod. 67:1252-55. 2004.

Pettit GR, Zhang Q, Pinilla V, Herald DL, Doubek DL, Duke JA. Isolation and Structure of Gustastatin from the Brazilian Nut Tree *Gustavia hexapetala*. J Nat Prod. 67:983-85. 2004.

Pettit GR, Melody N, Herald DL. Antineoplastic Agents 511. Direct Phosphorylation of Phenpanstatin and Pancratistatin. J Nat Prod. 67:322-27. 2004.

Pettit GR, Meng Y, Gearing P, Herald DL, Pettit RK, Doubek DL, Chapuis JC, Tackett LP. Antineoplastic Agents 522. *Hernandia peltata* (Malaysia) and *Hernandia nymphaeifolia* (Republic of Maldives). J Nat Prod. 67:214-20. 2004.

Pettit GR, Xu JP, Chapuis JC, Pettit RK, Tackett LP, Doubek DL, Hooper JNA, Schmidt JM. Antineoplastic Agents 520. Isolation and Structure of Irciniastatins A and B from the Indo-Pacific Marine Sponge *Ircinia ramosa*. J Med Chem. 47:1149-52. 2004.

Donato F. Romagnolo, Ph.D.

University of Arizona
Award Amount FY06: $49,434

Tobacco Metabolites Induce Negative Regulation of BRCA-1
Through a p53-Dependent Mechanism

Interventions between dietary xenobiotics and nutrients influence cancer risk by modulating overlapping biochemical pathways leading to repression of functions controlled by tumor suppressor genes, activation of tumor promoters, or both. Epidemiologic studies in humans suggest that tobacco smoke and diet are important vehicles of exposure to various xenobiotics, including polycyclic aromatic hydrocarbons (PAHs) and dioxins. The activation of the aromatic hydrocarbon receptor (AhR) pathway by PAHs and dioxins stimulates the expression of several genes including cytochrome P450s, which metabolize PAHs to highly mutagenic compounds that cause fixation of mutations in the p53 gene and repress the expression of the tumor suppressor gene-1 (BRCA-1). We report that 1) estrogen activation of BRCA-1 expression is mediated by a transcription complex that comprises the estrogen receptor-alpha and the cofactor p300. [This complex is recruited to an AP-1 element.]; 2) the unliganded AhR acts as a cofactor for the estrogen receptor alpha; and 3) activation of the AhR by dioxins leads to repression of estrogen-induced BRCA-1 activation. This repression leads to the recruitment of histone deacetylases to the BRCA-1 promoter.

Publications:

Jeffy BD, Hockings JK, Kemp MQ, Morgan SS, Hager JA, Beliakoff J, Whitesell LJ, Bowden GT, Romagnolo DF. An Estrogen Receptor-Alpha/p300 Complex Activates the BRCA-1 Promoter at an AO-1 Site that Binds Jun/Fos Transcription Factors: Repressive Effects of p53 on BRCA-1 Transcription. Neoplasia. 7(9):873-82. 2005.

Hockings JK, Thorne PA, Kemp MQ, Morgan SS, Selmin O, Romagnolo DF. The Ligand Status of the Aromatic Hydrocarbon Receptor Modulates Transcriptional Activation of BRCA-1 Promoter by Estrogen. Cancer Research. 66:2224-32. 2006.

Romagnolo DF, Degner SC, Kemp MQ, Hockings JK, Selmin O. Role of Dietary Xenobiotic-Gene Interactions in Carcinogenesis: Protective Effects of Nutritional Factors. Curr Nutr Rev & Food Sci. 2:205-14. 2006.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY06: $50,000

Eluding Drug Resistance in Cancer Chemotherapy

Drug resistance makes cancer cells immune to treatment with drugs that initially retard tumor growth. Antitumor drugs typically act by binding to and blocking the functional region of biomolecules required for cell growth. In cells, drugs readily interconvert between bound and unbound states. Resistant cancer cells employ pumps to expel the drug from the cell when the drug is unbound. Covalent bonding of the drug to its target biomolecule can prevent expulsion. We have designed, prepared and/or tested two dozen new compounds comprising five major groups of chemically reactive moieties that can covalently bond to their target. The compounds were tested for effectiveness on biomolecules and were screened at the National Cancer Institute for cancer cell growth inhibition in culture, and their mechanism of action was also studied. Many were found to inhibit cancer cell growth and/or to initiate cancer cell self-destruction, which might lead to new cancer treatments.

Nafees Ahmad, Ph.D.

University of Arizona

Award Amount FY06: $50,000

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

Infants infected with HIV-1 have a more rapid and fatal HIV disease course than infected adults. Our previous study has demonstrated that a minor genotype of HIV-1 with R5 phenotype from infected mothers is transmitted to their infants and initially maintained in the infants with the same properties. Several other regions of HIV-1 may be important in targeting strategies for prevention and treatment. One such critical region is the Rev Responsive Element (RRE) that interacts with HIV-1 Rev protein to export structural proteins mRNAs from nucleus to cytoplasm, an absolute requirement for the viral life cycle. However, generation of mutations in RRE during HIV-1 infection that may alter Rev-RRE interactions and affect viral replication has not been analyzed. In this study, we have characterized the primary sequences of RRE, including *in vivo* genetic variation and functional motifs required for Rev-RRE interactions as well as evaluated the RNA secondary structures of RRE derived from five mother-infant pairs involved in vertical transmission. Multiple (157) RRE sequences derived from five HIV-1 infected mother infant pairs showed that primary nucleotide sequences of the designated stems and loops of RRE were highly conserved with a low degree of viral heterogeneity and estimates of genetic diversity following vertical transmission. We also analyzed the effect of *in vivo* sequence variation on primary RRE sequences and secondary RRE structure. The RRE sequences from mothers and infants folded into stem-loop structures as predicted for RRE secondary structure and retained all the essential stem-loop formation required for Rev-RRE interactions. In addition, a primary 9- nucleotide (5' -CACTATGGG - 3') RRE sequence in the stem-loop B that is required for optimal Rev recognition and must be presented as a stem-bulge-stem structure was highly conserved in most of the sequences. The domain required for RRE-host protein interactions were also conserved in most of the RRE sequences. These data demonstrate that the primary RRE sequences in the context of secondary structures were maintained and the functional domains required for Rev-RRE interactions conserved in mother-infant pairs following vertical transmission and are consistent with a crucial role of this cis-acting RNA element in HIV-1 pathogenesis.

Publications:

Ramakrishnan R, Ahmad N. Derivation of Primary Sequences and Secondary Structures of Rev Responsive Element from HIV-1 Infected Mothers and Infants Following Vertical Transmission. Virology. (in press).

Dominick DeLuca, Ph.D. University of Arizona
Award Amount FY06: $70,000

Nicotine on T-Cell Development

Over 20 percent of pregnant mothers enrolled in Arizona's pregnancy programs continue to smoke throughout their pregnancy. The central hypothesis of this project is that tobacco use and nicotine exposure affect immune system development. To test this idea, we have tested cultures of mouse thymus tissue colonized with human T-cell precursors. We have found that low doses of nicotine inhibit the growth of T-cells, and that as one adds more nicotine, the production of T- cells increases. However, as the dose of nicotine increases further, the production of T-cells goes down again. Also, the production of T-cells is greater if the nicotine is given early in the culture period (2d) rather than late (12d). We are interested in exploring the concept of adding smoking material even later in T-cell development (25, 30, and 35 days) to see if nicotine effects suppress the growth of T-cells at those times as well.

John J. Marchalonis, Ph.D. University of Arizona
Award Amount FY06: $166,250

Immunomodulatory Autoantibodies to T-Cell Receptor in Rheumatoid Arthritis

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are classic autoimmune diseases associated with elevated levels of autoantibodies. Both are prevalent in the state of Arizona and RA has an abnormally high incidence of approximately 5 percent of arthritis cases reported in the Tucson area. RA patients have significantly elevated levels of autoantibodies directed against recognition molecules (T-cell receptor or TCR) of their own thymus-derived lymphocytes (T-cells). Using blood from RA and SLE patients, we have generated monoclonal autoantibodies (mAAb) that bind TCR and have shown that these are novel recognition molecules that have the potential to modulate the T-cell arm of the immune system. Surprisingly, administration of appropriate TCR variable region peptides to immunosuppressed mice enhanced the production of TH1 cytokines and restored levels of T-cell immunity. Our studies disclosed an unexpected immunoregulatory mechanism that involves a stimulatory for TCR-derived variable region peptides and a regulatory function for spontaneously arising autoantibodies.

Publications:

Adelman MK, Yocum DE, Marchalonis JJ. Endogenous Retrovirus as Etiological Agents in Systemic Lupus Erythematosis. **Infection and Autoimmunity.** Schoenfeld and Rose eds. Elservier Press, Amsterdam. 271-88.

Adelman MK, Schluter SF, Marchalonis JJ. The Natural Antibody Repertoire of Sharks and Humans Recognizes the Potential Universe of Antigens. **The Protein Journal.** 23:103-18

Sepulveda RT, Marchalonis JJ, Watson RR. T-Cell Receptor V Beta 8.1 Peptide Reduces Coxsackievirus-Induced Cardiopathology During Murine Acquired Immunodeficiency Syndrome. Journal of Cardiovascular Pharmacology.41:489-97.

Schluter SF, Adelman MK, Taneja V, Yocum DE, Marchalonis, JJ. Natural Autoantibodies to TCR Public Idiotopes: Potential Roles in Immunomodulation. Cellular & Molecular Biology. 49:193-207.

Paul F. Torrence, Ph.D.

Northern Arizona University
Award Amount FY06: $49,940

Nucleic Acid Therapeutics for West Nile Virus Infections

This research was carried out to try to improve upon novel compounds recently discovered in our laboratories at NAU. These discoveries involved compounds active against viruses of the smallpox family, namely vaccinia virus and cowpox viruses. Smallpox has re-emerged as the number one most potentially devastating bioterrorist treat, and new drugs are needed to treat this horrible disease. The above viruses are closely relayed to smallpox and are used as surrogate viruses, since the actual smallpox virus itself is carefully secured at the CDC in Atlanta and experiments with it are both difficult and dangerous. We have executed chemical changes to the structures of these so called lead compounds. All new products were screened against the smallpox-related viruses as well as additional virus of public health interest. Unfortunately, none of these new entities were sufficiently active against any of the target viruses. This disappointment is tempered by what we learned about the relationships between structure and antiviral activity. This latter information can be used to improve the design of future possible beneficial changes to improve activity.

Publications:

Fan X, Zhang X, Bories C, Loiseau PM, Torrence PF. The Ugi Reaction in the Generation of New Nuleosides as Potential Antiviral and Antileishmanial Agents. Bioorg. Chem. (in press).

Larry David Sparks, Ph.D.

Sun Health Research Institute
Award Amount FY06: $164,667

Water Quality and Cholesterol - Induced Pathology

Increased circulating cholesterol levels are associated with serious medical concerns and represent a mounting problem as elevated cholesterol becomes more widespread among the world population. Besides an obvious increase in coronary artery disease and stroke, emerging data suggest an increased risk of cognitive impairment with increased circulating cholesterol. Furthermore, excess cholesterol promotes production of the Alzheimer's disease (AD) neurotoxin, amyloid beta (Aß).

We identified a further link between copper, cholesterol and cognition in the cholesterol-fed rabbit model of AD. It was shown that dietary cholesterol induces central production of Aß and that copper determines if overproduced Aß is cleared to the blood or accumulates in brain. Levels of copper ion were manipulated by adding trace amounts of the metal to an animal's distilled drinking water. Memory deficits (80 percent) were associated with brain Aß deposits in cholesterol-fed animals administered 0.12PPM copper water, compared to cholesterol-fed animals drinking unaltered distilled water where there was minimal accumulation of Aß.

Cholesterol and copper may independently influence copper levels in blood and brain. Adding copper to the water of animals fed normal chow increases blood and brain copper levels compared to animals fed normal chow and drinking distilled water. Copper levels are increased in the blood and unchanged in the brain of cholesterol-fed animals drinking distilled water, while levels are decreased in brain and increased in the blood of cholesterol-fed animals drinking copper supplemented water. Copper may inactivate Aß, suggesting low brain copper could enhance Aß induced degeneration in AD.

We have previously reported elevated circulating copper and cholesterol levels in low-function controls (LFC), MCI and AD compared to high-function controls (HFC). We evaluated brain copper, zinc and iron levels in HFC, LFC, MCI and AD, and Parkinson's Disease (PD) patients with and without dementia.

SECTION B

CONTINUING CONTRACTS

Medical Research

Year Two

FY 2006

Dianne Lorton, Ph.D.
Sun Health Research Institute
Award Amount FY05: $50,000

Sodium Narcistatin in Treatment of Rheumatoid Arthritis

Narciciasine, an isocarbostyril isolated from the bulbs of the tropical spider lilly *hymenocallis littoralis* (Amaryllidaceae), is sparingly soluble in most organic and aqueous solvents, which limits its therapeutic potential. Sodium narcistatin (SNS), which is a water-soluble bioavailable phosphorylated prodrug of narciclasine, was tested using the adjuvant-induced arthritis (AA) rat model of rheumatoid arthritis (RA), to determine if SNS could reduce joint inflammation and destruction in RA. Treatment with SNS significantly decreased the dorsoplantar footpad soft tissue inflammation (approximately 70 percent) compared to saline-treated arthritic rats. Further, radiographic analysis showed SNS treatment reduced joint destruction by 50 percent compared to the saline-treated arthritic rats. The SNS-mediated anti-inflammatory and joint-sparing activity appears to parallel its ability to inhibit cellular proliferation. SNS is well tolerated and demonstrated little toxicity. These data suggest that the anti-proliferative properties of SNS may prevent expansion of precursor immune cells known to play a significant role in disease pathology.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY05: $164,500

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

The overall goal of this inter-institutional and multi-investigator collaborative project is to discover novel anticancer, anti-HIV and anti-infective drugs from endophytic fungi, fungi that live in the intercellular spaces of desert plants. During the past two years over 200 endophytic fungi have been cultured, their extracts prepared, and screened for their potential anti-cancer, anti-infective, and anti-HIV activities. Anticancer activity was elevated in NCI-H460 (non-small cell lung) and PC-3M (prostate) cancer cell lines, and target-oriented *in vitro* bioassays for activation of heat shock response and for the inhibition of the migration of metastatic cancer cell line, PC-3M. Bioactivity-guided fractionation of an extract active in cell migration inhibition assay yielded a small cyclic peptide identified as beauvericin. Prior to testing of fungal extracts for anti-HIV activity in virus infected T-cells, they were evaluated in A30.1 lymphocyte cell line for their toxicity towards this cell line. Out of the extracts tested in this primary assay, 100 were selected for evaluation in anti-HIV assay, and of these 20 were found to have some degree of antiviral activity. Investigation of one such extract resulted in the isolation of funalenone with moderate anti-HIV activity. Of the 248 extracts tested in anti-microbial assay, 43 showed inhibition of growth of at least one of the six pathogenic microorganisms used. If extracts active in above assays contain compounds that can inhibit the growth of solid tumors such as lung, breast, colon and prostate cancers, and/or are capable of inhibiting the human immune deficiency virus (HIV) and pathogenic microorganisms, our results will have an impact on the health of Arizona's population.

Publications:

Gunatilaka LAA. Natural Products from Plant Associated Microorganism: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. J of Nat Prod. 69:509-26. 2006.

Zhan J, Gunaherath GMKB, Kithsiri-Wijeratne EM, Gunatilaka LAA. Asperpyfone D and Other Metabolites of the Plant Associated Fungal Strain *Aspergillus tubingensis*. Phytochemistry. 2006 (in press).

Laurence Hurley, Ph.D.

University of Arizona
Award Amount FY05: $49,746

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

We have identified a signaling pathway that is important in the survival of pancreatic cancer cells. In this pathway the key molecular switch involves an unusual DNA structure and an enzyme that remodels the DNA to activate this signaling pathway. During the previous grant period we have shown that the G-quadruplex-interactive drugs that bind to the promoter PDGF-A can modulate gene expression. We have also shown the importance of binding of the SHS G-strand to the looped-out C-rich strand of the promoter.

Tamara King, Ph.D.

University of Arizona
Award Amount FY05: $164,500

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

This year we examined bone changes following injection of osteoblastic/osteolytic prostate cancer cells into the mouse femur. This will allow us to test potential non-sedative analgesic drugs in two different forms of bone cancer that may produce pain that is differentially responsive to analgesic drugs. We also demonstrate that PC3N prostate cancer cells express message for several nicotinic acetylcholinergic receptor subunits. This is an important step in characterizing tumor responses to nicotine, a potential non-sedative analgesic drug to be tested as one of the research goals of this project. The experiments from this past year, in addition to ongoing studies, will determine whether non-sedative analgesics can alleviate bone cancer induced pain as effectively as the currently used opioid analgesics. Moreover, the ongoing studies examining bone cancer induced neuroplastic changes may reveal novel targets for the developments of alternative analgesic drugs with fewer side-effects than currently available drugs.

Emmanuelle Meuillet, Ph.D.

University of Arizona
Award Amount FY05: $164,500

Novel Inhibitors of Akt as Anticancer Drugs

Cancer cells are able to survive under adverse conditions where normal cells will die through a process of apoptosis. This feature allows cancer cells to thrive where normal cells cannot and also makes cancer cells resistant to cell killing by chemotherapy. Inhibiting the signaling pathways that promote cancer cell survival offers a rational and attractive way of selectively inhibiting cancer growth. The protein Akt is a key player in this cell survival signaling pathways in cancer cells. It therefore is an attractive target for the development of drugs to promote death specifically in cancer cells. We have adopted a novel and unique approach to interfering with the signaling by Akt. Our team has designed, produced, and tested novel inhibitors of the function of this protein. These new compounds will be developed further to improve their efficacy as anti-cancer agents.

Joyce A. Schroeder, Ph.D.

University of Arizona
Award Amount FY05: $49,718

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

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

Steven P. Stratton, Ph.D.

University of Arizona
Award Amount FY05: $164,366

Phase II Trial of Topical Perillyl Alcohol in Sun Damaged Skin

Skin cancer is by far the most common cancer (with more than 1.3 million new cases expected in 2003 in the U.S.) and is a tremendous public health problem, especially in Arizona and the southwestern United States where sun exposure is high. As reported by the Southeastern Arizona Skin Cancer Registry, rates of non-melanoma skin cancer in Arizona are among the highest in the world and are 4-6 fold higher than in the general US population. Incidence rates for melanoma, the most deadly form of skin cancer, are rising faster than almost any other cancer. Topically administered chemopreventive drugs that actually stop or reverse the growth of precancerous lesions in the skin may reduce this burden. Perillyl alcohol is a molecule found in the essential oils of lavendin, peppermint, spearmint, cherries, celery seeds, and other edible plants. We have shown that pure perillyl alcohol effectively reduces the incidence of skin tumors when applied topically to the skin in preclinical models of both melanoma and non-melanoma skin cancers. We have recently performed a Phase I clinical trial of a cream formulation of topical perillyl alcohol developed by our group. Results of this study indicate that this formulation is safe when applied twice daily for 30 days. Further clinical testing is now warranted to determine if perillyl alcohol applied directly to sun-damaged skin can reverse such damage. The objective of this research is to perform a randomized, placebo-controlled, double-bind, Phase 2a dose-finding clinical trial of topical perillyl alcohol in subjects with moderately to severely sun-damaged skin. The hypothesis being tested is that topical perillyl alcohol, when applied twice daily for three months, can successfully reverse sun damage in skin in a dose-dependent manner as evidence by histopathologic normalization. As secondary endpoints, we will also determine if topical perillyl alcohol can significantly alter previously-studied surrogate endpoint biomarkers of neoplastic changes, including optical coherence tomography (OCT) of skin, as well as p53 expression, c-Fos expression, and apoptosis (as measured by expression of activated caspase-3) in skin biopsy tissue. In addition, karyometric analysis of nuclear chromatin patterns in skin biopsy tissue will be measured. Establishment of valid biomarkers is vital for demonstrating the activity of this and other drugs in future studies. Safety, tolerance, absorption, and formulation stability will also be monitored. Ancillary studies dependent on performance of this trial (but funded from other sources) will include comparative genomic hybridization analysis of skin biopsy samples for gene copy number changes (in collaboration with the Translational Genomics Research Institute [TGEN] in Phoenix, Arizona) and nutritional correlates and dietary assessment. The protocol has been approved by the University of Arizona Institutional Review Board (IRB) and successful recruitment of eligible subjects to this study is ongoing. Accrual will be complete in early 2007.

Robert P. Erickson, M.D.

University of Arizona
Award Amount FY05: $88,769

Identification of Genes Involved in Lymphedema by Single Nucleotide Polymorphism Mapping

Genetically caused lymphedema (swelling of the limbs) is a rare disorder compared to post-breast cancer lymphedema of the arms. By studying these rare disorders, one hopes to find information that will lead to prevention and cure of more common varieties of lymphedema. Our second year goal was to refine the chromosomal region on chromosome 18, which was previously implicated in our genetic studies of lymphedema among Arizona families. We also planned to sequence more candidate genes in regions identified from refined mapping. During this work period, we have completed sequencing of four genes in the identified region of chromosome 2 in family 5. The candidate gene that we had previously found did not segregate with lymphedema in this family. We have found one other presumably recessive mutation which also does not segregate with lymphedema. We have further refined regions on chromosomes 11,18 and 3, studied in Arizona families.

Douglas Lake, Ph.D.

University of Arizona

Award Amount FY05: $39,600

Dendritic Cells and Immunity of Valley Fever

We are studying the initial interactions of the immune system with the fungus that causes Valley Fever. Dendritic cells are sentinel cells of the immune system and are the first to encounter the fungus. In year 2 of the funding period we investigated the interactions of dendritic cells (DC) with killed spherules. We will work with live spherules next, but since cocci is on the CDC select agent list for bioterrorism, working with live spherules pose some logistical barriers. We showed that whole spherules stimulate peripheral blood mononuclear cells (PBMC) from immune donors, similar to our previous results with T27K. We also showed that immature DC phagocytosed multiple spherules in a time and temperature dependent manner. Binding of spherules to DC was blocked by mannan, suggesting involvement of the mannan receptor. Binding of spherules to DC led to internalization of multiple spherules and subsequent maturation and processing of spherules followed by presentation to immune and non-immune donors, as demonstrated by strong proliferative responses by T-cells and to a lesser degree in non-immune donors.

Eric J. Guilbeau, Ph.D.

Arizona State University
Award Amount FY05: $50,000

Biosensor for Measurement of Breath Acetone

This research is aimed at developing a novel, inexpensive and easy to use sensor that can be employed by individuals with diabetes to monitor their breath acetone concentration. The sensor works by measuring the heat that is generated when acetone reacts with another chemical on the surface of a very sensitive temperature measuring device called a thermopile. The research is significant because a large number of individuals in the U.S. suffer from type 2 diabetes. Individuals with type 2 diabetes are susceptible to a condition called diabetic ketoacidosis (DKA). Under this condition high amounts of acetone are released into the blood stream and unless corrected, the individual may die. During the past year, we continued to successfully fabricate a number of sensor prototypes and used these prototypes to measure acetone concentrations comparable to those in the breath of individuals with diabetes. Refined mathematical models were used to confirm the experimental sensor response and the theoretical basis for the sensor's operation. Studies are now being initiated to identify whether or not other substances in the breath might interfere with the sensor's ability to measure acetone.

Marek Jan Romanowski, Ph.D.

University of Arizona

Award Amount FY05: $50,000

Contrast Agents for Optical Coherence Tomography

The overall goal of this project is to develop a new class of contrast agents for skin cancer research and noninvasive diagnosis of skin lesions. The proposed contrast agent will be used in conjunction with optical coherence tomography (OCT), a biomedical imaging technique for visualization of living tissues. We seek to develop this contract agent by forming dense arrays of gold nano particles on the surface of liposome, a biocompatible sphere of diameter ca. 100nm. In year 2 of this project we successfully generated and characterized liposome-supported gold nano shells, a new class of structures strongly interacting with light, tunable in the near-infrared spectral range. These plasmon resonant structures are supported on a biodegradable soft template appropriate for applications *in vivo*. Independently, we demonstrated contrast enhancement in OCT images using nano rods of gold and we introduced a new technique of differential OCT imaging which allows for further improvement of image contrast.

Burris Duncan, M.D.
University of Arizona
Award Amount FY05: $168,806

Acupuncture as Complementary Therapy for Cerebral Palsy

Cerebral palsy (CP) is the most frequent cause of childhood disability in the U.S. In Arizona, the 1,601 children and 1,818 adults with CP enrolled with the Division of Developmental Disabilities (DDD) during 2002 received $79.3 million in services.

The brain injuries that cause CP are non-progressive, but the motor problems often worsen over time resulting in serious disabilities. Current U.S. standards of care for CP result in less than desired outcomes. This project investigates the standard of care in China where far superior outcomes are reported.

ABRC has taken a leadership role in providing initial funds for this study that promises to have a significant impact on national CP healthcare practice and policy. If our hypotheses are confirmed, it offers the prospect of improving the lives of children with CP, preventing many of the accompanying complications, and giving these children a greater opportunity to realize their individual potential.

Michael R. Sierks
Arizona State University
Award Amount FY05: $124,941

Morphology Specific Antibodies for Treating Parkinson's

We have isolated scFv fragments that specifically bind either the fibrillar or oligomeric form of α-synuclein. The anti-oligomeric scFv is of particular interest for Parkinson's Disease since this form has been shown to be particularly toxic to cells. The α-synuclein protein is abundantly produced in nerve cells, and it would be very desirable to obtain antibodies that can bind only the toxic oliogomeric forms of α-synuclein without interfering with the other normal necessary functions of the monomeric forms. We showed that the anti-oligomeric scFv does not bind other morphologies of α-synuclein and that it can inhibit toxic aggregation of α-synuclein when co-incubated with a solution of monomeric α-synuclein. We also showed that the anti-oligomeric scFv can inhibit toxicity of preformed oligomeric α-synuclein aggregates. Therefore, this scFv has potential value both as a possible therapeutic and a diagnostic for Parkinson's Disease. We are continuing to develop the anti-oligomeric scFv as well as isolating additional scFvs to other forms of α-synuclein.

Timothy L. Vail, Ph.D.

Northern Arizona University
Award Amount FY06: $39,492

Paramagnetic Nanoparticle Immunoassay for Food Pathogen Detection

Recent national outbreaks of foodborne illnesses demonstrate a need for rapid, sensitive, and specific methods to test for the presence of disease-causing bacteria in food supplies. Although such tests exist, their sensitivity comes at the cost of increasing the time to get a result. Conversely, rapid assays (less than 10 minutes to result) typically lack sensitivity. Thus, there exists a need for the continued development of highly sensitive and accurate assay technology. This research seeks to further the state of the art of assay technology through the research and development of prototype rapid assay using nanometer-sized paramagnetic particles onto which fluorescent dyes have been bound. To date, we have demonstrated the ability to create silica-coated paramagnetic nanoparticles with incorporated fluorescent dye (FITC), or quantum dots, through a multi-step process of chemical modification. We have attached a linker protein (avidin) to the surface of the particles and have shown increased stability via chemical cross-linking. Current experiments underway include the incorporation of fluorescent quantum dots as an alternative fluorescent marker. We have initiated a series of prototype immuno-chromatographic assays to demonstrate the functionality of the nano particles. The next steps will include the creation of functional particles that are specific for the causative agent of one type of food poisoning (*Listeria monocytogenes*). This system is also being used as a means to create quantitative, rapid assays that are being tested for the detection of endocrine disrupting compounds in wastewater. We have filed a provisional patent with the U.S. Patent Office on the design of a hand-held, field portable integrated assay and radial cassette reader. The ultimate goal is a miniaturized assay system that will probe for multiple types of disease-causing organisms simultaneously and provide testing results in a very short time. This system is intended to be adaptable to a wide variety of public health, medical, veterinary, and environmental applications.

Publications:

Vail TL, Hyslop S, Buschman S. Creating Multitasking Nanoparticles for Diagnostic Applications. Proc. 14th International Conference on Composite/Nano-Engineering. 14:690-91. 2006.

SECTION C

CONTINUING CONTRACTS

Medical Research

Year One

FY 2005

Danny Brower, Ph.D.

University of Arizona
Award Amount FY06: $49,995

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

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

Katerina Dvorakova, Ph.D.

University of Arizona
Award Amount FY06: $153,922

Barrett's Esophagus Esophageal Adenocarcinoma and Apoptosis

Barrett's esophagus (BE) is a premalignant lesion that arises in the esophagus as a consequence of chronic heartburn. BE is associated with a nearly 30-fold increased risk for the development of esophageal carcinoma. However, the mechanism of BE development is unknown.

The major focus of our studies is to identify molecular pathways that are responsible for the development of BE and esophageal adenocarcinoma. Our studies indicate that exposure to bile acids and gastric acid, two major components of the refluxate, may induce oxidative stress and oxidative DNA damage as well as increased activation of anti-apoptotic pathway and increased expression of proteins associated with apoptosis resistance. These include Bcl-xL and Mcl-1. Furthermore, we developed cells resistant to low pH and bile acids. We found that these cells are under oxidative stress and express antiapoptotic proteins. The data from these studies were presented at several national and international meetings.

Scot W. Ebbinghaus, M.D.

University of Arizona
Award Amount FY06: $50,000

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

Our major goals are to understand how an important leukemia gene called MYB is controlled and discover drugs that could be used for leukemia treatment by shutting off MYB expression. We found that the "on-off switch" for MYB expression contains a repetitive DNA sequence, a GGA repeat region which controls MYB expression. We also discovered that a protein called MAZ binds to the GGA repeat region to repress MYB expression. MYB expression is repressed by MAZ when leukemia cells are treated with Gleevec7®, a targeted therapy for leukemia. This finding suggests that drugs that bind directly to the GGA repeat region could be used to silence MYB expression to treat leukemia, and we are testing drugs that can bind to a unique DNA structure, discovered in our laboratory, formed by the GGA repeat region of the MYB gene.

Leslie Gunatilaka, Ph.D.

University of Arizona
Award Amount FY06: $150,000

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

The majority of anticancer drugs in use today are from natural sources. Our preliminary studies have shown that Sonoran desert organisms are rich in biologically active compounds. The overall goal of this inter-institutional multidisciplinary project is to investigate Sonoran desert organisms for cell motility (migration) inhibitors and to conduct structure-activity relationship (SAR) studies of beauvericin, a fungal metabolite encountered in a previously funded ABRC project, with the broad long-term objective of discovering and developing novel anticancer agents to treat solid tumors. During the course of the first year of this project several plant and microbial extracts were evaluated for inhibition of cell-motility and one microbial and two plant extracts active in an assay for cell-motility inhibitors were subjected to bioactivity-guided fractionation to obtain two step imidone derivatives and several iridoids inhibiting the migration of metastatic PC-3M cells. Further studies with beauvericin showed that it has anti-angiogenic activity and inhibits migration of three additional tumor (breast, pancreatic, and glioma) types, and that proliferation of these tumor cells was unaffected except in the glioma cells; gene expression analysis is currently in progress. In a precursor-directed biosynthetic approach to prepare beauvericin analogs for SAR studies out of the 21 precursors tested, three amino acids were incorporated providing nine new unnatural analogs, six of which have been isolated and characterized. Four of the isolated compounds inhibited cell migration and SAR studies of these analogs are currently in progress.

Publications:

Zahn J, Burns AM, Lui MX, Faeth SH, Gunatilaka LAA. Search for Cell Motilitiy and Angiogenesis Inhibitors with Potential Anticancer Activity: Beauvericin and Other Constituents of Two Endophytic Strains of *Fusarium oxysporum*. J of Nat Prod. 2006 (in press).

Bashyal BP, McLaughlin SP, Gunatilaka LAA. Zinagrandinolides A-C, Cytotoxic d-Elemanolide Type Sesquiterpene Lactones from *Zinnia grandiflora*. J of Nat Prod. 2006 (in press).

Laurence H. Hurley, Ph.D.

University of Arizona
Award Amount FY06: $183,010

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

The overall objective of this project is to characterize the structure of the i-motif in the silencer element of the c-Myc promoter and also its drug complexes, which will then be used as basis for drug design and development. The long-term objective is to identify a small molecule that will selectively modulate c-MYC gene expression and then work to identify a clinical candidate molecule. The 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,
3. to define the overall structure of the silencer element in the c-MYC promoter and its complexes with agents that modulates c-MYC gene expression, and
4. lead identification and subsequent optimization and drug development.

During the time period covered by this report, we have made progress on specific aims 1 and 2. In specific aim 1 we have elucidated the folding pattern of two equilibrating species.

Lonnie P. Lybarger, Ph.D.

University of Arizona
Award Amount FY06: $50,000

CD8 T-Cell Priming Using Engineering MHC Class I Molecules

The goal of this research project is to evaluate the utility of a promising new vaccine technology for the generation of anti-cancer immune response. Cancer is a leading health concern in Arizona, with approximately 24,000 new cases in Arizona in 2004. The immune system, through MHC class I molecules, can detect and eliminate tumor cells. Here, we have developed a novel strategy to engineer MHC class I molecules to be especially potent in terms of their ability to stimulate anti-cancer T-cells. This project will compare the ability of these engineered molecules to prime anti-cancer immune responses versus more conventional vaccine approaches in a mouse model that mimics human cancers. We have now generated several new MHC class I reagents for this study, and they all have the desired properties. We are now ready to begin the *in vivo* experiments to test their anti-cancer properties when used as vaccines.

Estvan Molnar, Ph.D.

University of Arizona
Award Amount FY06: $49,885

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

The objective of the project is to discover novel natural products in a unique collection of bacteria at the Natural Products Center, University of Arizona for the development of anticancer drugs. These bacteria originate from the rhizosphere of desert plants growing under extreme environmental conditions. During the first project year we have established the screening workflow by determining optimal extract concentration for screening and selecting four culture media from the initial 26. We have fermented 306 bacterial strains on several media to provide 1628 extracts and assayed these in a mechanism-based and a general cytotoxicity assay. We have not yet found extracts active in the mechanism-based assay, but identified 50 bacterial strains that provided 181 cytotoxic extracts. These extracts could contain novel cytotoxic metabolite that could serve as lead compounds for anticancer chemotherapy. These might improve the health prognosis of cancer patients in the state of Arizona and worldwide.

Paul B. Myrdal, Ph.D.

University of Arizona
Award Amount FY06: $48,522

Lung Cancer Chemoprevention Via the Inhalation Route

This research program was designed to identify and explore the 5-lipoxygenase pathway, a potential molecular target for the chemoprevention of lung cancer which is responsible for 28 percent of all cancer related deaths in Arizona. To date, several key steps have been made towards this goal. A formulation for the 5-lipoxygenase inhibitory compound, Zileuton, has been developed and tested *in vitro* to establish a sustained release profile. Aerosol delivery equipment has been successfully modified in order to deliver three dose levels of Zileuton. These levels have been administered and results of analyses to determine the compound's ability to modulate the pathway of interest and illuminate any global effects to the lipoxygenase pathway are pending. The sole item remaining to complete the last segment of this phase of the program is to evaluate the sustained release formulation *in vivo*. The final aim of this project will be completed during the final year of funding.

Adrienne C. Scheck, Ph.D.

Barrow Neurological Institute
Award Amount FY06: $50,000

Molecular Analysis for the Diagnostic Identification of
Clinically Aggressive Meningiomas

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

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

University of Arizona
Award Amount FY06: $50,000

Deregulation of Translation Initiation by eIF3f in Melanoma

The incidence of malignant melanoma is continuing to increase in Arizona. This project focuses on the role of eIF3f in melanoma tumorigenesis. The hypothesis of this projects is that eIF3f is a translation inhibitor; disruption of eIF3f function contributes to melanoma tumorigenesis by deregulating translation and apoptosis. Specific aim 1 is to evaluate eIF3f gene abnormalities in melanoma. We can demonstrate loss of eIF3f gene in melanoma by LOH and gene copy analysis. Mutation analysis also indicates alterations at the promoter region of eIF3f gene in melanoma cells. Specific aim2 is to characterize the mechanism of eIF3f-mediated regulation of translation and apoptosis. Ribosome profile and Northern blot analysis revealed that eIF3f degrades rRNA and reduces ribosomes. We propose that eIF3f may play a role in ribosome degradation during apoptosis. Accomplishing the objectives of this award will improve the understanding of melanoma tumorigenesis and provide foundation for developing novel treatment for melanoma.

Publications:

Shi J, Kehle A, Hershey JW, Honchak BM, Warneke JA, Leong SP, Nelson MA. Decreased Expression of Eukaryotic Initiation Factor 3f Deregulates Translation and Apoptosis in Tumor Cells. Oncogene. 25:4923-36.

Shi J, Honchak BM, Hershey JWB, Nelson MA. Aberrant Expressionof Eukaryotic Initiation Factor 3f in Melanoma. Proceedings of the American Association for Cancer Research. 46:2889. 2005.

Shi J, Hershey JWB, Melson MA. Phosphorylation by Cycllin-Dependent Kinase 11 Enhances the Translational Inhibitory Function of the f Subunit of Eukaryotic Initiation Factor 3 During Apoptosis. Proceedings of the American Association for Cancer Research. 2006.

Daekyu Sun, Ph.D.

University of Arizona
Award Amount FY06: $48,984

Targeting Tumor Angiogenesis using G-quadruplex Interactive Ligands

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

Johanna K. Wolford, Ph.D. Translational Genomics Research Institute
Award Amount FY06: $50,000

Diabetic Kidney Disease in American Indians

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

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

Mayo Clinic Scottsdale

Award Amount FY06: $250,000

Arizona Parkinson's Disease Center

The overall goals of the APDC are to develop clinical biomarkers for Parkinson's Disease and PD with dementia (PDD) as well as find targets for novel treatment strategies. To this end the program is divided into a clinical core which prospectively examines PD and control subjects enrolled in the brain donation program, a neuropathology core that performs the autopsies and provides CSF and brain tissue to the laboratory scientists, and 4 projects investigating the pathophysiology of PD and PDD. To date >5,000 clinical evaluations of >800 subjects have occurred. In the past year 11 PD and 11 control subjects came to autopsy. Projects 1 and 2 have found changes in the α-synuclein and DJ-1 proteins, respectively. Project 3 has found dysregulation of multiple sets of genes when comparing brain tissue of control, PD, and PDD cases. Project 4 has developed the methodology for CSF protein analysis. The first year's goals of our program have been met with plans for the next two years of funding being on target. Four papers have been published and 11 presentations made. Additionally, we are pleased to report that additional funding from the Michael J. Fox Foundation has been obtained.

Publications:

Sabbaugh MN, Silverber N, Bircea S, Majeed B, Samant S, Caviness JN, Reisberg B, Adler CH. Is the Functional Decline of Parkinson's Disease Similar to the Functional Decline of Alzheimer's Disease. Elsevier. 2005: 311-15.

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

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

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

Alzheimer's Disease (AD) is the most common cause of dementia in the elderly, affecting up to 15 percent of people over the age of 65 and nearly half of all individuals by the age of 85. Given the fact of Arizona has the second highest population of elderly people in the U.S., AD is very prevalent in our state. Increasing evidence indicates that postmenopausal women have a higher risk of developing AD than age-matched men. This increased risk may be due to the loss of estrogen in the postmenopausal women. We recently discovered β-secretase (BACE) enzymatic activity is significantly increased in AD brains. However, it is not known whether estrogen plays a role in BACE activity. Our ultimate goal is to identify the key mechanisms of estrogen action in AD pathogenesis and provide scientific evidence for developing novel alternative estrogen therapies to prevent and even possibly treat AD.

T. Philip Malan, M.D.

University of Arizona
Award Amount FY06: $50,000

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

Opioids, drugs like morphine, are the most effective and commonly used drugs for the treatment of moderate-to-severe pain. Despite their effectiveness, they also have properties that may worsen pain, potentially causing unintended harm to patients. We have been studying the mechanisms by which short-term opioid administration, such as is used to treat postoperative pain, produces a delayed increase in pain sensitivity. We have shown that short-term opioid administration produces a long-term increase in pain sensitivity. Disrupting selected sensory pathways leading from the spinal cord to the brain or pain-regulating pathways descending from the brain to the spinal cord abolishes opioid-induced pain hypersensitivity, showing that these pathways are crucial to this phenomenon. Further, activity of neurokinin receptors and of the regulatory molecule dynorphin in the spinal cord are required for the production of delayed pain hypersensitivity. These findings give important insights into this unwanted side effect of pain-treating drugs.

John J. Osterhout, Ph.D.

University of Arizona
Award Amount FY06: $49,999

Structure and Mechanism in Alzheimer's Disease

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

Joseph Rogers, Ph.D.

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

Translational Research on Aß Metabolism, from Synthesis to Clearance

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

Sanjay Ramakumar, M.D.

University of Arizona
Award Amount FY06: $141,969

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

Gene therapy is one of the most promising treatments of the 21st century; however, it is one of the most challenging. Despite research demonstrating appropriate targets for treatment, the consistent delivery of genes has been problematic. We have previously developed models of kidney disease caused by diseases such as diabetes and high blood pressure. Our laboratory models provide a basis for which treatments can be tested.

The fundamental goal of this project is to synthesize a nano-scale-gel particle that can act as a delivery vehicle for a small gene fragment. Once optimized, we plan to test specific genes that target inflammation in the kidney caused by poor blood flow.

In the first year, we have constructed the nano gel and begun optimizing the chemistry of the particle for animal conditions (temperature, pH). We have also successfully created a kidney cell culture line to test this gene delivery system and created a rabbit model of kidney damage with delivery methods for the nano gel.

Arthur F. Gmitro, Ph.D.

University of Arizona
Award Amount FY06: $50,000

Ultra Miniature Endoscopes for Biomedical Imaging

This research project is aimed at development and evaluation of an ultrathin endoscope that incorporates illumination and imaging into a single channel. In addition, the device will be capable of simultaneous white light and fluorescence imaging. The ultimate goal of this work is to provide an imaging catheter with more resolution elements per given area than is currently available. If successful, this technology will enable the development of catheters with outer diameters down to 0.35 mm. thus making imaging of more peripheral vasculature and distal airways possible. Specific objectives of the work are to complete development of the instrumentation, evaluate the imaging performance of the instrument, and show the feasibility of the using the instrument in a Barrett's Esophagus mouse model.

Publications:

Kano AL, Gmitro AF. Ultrathin Fiberscope Utilizing a Single Channel for Both Illumination and Imaging. Frontiers in Optics 2005. FTuG4 (Tucson, AZ). 2005.

SECTION D

NEW CONTRACT AWARDS

BEGINNING IN FY 2007

Craig A. Aspinwall, Ph.D.

University of Arizona
Award Amount FY07: $48,323

Stabilized Polymer Phospholipid Imaging Probes

Changes in cellular mass, through either loss or gain of cells, are associated with a number of disease states including Type 1 diabetes mellitus and cancer. The ability to detect the small changes in mass that occur at the onset of disease will markedly enhance the capabilities for diagnosis and prognosis of the disease. Current imaging technologies lack both the sensitivity and the spatial resolution required to detect the small changes early in the disease state. Further, the lack of chemical specificity prevents positive identification of the cell type that is changing. Thus, the capability to detect small changes in cellular mass with high sensitivity, specificity and spatial resolution is highly desirable in a number of disease studies.

We will develop a series of nanometer-sized fluorescent imaging probes that possesses unparalleled sensitivity, specificity and are small enough to allow sub-cellular spatial resolution. It is our hypothesis that these probes will allow imaging of the small decrease in cellular mass associated with Type 1 diabetes mellitus. The proposed probe geometry contains a highly fluorescent core surrounded by a chemically and environmentally stabilized phospholipid membrane. Key advantages of this probe geometry include a) higher sensitivity; b) enhanced biocompatibility, i.e. reduced biofouling; c) reduced toxicity; d) improved chemical and environmental stability; e) enhanced photostabiltity; f) enhanced spatial resolutions and g) improved selectivity (e.g. lower non-specific adsorption).

Yongchang Chang, Ph.D.

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

Mechanism of p1 GABAc Receptor Activation and Antagonism

γ-aminobutyric acid (GABA)-gated chloride channels (GABAa and GABAc receptors) play important roles in chemical signaling and control of electrical activity in the mammalian brain. They also are the major targets for many clinically useful neuroactive compounds. Dysfunction of GABA receptors is closely related to epilepsy and many other neurological and psychiatric disorders. Binding of GABA to its receptor is thought to induce a conformational change that leads to opening of a channel through which negatively-charged ions can pass into the cell (activation). Gaining insight into the structural basis of conformational changes thought to be involved in these processes is fundamental to understanding the mechanism of GABA receptor function. However, despite intensive studies in the past, structural basis involved in GABAa/c receptor function are still poorly understood. To this end, we have successfully established a technique to monitor conformational changes of specific residues in the receptor during channel activation. We will use it to delineate pattern of the structural changes during channel activation and differentiate agonist-induced conformational changes that are tightly or loosely coupled to channel activation by the allosteric antagonist picrotoxin.

The planned experiments will test the hypotheses 1) that initial interaction with GABA induces a rotation in part of the receptor that faces extracellularly, and that this rotation is coupled to a rotation of the region of the molecule lining the channel, which makes the pore wider, allowing ions to flow through it (activation) and 2) the agonist-induced conformational changes can be further divided into two categories that tightly or loosely coupled to channel activation. The objectives of the project are 1) to delineate the pattern of structural changes during channel activation and 2) to differentiate agonist-induced movements that are tightly or loosely coupled to channel function by an allosteric antagonist.

J. Richard Coast, Ph.D.

Northern Arizona University
Award Amount FY07: $43,347

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

With the increasing number of elderly people in the US, and particularly in Arizona, there is an increasing incidence of lung disease. One outcome of respiratory disease is weakness and fatigue of the muscles of breathing (diaphragm and others). These respiratory muscles are weakened through the extra work required to breathe, as well as a decrease in the diet that is often seen in people with lung disease. This severely limits the ability of the person to exercise, or to even participate in normal daily activities.

Training methods have been developed that help people strengthen their respiratory muscles, but they are often unpleasant for the person to undertake. They may result in a feeling of breathlessness, which is just the thing that lung disease patients are afraid of. If training methods can be developed which strengthen the respiratory muscles as well as minimize the discomfort and feeling of breathlessness of the person doing the exercise, these exercises may be better tolerated and eventually lead to an intervention for these patients. Through this project, we hope to determine exercises that can be tolerated by the very unfit and still lead to an improvement in respiratory muscle strength and endurance. If successful, the next step will be to work with physicians in a clinical test to see of these training methods work in lung disease patients.

The goals of this project are 1) to determine whether the diaphragm will fatigue in response to non-respiratory activities such as sit-ups, certain weight lifting exercises, and leg lifting exercise; and 2) to use these same activities to train the respiratory muscles and evaluate the fatigue of the diaphragm following this training. Such exercises, if shown to result in fatigue and training of the respiratory muscles, may lead to rehabilitation interventions for conditions in which improved diaphragm strength and endurance could be beneficial, such as pulmonary disease, certain spinal cord injuries, and possibly certain endurance exercise situations. The specific aims (questions) of the project are as follows:

a) Do non-respiratory activities involving the trunk muscles lead to fatigue of the respiratory muscles?
b) Can fatigue be decreased through appropriate (non-respiratory) training of the trunk muscles?
c) Does this training lead to an increased ability of the respiratory muscles to breathe during respiratory tasks?

The objectives of this project, derived from the specific aims are grouped as follows:

a) Evaluate fatigue of the respiratory muscles as a group and the diaphragm specifically. The fatigue measures will be assessed following tasks designed to specifically fatigue the respiratory muscles (inspiratory resistance). The measures will also be performed following a series of standard sit-up bouts and less strenuous exercises.

b) Perform sit-up and weight training exercises to train the trunk muscles for eight weeks. At intervals during and following the training, we will, again, evaluate respiratory muscle fatigue.
c) Perform specific respiratory muscle fatiguing tasks to determine whether the non-respiratory training of the respiratory muscle will have a carry-over effect on the ability to perform respiratory tasks.

The overall goal of this work is to further evaluate how different tasks contribute to respiratory muscle fatigue, and whether any of these show promise as additions to current interventions designed for subjects and patients whose respiratory muscle fatigue may limit activities of daily living. It expands upon work of the last 15 years in our laboratory that evaluates training and fatigue of the respiratory muscles.

Paul Coleman, Ph.D.

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

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

Arizona features some of the world's largest, most concentrated elderly populations – populations that are especially vulnerable to Alzheimer's Disease (AD). For this and other reasons, the ABRC-supported ***Arizona Biosciences Roadmap*** has singled out Neuroscience as one of three key platforms and, within that platform, AD research as a major focus. Also noted in the Roadmap and elsewhere is the urgent need for a reliable diagnostic and biomarker for AD, so that existing and emerging new treatments for AD can be initiated before irreparable damage is done. A diagnostic and biomarker would, in addition, dramatically facilitate clinical trials of promising new AD treatments. However, most AD diagnostic efforts, both in Arizona (e.g., the Arizona Roadmap's Bioimaging Program) and in the U.S. (e.g., the $60M NIH AD Neuroimaging Initiative), have focused on brain imaging methods for detecting and tracking AD. Though extremely promising, these methods are expensive and often invasive (e.g., PET).

There is now extensive evidence that manifestations of AD can be observed not simply in the brain, but also in multiple systems and organs throughout the body. Some of these systems, such as blood, constantly interact with the brain and appear to exhibit a number of alterations that may parallel, or at least provide an index of, the brain pathology of AD. For example, a project to assay red blood cells for a particular brain marker of AD that the cells may pick up was funded by the ABRC just a few months before this principle investigator's arrival in Arizona.

Preliminary data for the present application suggest that blood leukocytes may also show changes that are diagnostic for AD. Translating this discovery into the clinic as an inexpensive, minimally-invasive diagnostic and biomarker for AD is the overall aim of this proposal. Notably, the major expense of such a study – recruiting, evaluating, and obtaining samples from AD and other subjects – can be eliminated by using residual samples from the above-mentioned ABRC funded study of red blood cells enhancing the cost-effectiveness of the present experiments.

Overall, we seek to the test the hypothesis that peripheral blood leukocytes (PBLs) of AD patients exhibit changes in expression of specific subsets of cell stress and inflammation genes and that the pattern of such changes can be used to distinguish AD from normal elderly control and demented, non-AD subjects with very high sensitivity and specificity. Inclusion of putative early AD patients and a longitudinal design will also assess the method as an early diagnostic and biomarker. A key feature is the use of an analysis method, canonical multivariate analysis, that does not simply test for changes in expression of individual genes as previous approaches have done, but looks for patterns of change in the expression of multiple genes. Our preliminary data suggest that this method can very accurately pick out patients that have been diagnosed clinically with AD. Specific Aim 1 of the proposal, therefore, seeks to validate our successful but still very preliminary results in a larger cohort of living subjects. Specific Aim 2 seeks to confirm the results in rapidly-obtained blood samples from antemortem-evaluated, postmortem-confirmed subjects, where diagnostic accuracy is optimal. That is, lacking a reliable diagnostic such as the one we are trying to develop, diagnosis of AD in living subjects is only 85-90 percent accurate even in the best clinics. Postmortem confirmation is therefore the only present way to diagnose AD with certainty. Specific Aim 3 seeks to explore whether or not the method can be enhanced by focusing on a specific leukocyte subpopulation. If successful, the method should warrant further translation on a large-scale (e.g., partnership with industry).

John K. DiBaise, M.D.

Mayo Clinic Arizona
Award Amount FY07: $111,044

Transmucosal Delivery of Erythromycin to Treat Gastroparesis

Abnormalities in the speed with which material passes through the stomach after it is swallowed can lead to problems with food and medication absorption. Food may remain in the stomach for an extended period of time, a condition known as gastroparesis, causing a variety of symptoms and, when severe, resulting in weight loss requiring alternative means of feeding. A common condition associated with gastroparesis is diabetes. Diabetes treatment is often complicated in those with gastroparesis because of the difficulty in matching the timing of insulin delivery and food intake. Gastroparesis, one of the most feared complications of diabetes, is a particularly relevant problem for Arizona because of the high incidence of diabetes among the general population and the Native American population, particularly.

Medications given orally to improve stomach emptying, such as erythromycin, may be irregularly absorbed, making them less effective. Transmucosal (sublingual) administration has been successfully used to treat other conditions and may be a more effective alternative to treat gastroparesis as symptom relief should become independent of gastrointestinal absorption. This may lead to improved stomach function which could result in symptom and quality of life improvement and, possibly, an improvement in glucose control in diabetics.

The long-term goal of this research is to improve the treatment of gastroparesis. The immediate goal is to develop a transmucosal system that is able to deliver adequate amounts of erythromycin, a potent stomach stimulant, into the blood. This route of delivery of these medications may provide substantial benefit to gastroparesis patients. To accomplish these goals, this project involves three interconnected parts. In part 1, the ability of a novel substances to be used as a sublingual system for administration of erythromycin will be evaluated. In part 2, the ability of this new delivery system to allow erythromycin to be absorbed into the blood stream of a rat will be evaluated and will be compared to results from a commercially available sublingual delivery system, Carbopol 974P, that has not been previously used to administer erythromycin. Finally, in part 3, the Carbopol 974P delivery system will be studied in healthy humans to determined how well it works to transport erythromycin into the blood stream and how well it stimulates stomach emptying.

If successful, this project could improve the effectiveness of prokinetic therapy, reduce medical care cost and result in a significant improvement in the quality of life for millions of people. In addition, the preliminary data required to apply for long-term funding from the National Institutes of Health in order to complete studies delivering sublingual erythromycin (or other stomach stimulating agents) in both a gastroparesis animal model and humans with gastroparesis will have been collected.

Kathleen Dixon, Ph.D. University of Arizona
Award Amount FY07: $149,151

Imaging of Markers for Skin Cancer Risk

The incidence of skin cancer is continuing to rise in the United States and it is particularly high in the state of Arizona. One of the most important risk factors for skin cancer development is exposure to sunlight. Sunlight is made up of visible light and ultraviolet (UV) light; it is the UV light that can damage skin and cause cancer. The skin is made of several layers of skin cells, and UV light from the sun can cause changes in those cells. First, the UV light can cause damage to the genetic material (the DNA) in the cells. Although the cells have mechanisms (called DNA repair pathways) to repair the damaged DNA, sometimes if the damage is too great, not all of it is repaired. Then genetic changes can occur in those cells, and some of those genetic changes can convert the cell from a normal cell to a cancer cell (carcinogenesis). This process of cancer development occurs slowly over time and appears to require repeated exposure to the sun. Another effect of UV light is to stimulate the skin cells to divide (to increase in numbers). This effect of UV light is similar to the way cells are stimulated to divide by growth factors (like estrogen or pituitary hormone). These cellular changes start at the cell surface and the program of gene expression inside the cell is changed. This growth stimulation by UV appears to work together with the DNA damaging effects of UV in the process of skin carcinogenesis.

We are interested in finding ways to easily measure the effects of UV on skin cells for several reasons 1) to understand more about how sunlight exposure causes cancer, 2) to determine why some people are more likely to develop skin cancer than others, 3) to measure the biological response of cells to UV light in tests of agents that are being developed to prevent skin cancer and 4) possibly to develop easy clinical tests for skin cancer risk.

A statewide multidisciplinary collaboration for the image analysis of cellular responses to UV radiation is proposed. This work will focus on the identification of skin cancer susceptibility factors and the development of chemopreventive agents. This collaboration will include mathematicians from UA and biomedical scientist from UA colleges of Medicine and Science, the Arizona Cancer Center, and NAU College of Engineering and Science. The ultimate goal of this work is to provide tools that can be used in a clinical setting to monitor skin cancer susceptibility, progression, and responses to prevention/intervention strategies. Three specific aims are proposed:

Aim 1) Cellular responses to UV radiation-induced DNA damage. We propose to use microscopy and image analysis to visualize the changes that occur inside the cell nucleus after treatment with UV radiation.

Aim 2) Cellular early responses to UV radiation. We propose to use the fluorescent imaging techniques in the mouse skin for detection of certain changes in gene expression that are important in skin carcinogenesis. These quantitative assays will then be used to screen drug inhibitors of UV responses that might work to prevent cancer development.

Aim 3) Ultrastructural changes associated with cellular responses to UV radiation. We propose to use transmission electron microscopy (TEM) to characterize morphological changes in cells following UV radiation. These changes can the be used to evaluate the overall toxicity of UV radiation and potential cancer prevention agents.

Graeme J. Dougherty, Ph.D.

University of Arizona

Award Amount FY07: $49,535

Molecular Therapy of Bladder Cancer

Each year around 800 men and 200 women in Arizona will be diagnosed with bladder cancer. It is the 4th commonest male cancer and 9th commonest female cancer respectively, and although curable in its early stages, the price paid for this result is high. Cystectomy has been the gold standard in the US for treatment of muscle-invasive disease, and although outcome is generally good, the psychological impact of dealing with urostomy bags, as well as the constant worry related to infection and the development of kidney problems, should not be underestimated. Importantly, in high risk groups as many as 80 percent of patients that initially present with superficial pre-invasive disease will eventually relapse and progress to a muscle invasive stage that requires surgery. In order to avoid this situation, it is imperative that new ways be developed to treat early stage bladder cancer that are effective at preventing recurrence and progression of the disease.

The major objective of this study is to explore the therapeutic potential of a novel approach to the treatment of bladder cancer that exploits the differential secretion by the abnormal tumor cell population of a particular soluble molecule known as vascular endothelial cell growth factor or 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. The goal now is to define various patient specific variables that might impact on the efficacy of this promising therapeutic approach. Such studies are important as they will provide information that can be exploited in a future diagnostic setting in order to identify the subset of bladder cancer patients most likely to benefit from this novel treatment.

Lars Ewell, Ph.D.

University of Arizona
Award Amount FY07: $50,000

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

Brain tumors (gliomas) constitute a large branch of the cancer patient population with over 20,000 Americans diagnosed each year. Radiation treatment is considered the current "standard of care" for people diagnosed with this disease. It has been proven to extend the life and quality of life for people afflicted with this serious disease. However, although the radiation has proven effective at killing the cancerous cells in the brain, it also, unfortunately, kills normal brain cells as well. A side effect of the radiation treatment is a malady called Radiation Induced Necrosis (RIN). A difficulty in diagnosing RIN is that in a normal MRI scan it closely mimics what a recurrent brain tumor looks like. A recurrent brain tumor can be treated with additional radiation, while RIN will be made worse with additional radiation.

The main objective of this research is to attempt to solve the dilemma of how to tell the difference between RIN and recurrent gliomas. To this end, we will implement two new forms of imaging - Diffusion Weighted MRI (DWMRI) and Magnetic Resonance Spectroscopy (MRS). These two different and complementary forms of imaging should prove useful in solving this problem. DWMRI can measure how freely water flows. MRS can measure how certain metabolites in the brain are reacting to therapy.

The University of Arizona has a history of advances in biomedical imaging. In addition, with the recent purchase of the Novalis Brainlab therapy accelerator, the University Medical Center has the ability to deliver the most accurate form of radiation to the brain currently available. By combining these two leading edge cancer therapy and imaging technologies, we will be able to insure that the state of Arizona retains its eminence in the important research areas of cancer therapy and medical imaging.

Robert Gillies, Ph.D.

University of Arizona
Award Amount FY07: $250,000

Multimeric Ligands for Targeting Cancer for Imaging and Therapy

A major goal for diagnosis and therapy of cancer (and other disease) is to target the diagnostic imaging or therapeutic agents directly to the tissue or cell of interest without cross-reacting with other cells or tissues. Cell surface proteins, such as receptors, are attractive as targets as a) they already express highly selective binding for biomolecules known as ligands such as hormones, and b) they are accessible from outside the cell, meaning that targeting agents do not have to cross the cell membrane to interact. Although receptors are highly selective, they are rarely found to be associated with just a single abnormal cell type, but are found expressed in normal cells as well. Often times, however, the abnormal (target) cell will express more of the receptor compared to the normal controls. This proposal seeks to develop agents that can discriminate these target cells from normal cells in humans using a multimeric ligand approach. A multimeric ligand is a molecule that contains more than one ligand binding motif attached to a backbone linker, making a nano particle. These particles can contain multiple copies of the same ligand (a homo-multimer) or can contain copies of different ligands (a hetero-multimer) allowing cross-linking of two or more different receptors which affords much higher specificity.

This has relevance to Arizona in a number of ways. First, the current proposal is focused on Pancreatic Adenocarcinoma, a devastating disease with high mortality it this state and elsewhere. The state has leading edge research into this disease with Specialized Programs in Research Excellence (SPOREs) at both the Mayo-Scottsdale and at UA-Tucson and a pancreatic cancer program project (PO1) at TGen. Second, this technology was invented at the University of Arizona, and it remains a leader in the discovery and design of homo-and hetero-multimeric complexes. Finally, these ligands are used for both therapeutics and imaging, which are two engineering strengths in the State.

This research program is divided into four interacting projects to 1) discover and validate appropriate targets using DNA Arrays and Tissue Arrays, 2) discover novel binding ligands using high throughput screening, HTS, 3) investigate construction of multimerics using linear linkers and polymer chemistries and 4) provide a more sound theoretical basis for the future design and application of multimeric constructs. This program is highly interactive, multi-institutional, and multi-disciplinary. The overall goal of this work is to develop ligands that can specifically target pancreatic cancer in humans for the purpose of diagnostic imaging and delivery of therapy (molecular surgery). Although focused on Pancreatic Cancer, this technology can and will be expanded to targeting of other cancers such as ovarian, glioma, and melanoma.

John G. Hildebrand, Ph.D.

University of Arizona
Award Amount FY07: $49,998

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

Blood-sucking triatomine bugs, commonly known as kissing bugs, cone-nose bugs, or Mexican bed bugs, are important to human health in Southwestern USA because of the mild to severe allergic reactions their bites can cause and their role as potential carriers of the parasite that causes Chagas Disease. Kissing bugs could become a more common health problem in the Southwest owing to the fact that that human populations are continuously expanding into the natural habit of these insects. Indeed, Arizona is one of the states with the highest reported incidence of contracts between kissing bugs and humans. Although only a few cases of Chagas Disease have been reported in the USA, this disease can be deadly; it affects 18-20 million people in Mexico, Central and South America and results in about 14,000 deaths every year. The possibility of higher rates of infection by this parasite in Southwest USA, however, cannot be excluded given that both infected insects and mammalian hosts (e.g., pack rats and other rodents) are present. In addition, species of kissing bugs that are more efficient carriers of the parasite may disperse from areas where the disease is common (e.g., neighboring Mexico) and find their way to Arizona. In neither Arizona nor other parts of the USA are there up-to-date data on the species of kissing bugs present, their abundance, and their rates of parasitic infection. Moreover, there are no methods for trapping kissing bugs and thus reducing human exposure.

We will assess the risks that kissing bugs pose to human health in Southern Arizona and develop control methods to intercept those insects before they reach human houses. This will reduce contacts between kissing bugs and humans. In parallel, we will develop educational tools for increased, much-needed awareness in the general population about health risks associated with kissing bugs. To accomplish these goals we will 1) study which species of kissing bugs are present in the periphery of Tucson, an area of high incidence of allergic reactions from kissing bug bites; their geographical distribution; abundance; and the infection rate of the parasites that cause Chagas Disease; 2) study biological aspects of the insects that are relevant to their capacity to locate, contact, and bite humans transmitting the Chagas Disease parasite to humans (e.g., feeding habits, low domiciliation capability, preference for feeding on other vertebrates); 3) identify odors that are highly attractive to the insects using chemical analysis and studies of the insects smell function; 4) develop a trap baited with attractive odor blends identified under aim 3, which will be tested under laboratory and field conditions and will help to reduce contracts between kissing bugs and humans; 5) establish a referential website for educational and informational purposes with information specific for Arizonans; and 6) pursue a pilot program of educational outreach activities in selected school districts. Accomplishment of our goals will help reduce contracts between kissing bugs and humans and thus benefit Arizonans by reducing the risk of Chagas Disease and severe allergic reactions.

James B. Hoying, Ph.D.

University of Arizona

Award Amount FY07: $50,000

Genetic Diagnostics of Angiogenesis

Good tissue health depends on the presence of an extensive network of blood vessels to efficiently deliver blood to the tissue. Following tissue injury or in certain diseases, new blood vessels form through a process called angiogenesis. Following angiogenesis, the newly formed vessels must connect with each other and pre-existing blood vessels to form a new network capable of carrying blood. Knowing whether or not a tissue is forming a new vascular supply (called vascularization) is important in diagnosing disease conditions. For example, it is believed that a tumor will not grow beyond a certain size unless it can cause the growth of a new vascular supply. Unfortunately, there is not yet an effective means by which vascularization can be assessed. One of the best ways to identify a particular biological process such as vascularization is by assessing the expression of genes associated with the process. Such diagnostic markers can be measured and used as an index of tissue health. For example, high expression of the prostate specific antigen (PSA) gene is associated with an unhealthy prostate. We propose to develop a diagnostic for vascular health.

For any given process there are hundreds to thousands of genes expressed. Often, different tissues will express many of the same genes. Consequently, it can be difficult to identify one or two genes that uniquely identify a given process or activity. This is particularly relevant in vascularization where a great many genes are expressed in a complex manner. Therefore, we believe that useful diagnostic for vascularization will involve using combinations of expressed gene markers to say whether or not a vascular bed is growing new vessels (i.e., angiogenesis). The proposed studies will lead to the development of such a "panel" of diagnostic markers for use in diagnosing the state of vasculature in a tissue for clinical purposes. In addition, this diagnostic panel will greatly contribute to research investigating how blood vessels actually grow and form a new vascular bed.

To identify useful gene combinations, we have developed an experimental system that mimics or models the vascularization process in the laboratory. With this model system involving mice, we can recreate vascularization as it occurs in the human while still being able to manipulate the process experimentally and make useful measurements and characterizations. We will be using microarray analysis to make the many thousands of gene expression measurements in this model. Our experimental approach is to make measurements of expression for nearly 30,000 genes in our experimental model of vascularization to identify those genes whose expression matches with the different stages of vascularization. We will then use sophisticated, computer-based methods to determine what genes and what combinations of genes prove to be effective at identifying specific aspects of vascularization (e.g. angiogenesis vs network reorganization). Finally, we will take the genes identified to be useful and important and examine real tissues undergoing repair or growth to test the ability of these gene markers to diagnose vascularization.

Richard D. Lane, Ph.D. University of Arizona
Award Amount FY07: $150,000

Neural Basis of Vagal Tone Dysregulation in Depression

Major depressive disorder (MDD) is a very common and disabling condition that is associated with increased mortality. Although a number of antidepressant medications and psychotherapies are available, many patients do not respond and most do not recover fully. Depression is a major public health problem in Arizona. The FDA recently approved "vagus nerve stimulation" (VNS) as a treatment for severe, refractory depression. The mechanisms by which VNS works are not fully understood. The vagus nerve is a main conduit connecting the brain and body, and VNS consists of repeatedly stimulating the vagus nerve in the neck. Current evidence suggests that VNS works by altering activity in brain areas involved in regulating mood. This project, based on evidence that the vagus nerve plays an important role in regulating mood, aims to understand how vagal mechanisms may be operative in MDD independent of VNS. MDD is characterized by a type of hyperarousal in the body that may reflect inadequate compensation for the low energy and motivation observed in depression. Low vagus nerve activity (or low vagal tone) is associated with hyperarousal which is present in depression and returns to baseline with successful treatment. The purpose of the current proposal is to examine whether a standard measure of vagal tone, heart rate variability (HRV), can be used as a marker of depressive symptoms and the changes that occur in the brain as patients recover from depression.

Current evidence suggests that there is dysfunction of several subsectors of the anterior cingulate cortex (ACC) in patients with MDD. The dysfunctions in these brain areas (either over-active or under-active) are known to recover and return to baseline with successful treatment. Current evidence also indicates that vagal tone is decreased in patients with MDD and that vagal tone recovers and returns to baseline when patients recover from MDD. Thus, with recovery from MDD, both activity in ACC subsectors and HRV normalize. These findings may be linked, as studies in healthy volunteers indicate that HRV is correlated with activity in ACC subsectors. Taken together, these findings suggest that activity in ACC subsectors, depressive symptoms and HRV may change together as patients recover from MDD. However, no previous studies have examined the covariation of these variables at baseline or the degree to which they change together in patients being treated for MDD. A primary objectives of this research is to examine the covariation of these variables as depressive symptoms change. In this study we will treat some patients with medication and others with psychotherapy so that we can examine change in depressive symptoms independent of any particular antidepressant treatment. The goal of this research is to determine whether HRV can be used as an indicator of activity within the ACC. If it can, it would enable clinicians to use HRV to help make the diagnosis of depression, choose antidepressant treatment and track its progress. Improvements in these areas will lead to reductions in the prevalence of depression in Arizona.

Ana Maria Lopez, M.D. University of Arizona

Award Amount FY07: $150,000

Expedited Breast Care: A New Model in Breast Health

One million women in the United States have breast biopsies each year, most often based on mammography study results. Currently, Arizonans who have screening mammograms for breast cancer often wait a week or longer to find out the results of their biopsies. If they are diagnosed with a cancer, they then wait additional weeks to get an appointment with a surgeon and then with a cancer doctor to lay out the cancer treatment plan. The Arizona Telemedicine Program, in collaboration with the Navajo Nation and the University of Arizona College of Medicine faculty, established rapid result digital mammography services at Tuba City and three other Navajo healthcare sites over the past four years. Today, Navajo women routinely get the results of their digital mammography studies without leaving the clinic within 90 minutes. This has set a new standard for breast care mammography. The research problem addressed in this project will take the one-stop rapid clinic concept much further. The research is aimed at developing a model for breast health care. Women with a new diagnosis of breast cancer can have the abnormal area biopsied, can have the tissue diagnosed, and can then see the oncologist (teleoncologist) via videoconferencing, during a single clinic visit. Using advanced diagnostic and telemedicine technologies developed in Arizona and described in this proposal, the time it will take for a woman to get from having a mammography study to a clinical planning session with an oncologist is reduced from the best case scenario of three to four weeks, to less than 6 hours. This approach to breast care will benefit patients in both rural and urban settings.

The major goal of the research is to create a one-stop single day multi-specialty breast clinic. This new type of clinical service should increase the accessibility and convenience of breast cancer testing; improve patient outcomes for a disease that requires the involvement of multiple specialists including surgeons, pathologists, and oncologists and is very time consuming for the patients; and reduce costs.

The specific objectives are to:

a) establish an expedited breast disease clinic at a breast center in order to assess the time endpoints for breast clinical and laboratory services,
b) assess participant (i.e., patients, healthcare services providers) satisfaction with expedited breast services, and
c) evaluate and model cost differences between conventional breast care and the expedited breast care service health delivery system.

Stephen L. Macknik, Ph.D.

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

Blood Flow Measurements During Ictal Events: Implications For Neuroprotective Therapies

One of the crippling effects of epilepsy is the progressive development of brain damage which can eventually lead to permanent memory loss as well as death. Theories to explain epilepsy-related neural degeneration have focused mainly on metabolic damage to the seizure-affect parts of the brain. However, large variations in blood flow during seizures, caused by the spastic contraction and relaxation of the muscles that control blood flow within the vasculature of the brain, could also result in temporary blockages and overflows that could contribute to the brain damage in a way seen during certain types of strokes. These stroke-like effects of seizures are largely overlooked currently by the field, as a contributor to the long-term brain damage seen in epileptics. This may be because, while it is known that blood flow is higher during seizures than during resting periods, it is not known if seizure blood flow is truly abnormal. It is necessary to determine the degree of blood flow within the same capillaries, which has not been done. If seizures are correlated with abnormally high or low periods of blood flow, or both, we expect their long-term cumulative degenerative effects to be similar to those caused by iterative mini-strokes.

With Arizona's fast growing population, the impact of cumulative brain damage among the epileptic population has become a problem that cannot be underestimated. The effects of the problem on the cost of medical insurance and health care exacerbate an already serious problem for the Arizona economy, and it threatens the quality of life of many of Arizona's citizens. This project could lead to the rapid translation of the use of medicines already developed to protect the brains of stroke victims to a new use in protecting epileptics, thus ameliorating these problems significantly.

By showing that seizure blood flow extends beyond the range seen with normal functional brain responses, we will establish a strong basis to discover the mechanisms by which seizure blood flow drives neural degeneration. We, moreover, expect that therapies already developed to protect stroke victims from blood flow related neuron death will also protect epileptics, as seizures may (in a sense) be a type of mini-stroke.

The long-term goals of this research program are to establish the link between seizures blood flow and the long-term neural degeneration seen in epileptics, to determine the biological mechanisms by which abnormal blood flow causes neural death, and to also develop potential therapies (novel or borrowed from other therapy regimes such as stroke) to help ameliorate any potential blood flow related degenerative effects of epilepsy.

In the present project we propose the first step to directly compare normal blood flow withing the smallest blood vessels of the brain, capillaries. We will measure red blood cell velocity, volume, and total flux during seizures and during normal peak physiological function within the same capillary. The central hypothesis is that seizure blood flow will be abnormal in scope. The results will form the cornerstone for future research into the mechanisms of disease that link seizure blood flow with transient oxygen starvation and swelling within the brain, as well as the basis for treatments against seizure blood flow related to neural degeneration. To measure both seizure-generated and normal blood flow in the same capillary, we will employ a novel combination of two high-tech imaging methods *in vivo* in rats: intrinsic optional signal macroscopy and two-photon laser scanning microscopy. These two methods will allow us to image blood flow changes on the surface of the brain using intrinsic signal optical imaging, and to examine the flow using microscopic imaging (two-photon imaging).

Lawrence J. Mandarino, Ph.D.

Arizona State University
Award Amount FY07: $136,364

Metabolic Syndrome and Inflammation

Types 2 diabetes mellitus, obesity, heart disease, high triglyceride and cholesterol levels, and high blood pressure for diseases that often occur together and have been called the Metabolic Syndrome. These diseases are increasing dramatically in prevalence and are appearing in individuals at younger ages than ever before. The Metabolic Syndrome is especially prevent in Mexican American and Native American populations, although it occurs in all ethnic groups. Arizona is especially hard-hit by these diseases, in part because of our rapidly growing Mexican American population, but also in general. Estimates are that up to 40 percent of all health care costs are due to these diseases, and this percentage is even greater for public health care. These diseases are thought to stem from the common underlying problem of insulin resistance. Insulin resistance is a decreased ability of tissues to respond normally to insulin, the pancreatic hormone that lowers blood sugar. Although it is clear that insulin resistance is increasing in prevalence, what causes insulin resistance is unclear. There undoubtedly are as yet undefined genetic contributors, but here is also a very large acquired component. Only recently we and others have come to the realization that a chronic state of inflammation in the body may trigger insulin resistance. Our research is focused on how an oversupply of fat in the diet can produce inflammation and ultimately insulin resistance. Skeletal muscle is one of the most important tissues with respect to how insulin works in the body. Our research is designed to determine the mechanisms of how fat oversupply produces inflammation in muscle in human volunteers.

In experiments performed to determine how fat oversupply changes the activity of genes that are involved with how insulin works in muscle we discovered that fat oversupply produces temporary inflammation in healthy muscle that is characterized by a specific inflammatory pathway called the "NF-kB" pathway.

This pathway can be connected directly to the biochemical way in which insulin works. This finding opens a new bridge between inflammation and muscle insulin resistance. What is not known is whether fat is directly affecting muscle or producing its adverse effects by acting on other organs such as liver or fat, and those then release chemicals, called cytokines, that produce inflammation in muscle. The objective of the research described here is to use give fat intravenously to human volunteers and to follow the development of inflammation and insulin resistance in muscle over time. By comparing the time of appearance of cytokines in blood with the development of inflammation in muscle and looking at the production of cytokines in muscle itself, we can address our objective. Specifically, we propose to determine the time courses of fat-induced inflammation in muscle and blood cytokines. We will test the hypotheses that:

a) increases in circulating pro-inflammatory cytokines precede the development of inflammatory response in muscle, and there are no changes in expression of inflammatory cytokines in muscle itself,
b) the inflammatory response in muscle precedes insulin resistance, and
c) inflammation results in changes in serine/threonine phosphorylation of the insulin signaling protein Insulin Receptor Substrate (IRS)-1.

John J. Marchalonis, Ph.D.

University of Arizona

Award Amount FY07: $150,000

Modulation of Autoimmune Disease by Autoantibodies and Immunopeptides

Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and myasthenia gravis (MG) are autoimmune diseases associated with elevated levels of autoantibodies and autodestructive thymus-derived lymphocytes (T-cell). These diseases are prevalent in Arizona with RA having an especially high incidence of approximately five percent due to influx of individuals suffering from the disease and the high percentage of Native Americans who have increased susceptibility. The incidence of these diseases is higher in women than in men, with SLE showing greater than a 10-fold ratio of women to men. All three are painful and debilitating diseases that result in loss of productivity and considerable health care costs. In this project we develop unique approaches directed towards eventually controlling these and potentially other autoimmune diseases, including Juvenile Diabetes Mellitus, by a two-pronged strategy based upon the molecules essential for specific activation of inflammatory T-cells, T-cell receptors and major histocompatibility antigens, and by our ability to produce human monoclonal antibodies to critical regions of these two molecules. The long term goal is to be able to selectively suppress the autoimmune diseases without compromising the ability of the individual to respond normally to infections and vaccinations.

Under previous ABRC support, we established that individuals suffering from RA attempt to prevent development of the disease by generating autoantibodies against recognition portions of T-cell receptors reactive against their own tissues. We generated monoclonal anti-T-cell receptors autoatibodies from RA patients and found that these suppressed the production of inflammatory molecules by T-cells. The ability of T-cell receptors to recognized antigens is dependent upon the presentation of the antigens by major histocompatibility antigens (MHC). We have recently shown that individuals with SLE often produce high levels of autoantibodies directed against regions near the antigen-presenting site of the MHC molecule. We propose to build upon these results to develop a two pronged approach to the modulation or prevention of the development of human autoimmune disease by generating human monoclonal antibodies against MHC and TCR combining sites and testing them in a unique transgenic mouse model where the mouse MHC class II genes have been replaced by human MHC genes, rendering them susceptible to experimental development of collagen induced arthritis and myasthenia gravis. If successful, this approach will result in the generation of novel therapeutic reagents with broad usage in human autoimmune disease. Because of the specificity of the TCR and MHC, it should prove possible to suppress the development of autoimmune disease without destroying the capacity of the treated individual to respond normally to infections.

Jesse D. Martinez, Ph.D.

University of Arizona
Award Amount FY07: $49,999

Nutritional Modulation of Colorectal Cancer Risks

Colorectal cancer is second only to lung cancer in causation of death due to cancer, and epidemiological studies have implicated diet as the most important factor in determining risk for this disease. Because diet is the most influential factor that determines the risk and incidence of this disease, a large number of studies have been conducted to identify those factors that either increase colon cancer incidence or suppress it. One study showed that bile acids (BA), which are derivatives of cholesterol, are produced to the liver and excreted into the intestine during digestion to aid in the absorption of dietary fat. Thus high fat diets promote increased bile acid production and both clinical and animal studies indicate that bile acids are associated with increased colon tumorigenesis. Interestingly, some polyunsaturated fatty acids (PUFA) such as those found in fish oil have been shown to suppress colon tumorigenesis and may be useful as preventive agents. In these studies we will test whether fish oil/PUFAs can inhibit the tumor promoting activities of bile acids using both cells in culture and animal models of colorectal cancer.

Our objective in these studies is to determine whether dietary supplementation with fish oil can be used to counteract the tumor promoting effects induced by carcinogens and by bile acids. We will test the notion that these compounds can change the signals that lead to cell growth and that the polyunsaturated fatty acids in fish oil can act to suppress tumorigenesis in an animal model. Finding that PUFAs can suppress tumorigenesis would suggest that dietary supplementation which increases these may be useful in lowering the risk for developing colon cancer.

Raymond B. Nagle, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Translational Regulation of Protein Expression in Prostate Cancer Progression

Laminin-alpha3beta3gamma2 (LM-332) is a major component of the basal lamina surrounding normal prostate glands whose expression is lost in prostate cancer. LM-332 is essential for forming stable adhesion structures that inhibit cell migration, and thus, prevent cancer metastasis. Loss of LM-332 in prostate cancer, therefore, leads to a more metastatic phenotype. Our previous work indicates that LM-332 is controlled at the translational level, meaning the mRNA for the protein is present in prostate cancer, but not translated into functional protein. We also have evidence that a translation-inhibitor protein, 4EBP-1, is upregulated in prostate cancer. Determining how LM-332 expression is lost in prostate cancer progression is important for understanding the mechanism by which this cancer becomes invasive. In Arizona, prostate cancer is the most commonly diagnosed cancer in the male population and is the second most common cause of death in Arizona males related to cancer. Clearly, a need exists to understand the molecular events that occur during prostate cancer progression in order to develop novel therapies for the disease. A prostate cancer's ability to develop an invasive and metastatic phenotype is arguably the most important determinant in the clinical relevance of prostate cancer, and therefore, the study of factors involved in the development of this phenotype is of primary importance. By understanding how LM-332 is lost in cancer progression, steps can be taken to re-express or maintain LM-332 expression to prevent metastasis.

The objective of this study is to identify the mechanism by which LM-332 translation is inhibited in prostate cancer. There is a crucial need for increased information about the mechanism by which prostate cancer becomes invasive for some men. The loss of LM-332 expression is directly related to prostate cancer invasion due to the loss of stable adhesion structures. Understanding the mechanism of regulation of this key protein in prostate cancer may lead to improved treatments to inhibit a migratory phenotype of prostate cancer from forming. The hypotheses to be tested are 1)that LM-332 protein is lost due to a lack of translation and that prostate cancer overexpressess the translation inhibitor 4EBP-1, 2) that the translation initiator protein eIF4E is sequestered by 4EBP-1 because of a failure of 4EBP-1 phosphorylation, and 3) that upregulation of 4EBP-1 causes a loss in LM-332 translation efficiency.

Mark C. Preul, M.D.

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

A Novel Peptide Mimetic for the Immunotherapy of Brain Tumors

This year, approximately 20,000 people will be diagnosed with a primary brain tumor, and 11,000 people will die of this disease. It has been estimated that at least 300 of these cases will be from Arizona. Over 150 new cases were seen at the Barrow Neurological Institute alone last year. This figure is likely to worsen in Arizona as the incidence of gliomas (the most common form of brain tumor) in people over the age of 65 has increased at a rate substantially higher than that of the general population. Furthermore, brain tumors are the second leading cause of cancer death in children under age 15 and in young adults up tp age 34.

Brain tumors are difficult to treat. Surgery and radiation therapy are effective but limited due to the risk each places on the surrounding normal brain tissue. A "good" surgical result can still leave the patient with severe physical incapacity, and "successful" radiation therapy to the brain can have deleterious long-term side effects. To date, chemotherapy has had only minimal success. Patients diagnosed and treated for glioblastoma multiforme, the most virulent form of the disease, have a mean life expectancy of one year, and there is an 80 percent mortality rate within two years. Although currently available therapy (including surgery, radiation and chemotherapy) kills up to 90 percent of the existing tumor cells, these tumors typically recur within a year and are refractile to further chemotherapy. The rapid recurrence of these tumors following therapy suggests the presence in the primary tumor of a cell type that is intrinsically resistant to radio-and/or chemotherapy. Improvement in the survival of these patients requires the design of new therapies. One promising avenue of research is the activation of the body's immune system to fight the tumor.

Scientists at the Barrow Neurological Institute (BNI) and Arizona State University (ASU) are collaborating to apply new technology to research on immunotherapy of cancer with emphasis on cancers of the brain. It is thought that our immune system can recognize tumor cells; however, this has little effect on cancer because macrophages, cells vital to the immune response, are rendered inactive by enzymes secreted by the cancer cells. Scientist at ASU have recently developed a peptide mimetic of a protein that stimulates the activity of a macrophage activating factor (MAF). This work, and the available literature, had led us to the hypothesis that appropriate stimulation of the immune system can effect tumor growth *in vivo*, providing therapeutic efficacy that will lead to prolonged survival. To prove this hypothesis we have partnered the pre-clinical and clinical expertise at BNI with the peptide expertise available at ASU to determine the effectiveness of this approach in the treatment of human cancer, particularly those of the central nervous system. Our overall objective is to complete the studies needed to take this treatment approach into human clinical trials. To this end, we propose the following specific goals: 1) use cultured cells from human brain tumors to demonstrate that we can activate microglia (macrophages from the brain) to kill tumor cells; 2) demonstrate that these cells do not produce anything known to cause inflammation; 3) begin the pre-clinical animal studies to determine the maximum tolerated dose of peptide mimetic, the best mode of administration (intravenous, skin patch, etc.) and the half-life of the mimetic in the blood; 4) demonstrate the absence of immunotoxicity and determine if this peptide mimetic is effective in the treatment of brain tumors; and 5) begin the large animal study required to obtain an IND to allow us to proceed into clinical trials.

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

University of Arizona
Award Amount FY07: $49,644

Pathophysiology of Menopause: Effects on Estrogen Withdrawal on Thermoregulation

Hot flushes represent a disorder of the brain's regulation of body temperature (thermoregulation) caused by the menopausal loss of estrogen. This disorder also occurs in older men, in young women with ovarian failure, and in patients receiving therapy for breast or prostate cancer. Hot flushes may last for up to five years and have a negative impact on the quality of life in many individuals. The most effective treatment of hot flushes is estrogen therapy, but recent studies have indicated that this treatment has significant health risks. Despite the vast numbers of individuals experiencing hot flushes, there is little understanding of what causes hot flushes and few laboratories devoted to studying this phenomenon in animal models. This project is designed to start a collaborative research program between scientists in Tucson and Phoenix with expertise in reproductive biology and thermoregulation. Our goal is to provide basic information on how estrogen withdrawal effects the regulation of body temperature. Increased understanding of the cause of hot flushes would greatly facilitate the development of effective treatments. This information is particularly relevant to the state of Arizona where there are 900,000 women over the age of 45 who have been or soon will be affected by menopause. In fact, this information would be beneficial for all women who will eventually reach menopausal age.

A menopausal flush is a disorder of body temperature regulation caused by estrogen withdrawal and characterized by an inappropriate activation of the physiologic mechanisms to remove body heat. In a flush episode there is dilatation of blood vessels (vasodilatation) which appears as flushing and can be measured by changes in skin temperature. In rats vasodilatation of the tail is a major mechanism to remove body heat, and this response can be studied by measuring tail skin temperature. In this project, we will initiate a collaborative effort between experts in the fields of reproductive neuroendocrinology and thermoregulation to study the effects of estrogen withdrawal on tail vasodilatation in rats. We propose experiments that are based directly on the observations of flushing in postmenopausal women. Body warming provokes flushing in humans; therefore, we hypothesize that estrogen withdrawal makes brain thermoregulatory networks more sensitive to increases in room temperature. We will test this hypothesis by studying the effects of estrogen withdrawal on the pattern of tail vasodilatation after raising the room temperature. We will also determine if environmental warming of estrogen-deprived rats will change the patterns of brain activation in cell groups that are thought to be triggers for hot flushes in women. These experiments will shed light on how estrogen withdrawal affects body temperature regulation and, thereby, contribute to our understanding of the cause of hot flushes.

Seth D. Rose, Ph.D.

Arizona State University
Award Amount FY07: $50,000

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

It is estimated that cancer killed 9,710 to Arizonans last year with 23,560 new cases being diagnosed. This causes major suffering and has a significant economic impact. Successful drug treatments for a wide variety of cancer as an alternative to surgery and radiation remain elusive. Cancers that are initially responsive to drugs become resistant, and the drug treatments typically have unpleasant side effects. Therefore, a pressing need exists to find effective and nontoxic anticancer drugs.

Cancer consists of the unrestrained growth of abnormal cells and their spread throughout the body. Ordinarily, cells that become abnormal have checkpoints in their development when the cell examines itself for abnormalities. If abnormalities are found and cannot be repaired, a mechanism is initiated that shuts the cell down before the organisms is harmed from development of a tumor. Unfortunately, cancer cells can evade this system in a number of ways. One is to destroy the protein in the cell that brings about the self-destruction of the abnormal cell. The protein that would normally eliminate the cancer cell is taken out of commission, and the cancer cell is free to continue to grow, divide, and invade new tissues.

Another way cancer cells achieve their formidable ability to invade and proliferate is by altering the normal process of cell growth and division. Cells normally respond to growth-limiting messages from other cells, and they divide only when they receive signals from other cells indicating that division is needed. Cancer cells, instead, stimulate themselves to grow and divide independently of the normal mechanism that regulates growth, resulting in constant growth and division.

The cancer cell's ability to evade the process of self-destruction and to constantly stimulate its own division underlies the hallmark behavior of wild proliferation and spread of cancer cells.

Cancer cells evade the process by which damaged cells undergo a pre-programmed set of steps resulting in death of the abnormal cell to protect the organism as a whole. They do this by eliminating a key protein in the process, a tumor suppressor protein, by chemically tagging it for destruction. We plan to develop chemical agents that block the tagging of the tumor suppressor protein for destruction.

Cancer cells stimulate their own growth and proliferation. Due to their greater growth rate, they can be much more dependent on cellular material and dynamics than normal cells. Cancer cells are particularly vulnerable as they prepare for and enter cell division. One of the needs of cancer cells is to rearrange the protein framework required for separating chromosomes into the two newly formed cells. We plan to develop chemical agents that block the cancer cell's ability to build that framework so the cell cannot successfully divide.

Each of the above processes require a protein that utilizes a key amino acid, cysteine. The same chemical reactivity that the protein needs cysteine for can be the Achilles' heel of the cancer cell. We propose to develop chemical agents that certain cysteines in the cancer cell will convert into highly toxic substances right at the protein in the cancer cell. This innovative strategy is a potentially powerful and new way for knocking out key processes in the cancer cell's method of achieving unrestrained proliferation and invasiveness.

The specific goals of this work are to develop and exploit (through design, preparation, and testing of new chemical agents) this strategy designed to inhibit cancer cell proliferation pathways based on their need to stimulate their own growth, subvert tumor suppressor protein, and to pull chromosomes apart at cell division.

The overall guiding hypothesis is that effective, specific inhibition of cancer cell proliferation pathways can be achieved by use of substances that are converted to active, toxic forms by the action of cellular proteins themselves.

Philip M. Service, Ph.D.

Northern Arizona University
Award Amount FY07: $45,259

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

It is known that genes (as well as environment) influence life span in many species, including humans. Studies of mutations have shown that several genes, including some that are involved in insulin-signaling and in nerve transmission, can influence life span in several animal species. Such studies may inform us about the physiological processes that influence the rate of aging. Nevertheless, very little is known about which genes are actually responsible for the naturally occurring genetic variation in life span that is characteristic of humans and other animal species. Discovery of those genes will provide a door both to greater understanding of the causes of aging and to possible interventions in the aging process.

Identification of genes that are responsible for naturally occurring variation in life span is difficult and very much so in humans because of our long life span and the impossibility of conducting controlled experiments. Biologists frequently use other species, like mice, fruit flies, worms, and yeast as model organisms to investigate phenomena that are also found in humans such as in this case, aging. Fruit flies (*Drosophila*) have many advantages for the study of aging. They exhibit natural genetic variation in life span, their life span can be modified easily in the laboratory, and numerous genetic tools (including the *Drosophila* genome sequence) are available. Depending upon the level of stringency used, one-third to three-fourths of all known human disease genes have counterparts in *Drosophila*. That degree of similarity strongly suggests that genetic studies with flies will be relevant to the understanding of life span variation and aging in humans.

As the beginning of the baby boom generation nears retirement, the population of the United States is aging. Because Arizona is an attractive location for retirement, the impact of an aging population will be even greater here. The elderly have increased needs for health care and social services. Understanding the genetic basis of aging may enable us to improve the health and quality of life of our older citizens, and reduce demands on our health care and social services systems.

This research project will build upon earlier work that identified 10 chromosome regions that contain life span genes in flies. Each of these regions, however, contains many genes in addition to those that control life span. The goals of the present project is the increase the precision of our estimates of gene location so that we may take a significant step toward the ultimate goal of identifying the actual life span genes. Our experimental design should permit a five- to ten-fold increase in the precision of our estimates of gene location. Then, using the information from the Drosophila genome, we may be able to identify candidate life-span genes for further investigation, or we will propose one more round of high-precision mapping experiments. Once our estimates of gene location are sufficiently precise, we will use DNA-based methods such as sequencing to identify the molecular basis of life span variation.

Edward B. Skibo, Ph.D.

Arizona State University
Award Amount FY07: $50,000

Non-nucleotide Inhibitors of IMP Dehydrogenase

A 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. Consequently, much effort has been devoted to designing IMP dehydrogenase inhibitors over the past four decades. Many of the candidate IMP dehydrogenase inhibitors are nucleotides that can interfere with other aspects of purine metabolism. The strategies for designing non-nucleotide inhibitors of IMP dehydrogenase are to design a purine ring mimic tethered to amino acid residues that binds to type II enzymes. The role of the tethered amino acids is to compensate for the loss of binding due to the absence of the ribofuranosyl and phosphate moieties found in the natural substrate IMP. Preliminary studies have shown that potent non-nucleoside IMP dehydrogenase inhibitors can be designed in this way. The amino acid fragment is also postulated to assist in the active uptake of the inhibitor. Preliminary studies have led to the design of a melanoma - specific candidate drug that takes advantage of a phenylalanine-specific pump. The proposed project will pursue the design of cancer specific IMP dehydrogenase inhibitors using computer models and amino acid mediated cellular uptake processes. To be sure, the further development of these specific cancer drugs will be beneficial to the people of Arizona, particularly those afflicted with melanoma cancers.

The postulates governing non-nucleotide IMP dehydrogenase inhibitor designs are that the pyrrolo-benzimidazole ring system is a suitable purine ring mimic and that amino acids can be tethered to this ring to facilitate enzyme active site binding. The role of the tethered amino acids is to compensate for the loss of binding due to the absence of the ribofuranosyl and phosphate (nucleotide) functionalities. In addition, the peptide tether is proposed to facilitate the active uptake of the IMP dehydrogenase inhibitor into select histological cancer types. Preliminary studies have supported these postulates by affording a melanoma-specific IMP dehydrogenase inhibitor. The project will validate these postulates by carrying out three objectives. The first objective of the project is to design, synthesize, and screen potential type II IMP dehydrogenase inhibitors. The computer model used to design non-nucleotide IMP dehydrogenase inhibitors predicts that tight binding analogues, specific for type II IMP dehydrogenase, are possible when novel amino acid and other organic residues are tethered to the pyrrolo-benzimidazole ring system. The second objective is to carry out kinetic studies of the inhibition process so as to clarify the inhibition mechanism. Although amino acid-linked pyrrolo-benzimidazoles inhibit IMP dehydrogenase, mechanistic details of the inhibition (reversible or irreversible inhibition, or the presence of alkylation) are not yet known. The third objective is to document cellular uptake of these inhibitors employing ^{14}C-labeling. Meeting this objective will prove the postulated active uptake of peptide-liked IMP dehydrogenase inhibitors by histologic cancer types such as melanoma.

Lucy J. Treiman, Ph.D.

St. Joseph's Hospital

Award Amount FY07: $ 50,000

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

Febrile seizures are seizures that occur in normal children, ages 3 months to five years, at times of high fever. Children with febrile seizures develop chronic epilepsy four times more often than children who do not experience febrile seizures. Why febrile seizures increase the risk of epilepsy is not known. There are experimental models in rats that can be used to try to answer this question. What is going on in the brain to cause this increased susceptibility to seizures is not well understood. Some experiments have been done that show that brain cells are more excitable, even in adult rats, after having febrile seizures as infant pups. We think that such long-term changes in the brain, that may explain how febrile seizures cause subsequent epilepsy, are likely caused by changes in the way genes controlling brain development and brain excitability are turned on and off. If we can understand which genes are affected by febrile seizures, we may be able to develop ways to prevent the development of epilepsy in children who have febrile seizures.

This is an important issue for Arizona. There are approximately 435,000 children under five years of age in the state of Arizona. At least 5 percent will have febrile seizures and thus be at risk for the development of chronic epilepsy. If 4-5 percent of these children develop chronic epilepsy, this will translate to direct health care cost of at least $2,000,000 to $3,000,000 per year. Knowledge that could be translated into the development of prophylactic measures to prevent chronic epilepsy after febrile seizures has the potential of a substantial economic impact.

The longer-term goal of this research program is to identify treatment interventions that could be used to prevent chronic epilepsy from developing in children who experience febrile seizures. Thus far, attempts at preventing subsequent seizures by treatment interventions with antiepileptic drugs or antipyretic drugs (drugs to prevent fever, like aspirin or acetaminophen) have not been successful. The immediate goal of this research is to better understand the ways in which febrile seizures in normal children may cause chronic epilepsy. In this model, 10 day old rat pups are exposed to a stream of warm air for 30 minutes. This has the effect of raising their body temperature to the point that they experience a series of seizures. We will expose a second group of rats to the stream of warm air, but prevent seizures by giving them an antiepileptic drug before exposing them to the warm air stream. Another control group will be a group of rats will be exposed to a stream of air at room temperature, so their body temperature will not rise and they will not have seizures. We will compare the three groups described above by exposing each group at 10 weeks of age, the adult age for a rat, to a chemical called kainate which can cause seizures in the rat, using a dose that is lower than the one usually required to cause seizures. We expect the rats that had febrile seizures to have seizures when given the low dose of kainate as adults. The rats that did not experience febrile seizures will not have seizures when exposed to the low dose kainate. In this way we will verify that this model of febrile seizures works in our laboratory and that febrile seizures in 10 day old rat pups make adult rats more susceptible to epileptic seizures.

Tsu-Shuen Tsao, Ph.D.

University of Arizona
Award Amount FY07: $ 49,999

Biological Function and Chemistry of Adiponectin Oligomerization

The Center for Disease Control has estimated that 18 million people, 6 percent of the population, have diabetes in the United States. In the state of Arizona, with its large population of the elderly and Hispanic and Native Americans, this critical public health concern is even more severe. Nearly one fifth of all people age 60 years or older has diabetes. In southern Arizona, an astonishing 28 percent of Native Americans have diabetes. The most common form of diabetes, type II, is characterized by the patients' bodies not responding effectively to the hormone insulin. Specifically, diabetic patients do not absorb glucose from the bloodstream as well as non-diabetic individuals in response to insulin. Common anti-diabetic drugs Actos™ and Avandia™ work by improving the body's response to insulin. A common side effect of these drugs is weight gain because, in addition to lowering blood glucose, insulin also promotes fat storage. Adiponectin is a hormone secreted from fat tissue that can circumvent this problem. It enhances insulin's ability to lower glucose levels but, unlike insulin, stimulates fat burning. Diabetic patients have decreased circulating levels of adiponectin. Among the Pima Indians of Arizona, low levels of adiponectin are associated with increased risk of developing type II diabetes later in life. The mechanisms by which adiponectin enhances the effects of insulin remain unclear. Elucidating this mechanism is complicated by the fact that adiponectin exists in the bloodstream as three distinct forms that differ in size. The relationships of these different forms of adiponectin to insulin's metabolic actions are poorly defined. This is a major obstacle in developing adiponectin into a drug to treat diabetes.

The ultimate goal of this project is to understand the mechanisms by which adiponectin enhances insulin action and how decreased adiponectin levels contributes to type II diabetes. One of the ways Actos™ and Avandia™ are thought to improve the body's response to insulin is by increasing the levels of adiponectin, particularly the largest isoform, suggesting that it has the potential to be used therapeutically. Additional support for this idea came from an observation linking increased levels of the largest adiponectin isoform to weight loss. This is important because obese individuals often have lowered response to insulin, which can be improved by losing weight. Lastly, the largest adiponectin isoform has been shown to be extremely stable in circulation, making it an ideal candidate to be developed into a drug that does not require frequent injections. This proposal contains experiments designed to better understand the composition, assembly, and biological processing of the large form of adiponectin so that it can be better developed as a drug. The first major aim of this proposal is to define the composition of the largest adiponectin isoform. The second major aim is to determine how it is assembled. While the largest adiponectin isoform is the most stable, the smallest isoform is more effective in enhancing the body's response to insulin. For this reason, it has been hypothesized that the largest isoform is converted to smaller forms in circulation. The third major aim is to test whether and how this process occurs.

Marlys H. Witte, M.D.

University of Arizona
Award Amount FY07: $49,672

Massage Therapy in Childhood Lymphedema (CLE)

The swollen or unequal legs or arms of children are most commonly due to a birth defect of their lymphatic system (termed "congenital lymphedema") that alters the removal of excess tissue fluid (lymph) by lymphatic vessels. These children often suffer severe physical and psychological disabilities that are occasionally life-threatening. At least several hundred and, more likely, thousands of Arizona children are affected by CLE, and 10 percent or more involve multiple family members. These defects can be accompanied by associated birth defects, infection, organ dysfunction, and malignancy. The understanding, evaluation, rational classification, and treatment of CLE and its sequelae have been neglected. Both non-operative and operative management has been particularly vexing in the infant and growing child in the absence of evidence-based standardized protocols with demonstrable efficacy. This project 1) examines the feasibility of a simplified method of massage alone rather than more complex, labor-intensive, demanding protocols for lifelong management of this chronic condition; 2) attempts to determine which groups of well-defined children with limb lymphedema respond to this regimen and what are the factors which influence this response; and 3) evaluates how well the child/family comply with treatment, and what long-term benefits and satisfaction results. This preliminary study should provide the background and rationale for a more rigorous standardized multi-center controlled international trial, coordinated in Arizona through global telecommunication, comparing various treatment regimens for relative short-term and long-term efficacy and acceptance in children with lymphedema, their families, and other care-givers.

The primary goal of this research is to test the ability of a specific form of massage to reduce swelling in the limbs of children with lymphedema. This is directly relevant to hundreds, if not thousands, of disabled Arizona children and will also impact hundreds of thousands of disabled children worldwide. Through careful study and analysis, quantitative outcomes such as reduced limb volume, impaired bioelectrical impedance, and associated increased mobility along with qualitative outcomes such as improved psychosocial adjustment will be assessed after short- and long- term treatment. The objective is to determine the percentage of children who respond to this simpler, less demanding therapy, the characteristics of those who do and do not respond (predictive factors), and the usefulness of this therapy over an extended (6 month) period for both limb volume reduction and improved quality of life.

George T. Wondrak, Ph.D.

University of Arizona
Award Amount FY07: $50,000

Melanoma Cell Survival Signaling by Glycolytic Intermediates

The rising incidence of nonmelanoma and melanoma skin cancers in the state of Arizona is a public health problem of increasing concern. Metastatic melanoma, a highly aggressive tumor that originates from pigment producing cells in human skin, poses a formidable therapeutic challenge with poor treatment options. In the state of Arizona, melanoma accounts for only 5 percent of all skin cancers but causes almost 80 percent of all skin cancer deaths. My recent published research suggests that a pathological process called carbonyl stress may represent an Achille's heel of human melanoma cells. Based on my preliminary experiments with human melanoma cells, I have designed a novel class of prototype drugs that block cellular carbonyl stress and effectively kill melanoma cells without harming normal cells. This research project aims to elucidate the detailed molecular mechanism by which these agents kill melanoma cells in order to develop novel potent anti-melanoma therapies.

The hypothesis of this project is that human melanoma cells are resistant to stress and anti-cancer drug treatment because they use small sugar breakdown products, formed from glucose during energy production, as novel survival factors. These reactive survival factors act by chemical modification of cell proteins that regulate cell death pathways. Therefore, agents that can neutralize the activity of these melanoma survival factors are potentially powerful anti-melanoma drugs.

The first aim is to detect and quantify the sugar-derived survival factors in human melanoma cells, target identification; aim 2 is to elucidate the cellular mechanisms by which these survival factors render melanoma cells resistant to stress and anti-cancer drugs, target validation. Aim 3 is to test novel agents, carbonyl scavengers, that neutralize the melanoma survival factors and thereby kill melanoma cells without harming normal cells, target elimination. This research project addresses a major gap in our understanding of melanoma survival pathways, critical for the targeted elimination of melanoma cells in cancer patients. Research data obtained from this project will be used to lay the foundation for future competitive NIH grant applications that will lead to the successful discovery and development of better anti-melanoma drugs in my laboratory.

Yitshak Zohar, Ph.D.

University of Arizona
Award Amount FY06: $50,000

Novel Applications of Nano-technology: Micro-devices for Capture & Analysis of Circulating Tumor Cells

The goal of this research project is to create new technology that can capture specific populations of cells from complex mixtures of cells. An example of such a mixture is whole blood which consists of many different kinds of cells including red and white blood cells. To create this technology we propose to use micro-fabrication methods that have largely been developed for manufacturing the microprocessors in cell phones, personal computers and other sophisticated electronic devices. Our philosophy is to use these micro-fabrication techniques, which are rooted in the semiconductor industry, in concert with methods of modern molecular biology. Our ultimate goal is to create a micro-device that can selectively target and extract circulating metastatic tumor cells (CTCs) from the blood of cancer patients. The cancer cells thus extracted can then be studied and the resulting information used to tailor detailed cancer treatment strategies to the individual patient from whom the cells came.

Circulating prostate and breast cancer cells have cell-surface/cell-adhesion molecules, called cadherins, which are distinct from those of blood and immune cells. We will take advantage of these differences to capture the CTCs. Moreover, the proposed methodology for sorting cells is not limited to cancer and has a variety of applications relevant to biomedical issues facing Arizona. If successful, results obtained from these investigations will provide a new technology for isolation of various rare populations of cells including circulating (endothelial) stem cells. Once selected, a target population of cells can be recovered and grown in culture or analyzed directly for their "molecular signature" to formulate treatment strategies. Note that prostate cancer is now recognized as most commonly diagnosed visceral neoplasm in the male population of the United States. Also, nearly 183,000 new cases of breast cancer were diagnosed in the year 2000. Estimates for 2005 are that approximately 211,240 women will be diagnosed with the invasive form of the disease (American Cancer Society, 2005). Early detection and treatment of the cancer improve the possibilities for long-term survival of patients. Thus, better detection of metastatic disease is an important objective of cancer research.

This project involves three major steps, each of which will yield innovative results relevant to cancer detection and treatment. First, one must develop cell-capturing techniques and chemistries based on the properties of the cadherins on the surfaces of CTCs. Second, semiconductor-processing methods, known as micro-machining and surface modification, must be developed to capture CTCs at precise positions within a micro-device. Third, integration of the CTC capture mechanism into a complete micro-device that includes integrated networks of micro-channels, fluid reservoirs, driving electrodes, pumps, electrical circuits, etc.

SECTION E

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Principle Investigators