New York State Center of Excellence in Bioinformatics & Life Sciences
The University at Buffalo

I. MISSION AND OBJECTIVES

A. Mission Statement and Statement of Purpose

The overall mission of the New York State Center of Excellence in Bioinformatics and Life Sciences at the University at Buffalo is to study the mechanistic processes involved in human disease with the goal of developing diagnostic tools and therapeutic interventions, preventative treatment, and other disease management devices and processes to alleviate the suffering of humankind. As part of that primary mission, the New York State Center of Excellence in Bioinformatics & Life Sciences will also act as a facilitator of economic development via Government/Academia/Industry partnerships. This includes facilitation of the definition of the Western and Upstate New York bio-economy, a complex, highly competitive process that is being undertaken in many communities throughout the U.S.. The bio-economy issue is described more fully in section **V.A.**

B. Identified Goals and Objectives

1. To apply the concepts, methodologies, and technologies related to biomedical informatics, including public health epidemiology, computational biology, genomics, proteomics, structural biology, disease modeling, and biomedical engineering to significant human health problems.

2. To develop fundamental discovery-based understanding of human diseases such as cancer, infectious diseases, cardiopulmonary disease, and neurological diseases, necessary to effectively develop diagnostic modalities, drugs, behavioral modifiers, and other strategies to prevent, treat, and otherwise alleviate human suffering.

3. To proactively transfer this fundamental knowledge to the development of devices, therapeutics, algorithms, and other strategies to manage human disease.

4. To develop educational programs which will assist in the development of a cadre of scientists and technical personnel who will work in the area of life science research and the biotechnology industry.

II. GOVERNANCE

A. Governance Structure

The Governance Structure of the New York State Center for Excellence in Bioinformatics and Life Sciences at the University at Buffalo is illustrated in **Attachment 1**.

The Executive Council ("the Council") will be initially constituted as a panel comprised of COE partners and other community and industry representatives. The Council will be charged with providing strategic advice related to the growth and development of the Center, and will work to support the development and execution of a comprehensive long-range business plan for the Center's operation. The initial membership composition is outlined in **Attachment 2**. The UB Provost and the Executive Director of the Center of Excellence will receive counsel from the Executive Council, and its responsibility will be advisory in nature. Additionally, the Council will receive guidance from Federal and State officials in acknowledgement of their influence on the Center's funding and mission implementation.

The Scientific Advisory Board **(Attachment 3)** will consist of outstanding national and international scientists in the fields of bioinformatics and life sciences. The Scientific Advisory Board will meet biannually with the senior scientific leadership of the Center to review programs, progress and scientific strategy. The Academic Advisory Board **(Attachment 4)** will consist of individuals in the region with academic, educational, and workforce development responsibilities. This group will provide advice and assistance with development of undergraduate, graduate, certificate and K-12 programs, and will be comprised of cognizant UB Deans, academic leaders from UB and partner organizations, and specific subject matter experts in the area.

The Economic Development Advisory Board **(Attachment 5)** will provide expert advise and counsel on matters related to commercialization. This Advisory Board will assist with the implementation of Center discoveries and will be comprised of COE partner representatives, community, government and industry leaders.

III. ORGANIZATIONAL SUMMARY

A. Organizational Ownership/Affiliation

The University at Buffalo is the lead academic institution for the New York State Center of Excellence in Bioinformatics and Life Sciences. UB's academic partners include Roswell Park Cancer Institute and the Hauptman-Woodward Medical Research Institute.

B. Organizational Structure

An Executive Director will be responsible for the management of the Center and all of the Center's day-to-day activities, scientists, and staff. The Executive Director will report to the Provost/Executive Vice President for Academic Affairs of the University at Buffalo. A Chief Operations Officer for the Center will have fiduciary management responsibilities and oversee staff and facilities operations. The Operations Officer will also be charged with assisting the Executive Director with other Center administrative activities, including report writing, grant writing, and personnel administration. The Operations Officer will be assigned to and funded by the Center.

An Economic Development Unit will also be developed to support the overall mission of the Center, and will include formal relationships with BuffLink, UB STOR, and the UB Office for Government Affairs. The Economic Development Unit will assist the Executive Director with industrial partners and interactions with officials from local, regional, and state economic development agencies.

Initially, the organization will also include the following research units:

- Data Intensive Analytical Bioinformatics Core Group
- Drug Discovery & Delivery Group
- Cancer Biology Group
- Neurodegenerative Diseases Group
- Cardio-Thoracic Disease Group
- Pathogens & Biodefense Group

All administrative and research units will report directly to the Center's Executive Director (**See Attachments 1, 6**).

C. Organizational Locations and Facilities

The main site of the New York State Center of Excellence in Bioinformatics & Life Sciences will be the 400,000 square foot Life Sciences Complex located on Virginia Street in downtown Buffalo. The collaborating institutions on the Life Science Complex – University at Buffalo, Roswell Park Cancer Institute and Hauptman-Woodward Institute – will carry out a joint plan for the completion and operation of the necessary research facilities and equipment to support participating faculty and staff in the Center of Excellence. Ultimately, the Life Sciences complex will be comprised of three linked buildings which will house the Center for Genetics and Pharmacology managed by Roswell Park Cancer Institute, the Bioinformatics and Drug Discovery facility managed by UB, and the Center for Structural Biology managed by HWI. Although the Life Sciences Complex will be the main site of the Center of Excellence, certain Center members may also be housed outside of the Complex as necessary.

a. Policy for Shared Facilities/Laboratories Usage.

The Life Sciences Complex will consist of three components; the interior of each will be designed by the institution managing the space. The components will be joined by common space and shared services currently being planned to include, for example, the power plant, loading docks, conference and meeting space. Exterior architecture will be compatible with standards set by the Buffalo Niagara Medical Campus. Administratively, each institution will maintain its own identity, the integrity of its own research programs and interior space. The building construction will be funded from appropriations which will be administered by the Empire State Development Corporation (ESDC). ESDC will be the lead agency for the project and will coordinate state financing Project Management will be coordinated through the Dormitory Authority of the State of New York (DASNY). The partner institutions will carry no indebtedness (neither principal nor interest) related to the State appropriation.

UB will have a 99-year, renewable sublicense or land lease covering the footprint of the Bioinformatics and Drug Discovery Building. The license/lease will have covenants that restrict the use of building to life sciences, health sciences, and/or academic activities compatible with RPCI's and UB's biomedical research missions. DASNY has contracted with Francis, Cauffman, Foley, Hoffmann to design the Life Sciences Complex. There will be a single construction manager, design consultant and bidding process for the entire Life Sciences Complex. However, the design and planning allows one building to proceed at a faster pace than the other without impacting the overall project or project management.

Routine maintenance, housekeeping, security, grounds keeping, as well as utility costs for the entire complex are handled through a UB lease with RPCI to ensure consistency and cost effective delivery of services. Each institution will be responsible for capital expenditures related to infrastructure, renovations and exterior structural maintenance of their respective component. Building specific regulatory mandates will be the responsibility of the respective institution. RPCI will be responsible for regulatory compliance for the shared space/services. The responsible party will address immediately regulatory non-compliance issues that impact the functioning or health and safety of the other employees. The Life Sciences Complex will be maintained to the standards of Roswell Park Cancer Institute and the guiding principles of the BNMC master plan. Required future capital investments to renovate/upgrade shared space and services, including the power plant, will be joint responsibility of RPCI and UB. Capital investment in each component will follow the respective RPCI and UB capital budget planning process.

Each institution will manage the programs and functions within its own component. The UB lease with RPCI should take care of all but the most unusual circumstances. A small Operations Committee (selected users and respective administrators) will meet regularly to resolve any issues related to utilization of shared space, joint programming, and regulatory requirements (OSHA, environmental safety, etc.) If, for some unforeseen reason, issues cannot be resolved through normal business channels, UB, RPCI, and HWI agree to employ national standards.

D. Core Resources

The New York State Center of Excellence in Bioinformatics & Life Sciences includes six inter-related units, including core laboratories for molecular targeting; gene expression; proteomics; structural biology; disease modeling; epidemiology; pharmacodynamics; nanotechnology; imaging; and bioinformatics. The Center's research units will build upon key core resources at UB, Roswell Park Cancer Institute, Hauptman-Woodward Institute, and the Kaleida Health System.

a. Policy for Shared Resources Usage. The collaborating partners, UB, RPCI, and HWI will support and pursue significant programs of collaborative research in order to utilize the strengths of each institution to promote and invigorate the research at each institution. Each institution shall make in-kind contributions to these collaborative core research activities in terms of equipment, lab space, financial resources and staff, and agree to use best efforts to provide substantially equal levels

of support to the collaborative activities. Charge back protocols will be jointly initiated to help defer costs of core resources.

All discoveries, inventions, ideas and concepts, whether patentable or not, conceived, discovered, made or otherwise developed through the collaborative core research facilities of the Life Sciences Complex, through collaboration between research faculty at two or more of the partners shall be proprietary property of each institutions, and shall be subject to the policies and procedures governing proprietary information and material, and governing technology transfer activities, at each institution. The parties shall endeavor to agree in advance to the projected percentage participation of each institution in each individual research collaboration project, and that the proprietary interest of each institution shall conform to that specified percentage unless otherwise agreed by the parties in writing subsequently.

E. Other Organizational Assets - CUBRC

The Buffalo-Niagara Region is an ideal location to build a Life Sciences Biotechnology Cluster based on the area's existing rich academic and research resources – the University at Buffalo, the Roswell Park Cancer Institute, and the Hauptman-Woodward Institute. The Center will also benefit from its relationship with Calspan-UB Research Center, Inc. (CUBRC), an independent, not-for –profit corporation established under IRS code as a 501c(3) with a mission to generate technological and economic growth in Western New York and New York State. CUBRC sales in Bioinformatics and Life Sciences areas were estimated at $14 million in 2004, predominately, but not exclusively, in the Pathogens & Biodefense area where CUBRC possesses a TOP SECRET facility clearance. CUBRC has the ability to invest in the formation of life science start-up companies and to leverage a variety of relationships with existing large corporations and the U.S. Department of Defense. Among other Buffalo-Niagara regional assets to the Center's biotechnology initiative include over 100 active life science biotechnology firms, which consist of 50 medical device firms and 34 biotechnology pharmaceutical firms. In addition, Buffalo (described as the epicenter of the golden horseshoe) links over 800 life science companies and 24 research institutions from the upstate NY/southern Ontario region spanning from Syracuse to Toronto.

IV. PROGRAM AND OPERATIONS

A. Research & Development

The New York State Center of Excellence in Bioinformatics & Life Sciences will distinguish itself from the myriad of other programs that now exist throughout the world. The opportunity for this

distinguished presence in the field of bioinformatics will require a broad vision to capture our existing strengths in the areas of basic research and science, public health and translational biomedical sciences, and our potential strength in discovery and commercialization.

To accomplish this goal, the Center will be comprised of inter-related specialty laboratories that can function alone and in sequence to facilitate the process of disease mechanism and therapy discovery. As proposed, the following research groups include:

- Data Intensive Analytical Bioinformatics Core Group
- Drug Discovery & Delivery Group
- Cancer Biology Group
- Neurodegenerative Diseases Group
- Cardio-Thoracic Disease Group
- Pathogens & Biodefense Group

By combining the Center's research capabilities in information acquisition, storage, analysis, and presentation of informatics with the vast technological, industrial, economic and societal potential of high throughput biological sciences, bioinformatics will enable the development of state-of-the-art techniques for modeling and decoding of complex biological systems, leading to the identification of new medical treatments, efficient drug discovery and delivery techniques, improved therapeutics protocols, and fabrication of innovative medical devices and systems. Involved in these activities are approximately 100 scientists from UB, RPCI, HWI, Kaleida Health, Invitrogen Corporation, and GE Global Research. All current local Center Scientists have substantial research grant portfolios. In addition to these individuals, we anticipate a requirement for hiring an additional 20 new principal investigators both to expand existing areas of strength and to further develop vital new skills and technologies within the Center. Partnerships have been developed with Biogen, Hunter's Hope Foundation, Invitrogen Corporation, GE, and Oracle for the co-development of technologies and support for new faculty hiring

Specific bioinformatics technology development and prototyping themes supported by the Center include:

- Therapeutic Lead Identification – Genomics, Proteomics, Molecular Genetics, and Disease Biology;
- Target Validation – In-vitro and In-vivo Disease Modeling for Target Validation;
- Therapy Design and Development – Structural Biology, Combinatorial Chemistry
- Therapy Evaluation and Prototyping – Drug Scale-Up and Pre-Clinical Toxicology

- Clinical Studies and Implementation – Trait Analyses, Epidemiology, and Therapy Trials

As noted above, these themes will be applied within the six primary organizational Units of the Center to enable its mission. Brief descriptions of the Center Units are provided below, with detailed project descriptions given in **Attachment 7.**

1. **Data Intensive Analytical Bioinformatics Core Group – Dr. Norma Nowak, Director**

   This Unit within the Center will act as a fundamental technology resource for Center members and the collaborating scientific community. As designed and managed by Dr. Nowak, the Data Intensive Analytical Core will coordinate and expand the talents and resources of several existing units within the Buffalo research community that will enable all other research areas within the Center. It should be noted that this Unit will not simply act as a general core facility, but will conduct funded research and development related to the associated technologies, many of which already represent proprietary work by Center members. Scientists and technologies represented by this Unit of the Center of Excellence include:

Bioinformatics Sciences:

Analytical Bioinformatics
Alan Hutson, Ping Liang, Matthew Beal, Daniel Gaile,
Predictive Bioinformatics
Jeffrey Skolnick, Yaqui Zhou, Feng Qin, Sriram Neelamegham
High Performance Computing and Visualization
Russ Miller, Tom Furlani, Aidong Zhang
Biomedical Informatics
Barry Smith, Marnie Lavigne, Gina Fideles, David Ellis

Functional Genomics Sciences:

Biorepository & Population Health
Christine Ambrosone, Maurizio Trevisan, Rick Munschauer
Genomics
Norma Nowak, Marc Halfon, *Andreas Califano,* Stelios Andreadis, Mattheos Koffas, Manolis Tzanakakis
Proteomics & BioConformatics
Michael Lubman (TBH), Ken Blumenthal, *Vishu Lingappa**
Structural Biology
George DeTitta, Herb Hauptman, Wayne Schultz, Timothy Umland, Robert Blessing
Gene Targeting and Transgenics
Yuejin Yu

Bioengineering Sciences:

Imaging
Robert Zivadinov, Ken Hoffman, Steve Rudin, Robert Miletich,

Eva Sevick-Muraca(TBH)

Nanomedicine

M. Kahn, Alan Oseroff, Paras Prasad, *Michael Boothman (TBH)*

2. Cancer Biology Group – Dr. John Cowell, Dr. Michael Brattain, Directors

This Unit within the Center will identify targets to inhibit tumor growth and cell proliferation. The Cancer Unit is directed by scientists supported by Roswell Park Cancer Institute and will oversee the work of Center members located in the Genetics and Pharmacology wing of the Life Sciences Complex, as well as new members housed in the Bioinformatics and Drug Discovery wing. The Unit will focus on tumor growth related to Neurological cancer, Melanoma and Lung Cancer, Colon cancer, Prostate cancer, and Breast cancer. Scientists and project areas represented by this Unit of the Center of Excellence include:

Colon Cancer

Michael Brattain

Neurological Tumors

John Cowell, Norma Nowak, David Goodrich

Melanoma and Lung

Alan Oseroff, Carl Porter, Paras Prasad, Christine Ambrosone

Prostate Cancer

Donald Trump, Candace Johnson, Robert Straubinger, Jo Freudenheim, Irwin Gelman

Breast Cancer

Michael Brattain, Christine Ambrosone

Lung Cancer

Christine Ambrosone

3. Neurodegenerative Disease Group – Dr. L.N. Hopkins, Director

This Unit within the Center will investigate disease mechanisms of several neurological disorders, including Alzheimer's Disease, Multiple Sclerosis, Krabbe's Disease, Parkinson's Disease, and Neurovascular Diseases. In particular, this group will look at demyleniation, neuroinflammatory processes, amyloid plaque formation and loss of cellular function. This Unit will also coordinate molecular and genetic analysis with imaging informatics approaches as applied to neurological problems. Scientists and project areas represented by this Unit of the Center of Excellence include:

Molecular and Genetic Neurodegeneration

Zhen Yan, John Cowell, Jian Feng, Murali Ramanathan, Tony Auerbach

Demylenation and Neuroinflammation

Rick Munschauer, TBH- Hunter's Hope endowment

Neurovascular and Neuroimaging

Steve Rudin, Hui Meng, Ken Hoffman, Nick Hopkins, Lee Gutterman

Stem Cells and Neurodevelopment/Degeneration

Steve Pruitt, Parsa Kasemi, Jim Reynolds

4. Cardio-Thoracic Group – Dr. Bruce Holm, Director

This Unit within the Center will identify mechanisms by which inflammatory processes associated with clinical events progress to acute and chronic disease. One arm of the group studies aspiration events that can progress to pneumonitis and ventilator associated pneumonias in order to develop treatments and diagnostics related to critical care lung diseases. A second arm of this group also studies sudden cardiac failure due to arrythmias from a molecular and genetic approach. A third arm works translationally to develop risk profiles, identify genes and gene products useful to monitor progression and response to therapy, and to identify genes and gene products that are potential targets for intervention by genetic manipulation and/or drug or other intervention. . Scientists and project areas represented by this Unit of the Center of Excellence include:

Critical Care Therapeutics
Bruce Holm, Paul Knight, Thomas Russo, Tim Murphy, Norma Nowak, Daniel Gail, Alan Hutson
Cardiac Arrythmia and Failure
John Canty, Fred Sachs
Translational Epidemiology of Inflammation
Robert Genco, Maurizio Trevisan, Alan Hutson, Frank Scannapieco, Richard Donahue

5. **Pathogenesis and Biodefense Group – Dr. Anthony Campagnari, Director**
This Unit within the Center will investigate virulence factors for bacteria and viruses important in human disease and in biowarfare. Novel vaccines, antimicrobials, and diagnostics will be developed based on structrure-function understanding of these factors. Scientists and project areas represented by this Unit of the Center of Excellence include:

Bacterial Pathogens
Tony Campagnari, Thomas Russo, Tim Murphy, Mike Russell
Viral Pathogens
Iain Hay, Stanley Schwartz, M. Nair, Ed Niles, George DeTitta, Gene Morse
Diagnostic Tools
Tom McMahon, Richard Karalus, Tony Campagnari; Alex Cartwright, Albert Titus

6. **Drug Discovery/Delivery Group – Drs. William Jusko & Huw Davies, Director**s
This Unit within the Center will develop biologically relevant compounds and delivery systems that can be linked to effects on specific gene expression patterns that create new drug targets. Nanomedicine approaches are also pursued by this group, particularly related to cardiovascular disease and cancer. Scientists and project areas represented by this Unit of the Center of Excellence include:

Pharmacokinetics/ Pharmacodynamics:
William Jusko, Richard Almon, Robert Straubinger, Ted Grasela
Drug Delivery:
William Jusko, Robert Straubinger, Huw Davies
Nanomedicine:
Paras Prasad, Mohammed. Kahn, Alan Oseroff

B. Faculty

Currently, the Center's faculty includes 50 outstanding researchers assigned to the six principal organizational areas. These primary Center Members include scientists from Roswell Park Cancer Institute, Hauptman-Woodward Institute, and UB. In addition, adjunct Center faculty from outside the WNY region may be appointed. Many of the existing Buffalo Center members are presently collaborating on projects supported by the New York STAR Center in Drug Discovery and Therapeutics, the New York Center for Advanced Technology and Biomedical Engineering, CUBRC, and several externally sponsored research programs. New faculty will have expertise relevant to the six primary Center areas and will benefit from access to core research facilities, expertise, and resources of the Center. New members recruited into the Center will go through a search process pertinent to the required subject matter areas. It is expected that all new Center members will meet a minimum funding and productivity standard to be established by the Center Executive Staff. Review of existing members will be performed annually for determination of continuing membership. Actions that might ultimately impact space assignments or equipment availability will be discussed with the members' appropriate dean or supervisor before actions are taken.

Faculty will be funded by the institutional resources at the University at Buffalo, Roswell Park Cancer Institute, and Hauptman-Woodward Institute. A policy for joint faculty and adjunct-faculty appointments (salary, start-up package, space, computer and equipment usage, use of funds for operating expenses and supplies) will be developed. Center resources will be used to leverage both the science and commercialization efforts of existing faculty as well as recruitment of new joint faculty of the collaborating partners in the Center.

Additional faculty policies, including a Faculty Responsibility Policy – Percentage of Time Committed to Teaching, Research, and Service – and a Policy for Faculty Start-Up Funds will be developed. Certain new hires at UB, Hauptman-Woodward Institute, and Roswell Park will be eligible for start-up funds from the Center's resources, if the faculty member's research is essential to and compatible with the Center's funding requirements, and if salary support is provided from their home institution.

C. Workforce Training Programs

One of the Center's primary missions is to act as an educational resource to educate scientists, engineers, and researchers in all critical technology areas pertaining to bioinformatics. The University

at Buffalo, as a comprehensive research university, is uniquely qualified to develop and offer bachelors and masters degree programs in this transdisciplinary field of bioinformatics.

The University at Buffalo has established new bachelors and masters degree programs to support the Center's educational mission. These areas of study include:

- Bioinformatics
- Medical Informatics
- Pharmacometrics
- Biophotonics
- Computational Chemistry
- Molecular Chemistry

The UB School of Informatics has developed a Master of Arts in Informatics designed to develop competencies in communication, team building, critical thinking, information and knowledge management, organizational culture and strategy, and systems. The program is designed to prepare students for careers in designing and managing information systems. Additionally, the Department of Structural Biology (School of Medicine and Biomedical Sciences) and the Department of Biostatistics (School of Public Health) each offers academic training programs in bioinformatics related fields leading to the MS and PhD degrees

UB's Graduate School of Education is developing K-12 educational programs in support of achieving long-term work-force development goals (as identified by state and federal politicians). These educational programs will be designed to assist students in enhancing their foundational knowledge in the fields of mathematics and science in the effort to attract students to academic and professional careers in the field of bioinformatics. The development of K-12 educational programs will also provide the opportunity to gain support and develop relationships with the IBM Foundation and the Gates Foundation.

Short-term training/certificate programs are under development that will cater to regional industrial needs. Initially, this will be accomplished through a summer program aimed at students in grades 10 through 12. A three-credit hour course (3 hours per day for 3 weeks) will be offered at UB and will cover computational and molecular biological aspects of the subject, with an emphasis on data mining and model building. The course will also include a career component and will be taught by UB/RPCI faculty. Visits to UB's Center for Computational Research and local biotechnology companies will augment the course curriculum.

D. Development of Industrial Partnerships

An important aspect of the Center is its interactions with industrial partners. These interactions will facilitate commercialization efforts of its discoveries, will increase job creation in Western New York – through co-locating industrial scientists in Buffalo – and will also increase research opportunities through grants and contracts from these companies. Additionally, academia-industry partnerships are a key element to both effectively expand the research functionality of the University and its research partners, and to enable the Buffalo academic research institutions to act as local economic drivers.

To affect academia-industry partnerships, the interaction must be business-oriented with tangible goals and projects. These projects must be expandable and developed with a long-term outlook that gives rise to a "win-win" scenario for both the academic and industrial culture. Secondly, the Center must have an adequately staffed professional (in this case, scientific) team to provide constant follow-up on the various academia-industry projects initiated. The Center must continuously reinforce the notion that, when necessary, it can act rapidly and effectively in its interactions with the corporation. Only after the appropriate business-oriented relationship has been established with a major corporation will there be a true opportunity for cultivating large corporate "gifts."

Corporate Partners

To date, the Center has initiated a number of high profile, high-potential interactions with industry. The partners listed below were developed through the Bioinformatics initiative, but touch virtually all of the scientific departments and decanal units within the University at Buffalo.

Key Corporate Partners for this initiative include:

- Amersham Biosciences (now GE Healthcare)
- General Electric Global Research
- Silicon Graphics Incorporated
- Biogen
- Pfizer Pharmaceuticals
- Invitrogen
- Stryker Corporation
- Cognigen
- 3M (Diagnostics and Pharmaceuticals)
- Oracle

The Center also has non-partnership Corporate connections that include:

- Hewlett-Packard

- Dell
- Sun
- TATA Corporation
- Polaris Ventures

The Center will benefit, not only from regional activities of these companies, but from their national and international research and business opportunities as well.

V. COMMERCIALIZATION

A. Regional Economic Development – Creation of a Bio-economy

Buffalo has traction in creating a life sciences economy as evidenced by the many new private sector jobs in the life sciences that have been created over the last two years. Strong linkages and collaborations have been forged among the major academic, research, government, and economic development entities, focusing on expanding the life science economy. Buffalo has an organizational base that can provide an integrated approach for cutting edge economic development, and education and training to support an emerging life science economy.

However, the definition of the bio-economy to be pursued in WNY/Upstate NY is critical to the strategic approach that must be utilized by the Center of Excellence. Areas such as San Diego and Boston are firmly established and many other regions around the globe are pursuing bio-economies. In many of these cases the strategy is not defined and success is unlikely. The process of creating a *de novo* bio-economy is extremely difficult and highly complex. Conversely, adequately defined and capitalized (with both human and financial resources) initiatives can act as an economic driver with appropriate expectations. The bio-economy, by any definition, can provide an economic growth stimulus with appropriate expectations for a maximum of a few thousand direct "bio" jobs. However, the appropriate approach will also act as a driver for the generation of additional development of indirect jobs in construction, shipping, insurance, financial, and retail sectors.

Bio-economy development will include both therapeutics and diagnostics, and by definition can include medical devices, translational and discovery-based services, biosciences program management services, and pharmaceutical manufacturing. The successful approach will need to include expansion and recruitment of existing companies in addition to the development of new start-up companies. Biomanufacturing may be one of the major opportunities for the Western New York economy, including the production and sale of substrate devices and materials, as well as the actual product manufacturing itself. The Center of Excellence, with its NYS and WNY community partners, will

work with an appropriate national expert to define the best chronological development plan for the local bio-economy. The Center will then help to facilitate the implementation of this plan through its corporate and economic development partners.

The recent expansion and refining of the New York State Center of Excellence in Bioinformatics and Life Sciences will generate unprecedented economic opportunities for the Buffalo-Niagara Region and New York State, including:

- The creation of new start-up firms based on the Center's intellectual property with products including diagnostic tests, medical devices, software and ultimately – pharmaceuticals;
- The expansion of current companies in the Life Sciences industries;
- The attraction of existing companies into new locations in the Buffalo-Niagara region;
- The creation of new jobs in the Life Sciences at salaries above the Buffalo-Niagara regional mean;
- The increased private sector investment in the region; and
- The enhanced reputation of the Buffalo-Niagara region as a world leader in the Life Sciences.

B. Relationship between BuffLink and the Center

A main purpose of the Center is to act as an economic outreach resource for industries based on bioinformatics, including those industries engaged in information technology, biotechnology, and pharmaceuticals. It is envisioned that the Center will attract the full range of bioinformatics industries, and also produce start-up companies based on the intellectual property produced in the Center.

In order to achieve the Center's goal to revitalize the region's economy, the Center will incorporate BuffLink into its organization as the Center's Commercialization Office. In that regard, BuffLink will occupy space within the Life Sciences Complex and the BuffLink Chief Operating Officer will have a dotted-line reporting responsibility to the Center Executive Director. In addition, the Center Executive Director will be a member of the Bufflink Board of Directors. The purpose of formalizing the relationship between BuffLink and the Center is to accelerate the results from technology transfer and to commercialize the Center's cutting-edge research. Bufflink duties on behalf of the Center will involve marketing the Center to the Life Sciences Industry; provide CAP services in collaboration with UB and RPCI; assist in fund raising initiatives; and provide human

resource support to the Center Executive Director relative to corporate partnership development and daily operations. It is anticipated that this will lead to the successful creation of new enterprises, growth companies, and ultimately, to a critical mass of life science activities that will make the Buffalo-Niagara region a global competitor and innovator in the field of bioinformatics and life sciences.

C. Coordination of Commercialization Efforts

The Center and BuffLink will work with officials at the University at Buffalo (STOR), Roswell Park Cancer Institute, and Hauptman Woodward Institute and key organizations – including Buffalo Niagara Enterprise, Buffalo Niagara Partnership, Empire State Development Corporation, Erie County Industrial Development Agency – to facilitate and accelerate the results from technology transfer and commercialization of cutting-edge research by partner institution scientists leading to new jobs in Buffalo with existing biotech firms and ones being attracted to the area by its emerging life-sciences economy.

a. Ownership of Discoveries

Agreements for ownership and commercialization of discoveries made jointly by scientists from the University at Buffalo, Hauptman-Woodward Research Institute, and Roswell Park Cancer Institute will be developed. These agreements will frame protocols surrounding intellectual property, copyright, and revenues.

b. Confidentiality

The parties from each partner institution agree that all information which they receive or have access to relating to each institution, its research activities or its patients, shall be held in the strictest confidence by the other institution and shall not be disclosed to any third party without the prior written consent of the party owning such information. For purposes hereof, confidential information shall refer to all material, documents, data, manuscripts or other information relating to an institution's research programs, grants and/or trials, financial, technical, marketing, demographic, billing, referral, patient information (including names, identifying information, and medical record information) and other information owned by emanating from or discovered at that institution. Confidential Information does not refer to information which (i) becomes generally available to the public other than as a result of a disclosure by the recipient or its staff,

(ii) was known by the recipient prior to disclosure hereunder, or (iii) which became available to the recipient on a non-confidential basis from a source other than the institution that owns the information.

VI. GOVERNMENT/INDUSTRY RELATIONSHIPS

The New York State Center of Excellence in Bioinformatics and Life Sciences' future requires close partnerships, relationships, and support from government and industry. In an effort to maximize the potential of these partnerships, the Center needs to ensure that its government and industry efforts are carefully planned and tightly integrated.

The Center's Executive Director through consultation with the organization's governing boards and faculty will develop a legislative and industry priorities plan. Legislative and industry priorities will resonate with the Center's research, commercialization, educational, and workforce development goals. (The Center's legislative and industry priorities plan will also include a strategy for identification of philanthropic funding sources.)

The Center, in concert with the Office of Government Affairs at UB, will create a comprehensive, coordinated, and integrated plan that will allow the Center to realize its government and industry objectives. This plan will include all efforts related to the identification, application, acquisition and use of government funds. The plan will also include the deployment of funds post realization including post award monitoring and compliance procedures.

VII. PUBLIC RELATIONS:

Public relations efforts for the Center will be coordinated through BuffLink and the UB development and government affairs officers assigned to Center, according to standard UB policies. All public materials will be reviewed by the Executive Council prior to release. The collaborating institutions are developing a consistent message regarding the Life Sciences Complex (LSC) and its component programs of bioinformatics, genetics and pharmacology, and structural biology. Any media communication citing two or more institutions will be reviewed by the respective press offices for approval prior to release. LSC collateral material will be jointly developed and used by RPCI, UB, and Hauptman-Woodward Medical Research Institute for all related media communications and public presentations.

Philanthropy – UB, HWI and RPCI will establish a Philanthropy Protocol that will include a requirement for pre-approval of any solicitation (individual, corporate, governmental or foundation) that directly states or could be inferred to imply that two or more institutions will be the beneficiary of the funds.

VIII. GRANTS AND BUDGET

A. Research Grants

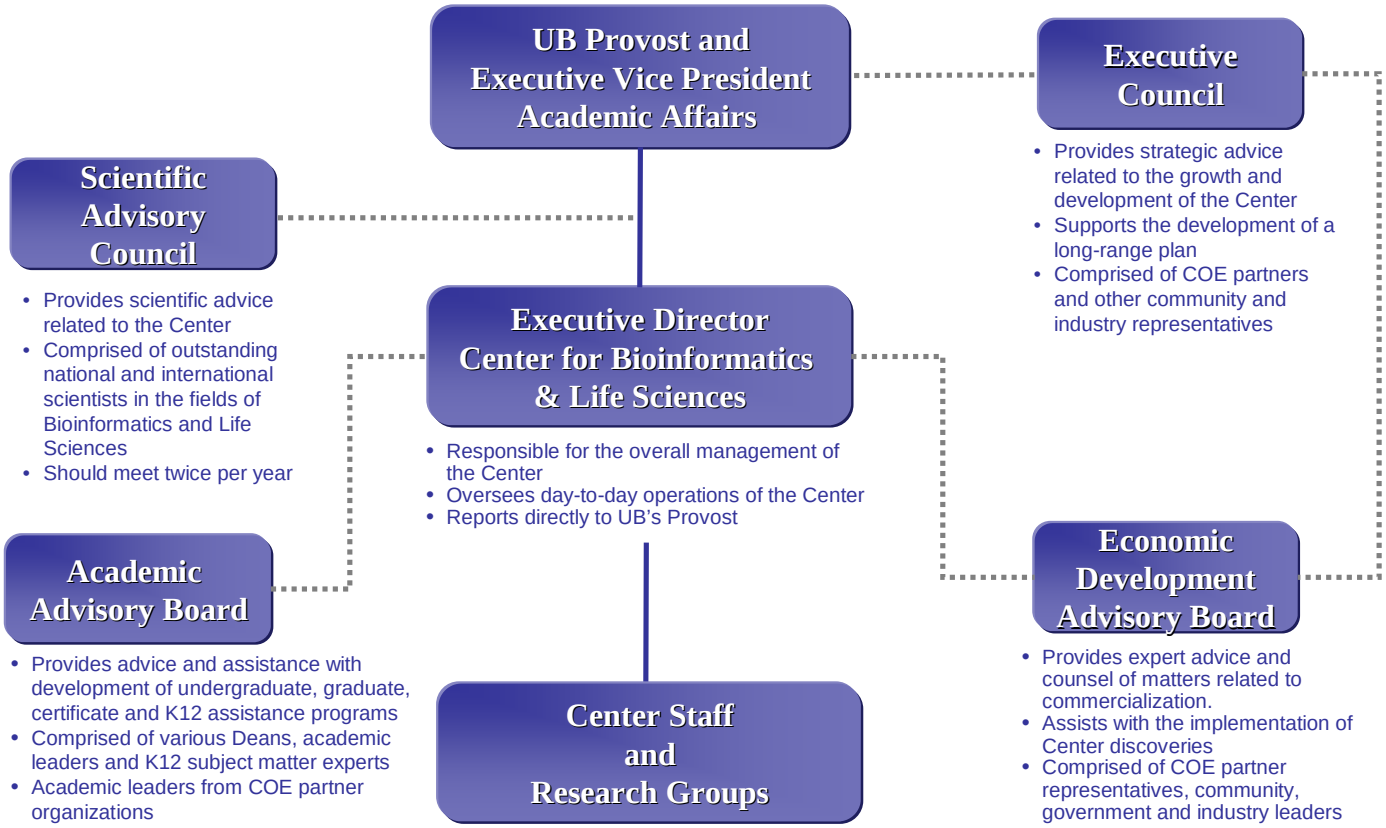
A grant routing and indirect cost-sharing policy on joint Center proposals from the University at Buffalo, Hauptman-Woodward Institute, and the Roswell Park Cancer Institute will be crafted by the appropriate administrative personnel at each of the partner institutions. Currently, existing Center members generate $25,296,802 in annual NIH research funding for the programs outlined above. The targeted recruitment plan for the first three years of this business plan sets an aggressive agenda that would work toward doubling the portfolio. Additionally, the affiliation of the Center with CUBRC as a 501C (3) also provides additional funding of $14,000,000 per year in Bioinformatics & Life Sciences areas, primarily from U.S. defense contracts. These funds are used to employ technical staff and purchase materials required to conduct the individual research projects within the Center.

B. Budget

Beginning with Governor Pataki's announcement of the Center of Excellence in 2001, over $200 million has been obtained to date from state, federal, and philanthropic sources for the program. These funds are being used to provide the basic infrastructure for the Life Sciences Complex and to recruit new entrepreneurial scientists into the region. The Governor, members of the Western New York State legislative delegation, Western New York federal Congressional Representatives, New York's US Senators, and the Oishei Foundation have all been instrumental in building the Center. These funds are in addition to the research grant funds that are used to run the individual research projects conducted by scientists within the Center. The remaining portion of the operating budget for the Center will be provided by the University and its research partners.

ATTACHMENT 1

Governance Model



ATTACHMENT 2

Executive Council Membership

- University at Buffalo, Provost
- Executive Director, NYS Center of Excellence in Bioinformatics & Life Sciences
- Roswell Park Cancer Institute, President and Chief Executive Officer
- Hauptman-Woodward Institute, Executive Director and Chief Executive Officer
- 3 additional members who meet the following:
    - The right values for WNY economic development
    - No business conflicts of interest
    - Passion for the work of the Center
    - Understanding of tech transfer
- Chairs of Academic and Economic Development Advisory Boards (non-voting)

ATTACHMENT 3

C. SCIENTIFIC ADVISORY BOARD: Potential Membership

- Russ Altman, MD, PhD – HHMI Investigator, Director of Bioinformatics, Stanford University
- Richard Frank, MD, PhD – Senior Clinical Scientist and Director of Molecular Medicine, GE Global Research
- Nathaniel Heinz, PhD – Professor of Genetics, Rockefeller University
- Donald Monroe, PhD – Director of Research & Development, Invitrogen Corporation
- Additional internationally known Corporate/Academic Leaders in Bioinformatics & Life Sciences
- Executive Director, NYS Center of Excellence in Bioinformatics & Life Sciences

ATTACHMENT 4

D. ACADEMIC ADVISORY BOARD: Potential Membership

- University at Buffalo, Dean, School of Medicine and Biomedical Sciences
- University at Buffalo, Dean, School Pharmacy and Pharmaceutical Sciences
- University at Buffalo, Dean, School of Engineering and Applied Sciences
- University at Buffalo, Dean, College of Arts and Sciences
- University at Buffalo, Dean, School of Public Health and Health Professions
- University at Buffalo, Dean, Graduate School of Education
- University at Buffalo, Dean, School of Informatics
- Academic leaders from COE partner institutions (4-6 people)
- Key Faculty members
- Executive Director Center of Excellence in Bioinformatics and Life Sciences

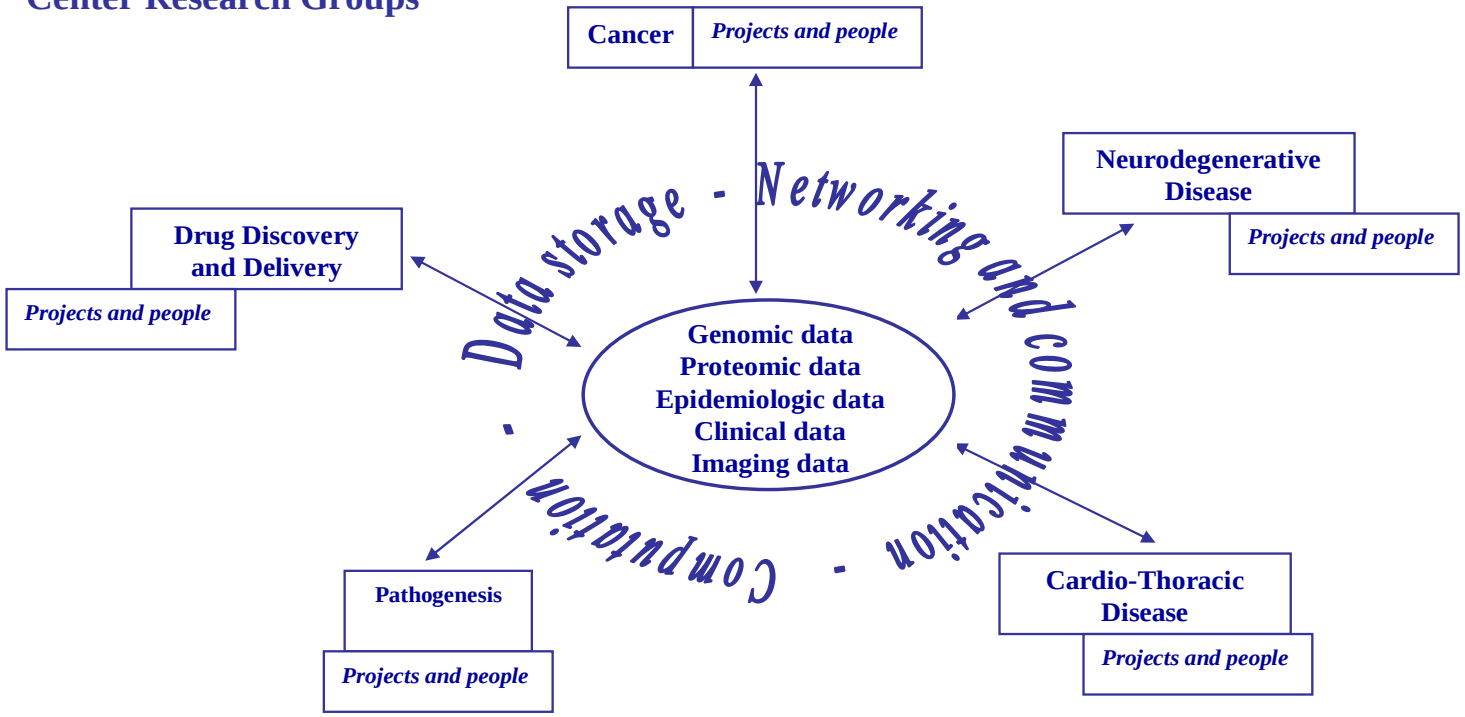
* Supported by a Curriculum Coordination Group (membership TBD)

ATTACHMENT 5

E. ECONOMIC DEVELOPMENT ADVISORY BOARD: Potential Membership

- University at Buffalo, Director, Office of Science, Technology Transfer, and Economic Outreach
- University at Buffalo, Associate Vice President for Government Affairs
- Buffalo Niagara Enterprise, President and Chief Executive Officer
- BuffLink representative
- Entrepreneurial Services Network of the Erie County Industrial Development Agency, President and Executive Director
- Empire State Development corporation representative
- Polaris Ventures, Managing General Partner (Mr. Terry McGuire)
- Mr. Peter Barton Hutt, Covington and Burling
- Ms. Maureen Suda, Private Consultant to Biotechnology Industry

Center Research Groups



ATTACHMENT 7

Center Of Excellence Unit Projects

I. Cancer (Selected examples of current faculty and funding)- Identify targets to inhibit tumor growth and cell proliferation.

A. Michael Brattain

1. Heterogeneity of Colon Cancer (5R01CA034432-20): This is a project that has been directed at understanding the role of aberrant TGFalpha signaling as a basis for disrupted growth regulation in colon cancer. We have found that TGFalpha is constitutively upregulated in highly malignant colon cancer cells, but is downregulated in Go premalignant and normal cells. This constitutive TGFalpha expression leads to a low, but constitutive activation of the EGFR, ErbB2 and downstream signaling including the PLCgamma, MAPK and PI3K pathways. These pathways generate growth factor independence as reflected by lack of exogenous growth factors for cell cycle re-entry and constitutive cell survival signaling. Colon cancer is one of the histological types of cancer showing a low rate of response to EGFR antagonism in the clinic as several small molecular weight tyrphostin derivatives as well as monoclonal antibodies are in various stages of development. The small molecular weight EGFR antagonists appear to be pan ErbB antagonists as well, but we have found that they fail to inhibit autophosphorylation of Y1248 by activated ErbB2 kinase in EGFR/ErbB2 heteromers. This is important because ErbB2 kinase, EGFR kinase independent signaling is prevalent in colon cancer cells and can lead to the activation of the PI3K or PLCgamma pathways and cell survival signaling. Thus, we have identified novel mechanisms of ErbB2 mediated signal diversification arising from aberrant autocrine TGFalpha which can circumvent the effects of this class of compounds in the clinic. Consequently, it is important to understand this signal diversification in colon cancer in order to optimize combination therapy with EGFR and ErbB2 antagonists. Specific Aims for the next cycle of the project are: 1. Determine the role of ErbB2 in the specification of essential signaling contributing to the malignant phenotype in colon cancer cells. 2. Determine how repression of ErbB2 dependent kinase signaling affects therapy by EGFR antagonists. 3. Determine mediators of cell survival signaling by ErbB family autocrine activation in colon carcinoma cells.

2. Autocrine TGF-A- Function and Control in Colon Cancer (5R01CA054807-14)**:** This is the competing renewal for CA548o7 Objectives during the past cycle of this project included the determination of a) autocrine TGF alpha function and b) mechanisms controlling TGF alpha expression in colon cancers. Accomplishments have included the determination that autocrine TGF alpha: in both early and late stage colon cancers functions primarily in the process of re-entering the cycling state from environmental conditions which limit cell growth (e.g.. growth factor deprivation) and does not appear to have a significant role in actively cycling populations (e.g., exponential growth in tissue culture). Late stage colon cancer cell lines differ from early stage cells in that they are completely independent of exogenous growth factors for re-initiation of DNA synthesis from growth arrest whereas early stage cells require an exogenous insulin-like growth factor l receptor (IGFIR) ligand for cell cycle re-entry. We have found that the growth factor

independence of late stage malignant cells arises from inappropriate TGF alpha: expression (not overexpression) and EGF receptor (EGFR) activation in growth arrested cells. This has the effect of producing constitutive EGFR activation, an event long associated with transformation. Early stage cells do not express TGF ALPHA: or show significant EGFR activation at growth arrest. IGFIR activation in early stage cells regenerates their autocrine TGF ALPHA: expression and EGFR activation events which we have shown to be necessary for DNA synthesis in these cells. Finally, we have found that autocrine TGF~ represses autocrine TGF~ expression in early stage cells. Inhibition of TGF~ function leads to enhanced expression of autocrine TGF ALPHA: and EGFR activation in growth arrested cells along with the acquisition of independence from exogenous growth factors and malignant progression to a late stage phenotype. Exogenous TGFj3 treatment also blocks IGFIR mediated induction of TGF ALPHA: mRNA in early stage cells. Several issues arise from these results. The first issue we will address is the mechanism by which IGFIR activation stimulates TGF ALPHA: re- expression from the growth arrested state. The second issue that will be addressed in the renewal project is the mechanism by which TGFj3 suppresses TGF alpha expression. The results from the last cycle of the project have shown that inappropriate expression of TGF alpha provides growth factor independence, but they have not addressed how inappropriate TGF alpha expression carries this out. Therefore, this issue will also be addressed in the renewal project.

3. Autocrine TGFBeta and Breast Cancer Cell Growth (5R01CA072001-08**):** State the application's broad long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this During the present cycle of funding we found that autocrine TGFbeta was induced by vitamin D3 analogs in ER+ breast cancer and pre-malignant mammary epithelial cells. Vitamin D3 analog response was 100 fold more resistant to inhibition in the absence of autocrine TGFbeta and preliminary evidence suggests that Smad3 activated by TGFbeta signaling enhances vitamin D3 receptor VDR directed transcription. However, while transfection of the type II TGFbeta receptor (RII) into RII null MCF-7 cells completely restores vitamin D analog sensitivity, transfection of Smad3 does not. This indicates that a second aspect of TGFbeta signaling is required in addition to Smad3. Thus, in Specific Aim I we will test the hypothesis that Smad3 interactions with the VDR are required for TGFbeta/VDR crosstalk, but that in addition to this Smad3 pathway, TGFbeta mediated activation of the MAPK pathway is also required. We and others have observed that vitamin D analogs cause apoptosis. The mechanism by which apoptosis is induced is largely unexplored, particularly with respect to the role of TGFbeta crosstalk. TGFbeta itself also induces apoptosis. Consequently, we will test the hypothesis that vitamin D3 analog induced apoptosis is mediated by the enhanced autocrine TGFbeta activity resulting from treatment with the drug. During the present period of funding we found that TGFbeta receptor expression in ER+ breast cancer cells is transcriptionally repressed. The repression can be blocked by treatment with the methylase inhibitor, 5 aza deoxycytidine. Surprisingly, this agent did not reverse methylation of the RII promoter, but instead increased the cellular level of Sp1 which is underexpressed in ER+ breast cancer cells. Sp1 is required for RII transcription. Interestingly, Sp1 transcription was not targeted by 5 aza deoxycytidine. Further, investigation showed that Sp3 transcription factor which represses Sp1 mediated transcription was elevated in ER+ breast cancer cell lines. Sp3 was shown to be a repressor of RII by southwestern, gel shift and blockade of RII promoter-reporter activity after Sp3 transfection. In addition Sp3 transcripts were decreased as a result of 5 aza cytidine treatment. Consequently, it appears as though Sp1/Sp3 homeostasis is responsible for repression of RII transcription in ER+ breast cancer cells. This hypothesis will be tested in Specific Aim III. The Specific Aims for the renewal project are: 1. Determine the mechanism of crosstalk between vitamin D3 analogs and TGFbeta in ER+ breast cancer cells, immortalized mammary epithelial cells and normal mammary epithelial cells. Determine whether vitamin D3 response in ER- cells is also associated with TGFbeta crosstalk. II. Determine the mechanism of induction of apoptosis by vitamin D3 analogs in the cell types listed in Specific Aim I and determine whether apoptosis inhibition is dependent upon autocrine TGFbeta. III. Determine how Sp1 and Sp3 homeostasis is controlled in ER+ breast cancer to regulate RII transcription.

B. John Cowell

1. Genetic Analysis of Myeloproliferative disease (5R01CA076167-05): Myeloproliferative disorders (MPDs) result from the abnormal proliferation of myeloid precursor cells in the bone marrow. Understanding the genetic events which result in MPDs will not only improve our understanding of the disease process, but will also provide insights into the normal developmental control in early progenitor cells. A variant form of this disease is associated with T-cell leukemia/lymphoma and peripheral blood eosinophilia. The clinical course of the disease is particularly aggressive with rapid progression of the disease to acute myelogenous leukemia or stem cell leukemia. The involvement of both myeloid and T-cell lineages in this disease strongly suggest a primitive origin for the cells involved, before the commitment to a particular lineage. These tumors invariably show a highly specific, reciprocal chromosome translocation involving chromosomes 8 and 13. This specific translocation is always involved with this biphenotypic tumor indicating that genes located at the translocation breakpoints play an important role in disease development. The genes involved in this rearrangement have now been identified as FGFR1 in chromosome region 8p11 and a zinc finger gene, ZNF198, of unknown function, in 13q12. The molecular conequences of this rerrangement have been shown to be identical in all of the four cases we have analyzed. As a result of the rearrangement a fusion gene is generated which is under the control of the ZNF198 promoter. This novel gene carries the zinc finger motif of ZNF198 fused to the tyrosine kinase domain of FGFR1. Because of the highly specific nature of this rearrangement and its consistent presence in all of the tumors analyzed to date, this rearrangement must clearly be important in leukemogenesis in these patients. Our goals therefore are (1) to transform normal cells using the fusion gene in order to establish a functional assay in vitro, (2) to create a transgenic mouse line expressing the fusion protein and so establish an in vivo model to study the biological consequences of the translocation and (3) since the fusion gene may act as a dominant-negative we will also investigate the normal function of ZNF198 in order to compare its activity with that of the fusion gene. As a result of our improved understanding of the genetic events which give rise to this MPD, it may eventually be possible to design novel therapeutic approaches to this disease directed against the aberrant gene(s) and its product.

2. Genetic Analysis of Neuroblastoma (5R01NS035791-08): Neuroblastoma (Nb) is the most common solid tumor in children. Despite intensive treatment regimens, however, Nb has a relatively poor prognosis in advanced stages and accounts for 15 percent of all childhood deaths due to cancer. Stage 4S disease is a special variant form of Nb which occurs in infants under the age of 12 months and, even despite a large tumor load and distant metastases, these tumors will often regress spontaneously. Identifying the genes which allow this rapid expansion and metastasis of neuroblasts will be invaluable in understanding the early stage of Nb tumorigenesis. Constitutional chromosome translocations have been instrumental in the identification of genes responsible for a wide variety of human genetic diseases, where it has been shown that the function of critical genes is affected in some way by the breakpoints. We have recently cloned the genes interrupted by a constitutional 1;10 chromosome translocation from a patient who developed stage 4S neuroblastoma. The 1p22 chromosome breakpoint occurs within the NB4S gene. This gene has two identifiable motifs: a TBC1 domain and a coiled-coil domain. Both of these motifs are indicative of protein-protein interactions. The gene on chromosome 10, TRNG10, is a member of an emerging class of genes which are transcribed but not translated. This gene is overexpressed in cancer cells compared with normal cells. As a result of the translocation the TBC1 domain of NB4S is truncated by in-frame fusion to TRNG10. Mutation analysis of NB4S does not indicate that it functions as a tumor suppressor gene. Transfection studies, on the other hand, indicate that a truncated form of NB4S is a dominantly transforming oncogene. To establish the function of the novel chimeric gene we will undertake a series of in vitro studies to characterize the dominant transforming activity, as well as the spatial and temporal expression pattern. We will also use immunoprecipitation and yeast-two hybrid analysis to define interacting genes that may be important for function. In an attempt to establish a mouse model for 4S neuroblastoma, we will express the chimeric gene in transgenic mice. As a result of an improved understanding of the genetic events that give rise to Nb, it may eventually be possible to design novel therapeutic approaches directed against the gene and its product, as well as to assess their value in the pre-symptomatic identification of at-risk individuals.

3. Molecular Stratification of Brain Tumors (1R21CA104504-01A1): Brain tumors are one of the more common cancers in individuals under the age of 35 and over the age of 60. For the vast majority of patients survival is still very poor. One of the major problems with stratifying patients who will respond to therapy or who have a better prognosis is the heterogeneity that often surrounds the histopathological classification of these tumors. Even highly trained neuropathologists often disagree on the diagnosis, which means that entering patients on specific trials can be hit-and-miss. Furthermore, within tumor types some patients do well and others do not. This observation suggests that there are underlying genetic events that determine the clinical outcome which cannot be classified on histopathology alone. What is clearly needed is a more objective way of stratifying brain tumor patients. Cytogenetic analysis of brain tumors has provided some clues as to the underlying genetic heterogeneity in brain tumors but the resolution of these approaches is inadequate to reveal the majority of genetic events that are present. It is likely, based on our limited knowledge to date, that brain tumors acquire small deletions and amplifications of critical genes during their development. Our hypothesis is that these changes could be the basis on which to subclassify brain tumors. The recent development of BAC arrays at RPCI now allows a high-resolution comparative genome hybridization (CGHa) analysis of tumor DNA which has the ability to detect deletions and amplifications currently down to 750Kb in a single hybridization experiment. We propose to use this novel technology to screen over 150 brain tumors for genetic changes and then relate the genetic changes to clinical parameters. Ultimately we will be able to develop a custom array which will allow an unbiased analysis of brain tumors in an attempt to predict clinical phenotypes such as response to therapy and prognosis.

C. Candace Johnson

1. Anti-tumor Mechanisms and Therapeutic Effects of Vitamin D (5R01CA067267-11): Vitamin D, 1,25 dihydroxycholecalciferol (calcitriol) has significant antiproliferative activity in vivo and in vitro. Calcitriol induces G0/G1 arrest, modulates p27 and p21, induces cleavage of caspase 3, MEK, and PARP, inhibits P-Akt and significantly increases MEKK-1. In vitro and in vivo, dexamethasone (dex) potentiates calcitriol-mediated antitumor activity through the vitamin D receptor (VDR). In a phase II trial in androgen-independent prostate cancer (AIPC) with high dose oral calcitriol and dex, we observed a 50 percent reduction in serum prostate specific antigen (PSA) in 28 percent of patients. Also, calcitriol significantly enhances the in vitro and in vivo antitumor efficacy of platinum analogues and taxanes. Enhancement of drug-mediated apoptosis by calcitriol is associated with an increase in PARP-, MEK- and caspase-cleavage, p73 and MEKK-1 with a decrease in P-Erk and P-Akt. Thus, we performed two phase I trials of calcitriol with either carboplatin or paclitaxel. From the pharmacokinetic (pk) data, increasing oral doses of calcitriol did not result in higher serum calcitriol levels; suggesting a potential decrease in bioavailability. In addition, calcitriol increases the expression of EGFR. When calcitriol is combined with the EGFR tyrosine kinase inhibitor, ZD 1839 ('Iressa'), a significant enhancement of antitumor activity is observed in vitro and in vivo associated with a significant decrease in P-Erk and P-Akt. Therefore, calcitriol has significant pro-apoptotic effects and can synergize with other cytotoxic modalities that potentially share the targets, P-Akt, P-Erk, MEKK-1 and/or p73. Therefore, we propose to examine the potential efficacy and mechanisms of calcitriol in combination with cytotoxic agents both clinically and pre-clinically by the following specific aims: 1) to determine the modulation of the molecular events of apoptosis in the calcitriol signaling pathway by platinum and taxanes in vitro and in vivo in tumor models; 2) to evaluate high dose intravenous (iv) calcitriol (QDx2, D1, 2), docetaxel (70mg/m 2, D2) and dex (QDx2, D1, 2) every 21 days in AIPC in a phase I/II clinical trial; 3)to determine the mechanisms involved in the enhanced antitumor activity of the tyrosine kinase inhibitor, ZD 1839 in combination with calcitriol in vitro and in vivo and 4) to examine in the Phase I setting ZD1839 250mg,QD) with escalating high iv doses of calcitriol weekly weekly in patients with advanced cancer.

2. Glucocorticoids and Vitamin D: Role in Anti-tumor Effects (5R01CA085142-05) Studies in our laboratory demonstrate that vitamin D or 1,25- dihy6droxycholecalciferol (calcitriol) has significant anti proliferative activity in vivo and in vitro. Calcitriol induces G0/G1 arrest, modulates expression of p27/p21, induces PARP cleavage, increases the bax/bcl-2 ratio and enhances the anti-tumor activity of cisplatin,

carboplatin and paclitaxel. Dexamethasone (dex) potentiates calcitriol-mediated anti- tumor activity and vitamin D receptor (VDR) ligand binding in vitro and in vivo whereas equivalent doses of methylpredinsolone (mp) do not. Both dex and mp enhance VDR protein expression; however, calcitriol plus dex results in an increase in retinoid X receptor (RXR) alpha levels and calcitriol/mp decreases RXRalpha. Calcitriol/dex suppresses activity mitogen-activated protein kinase (MAPK) activity while calcitriol/mp have no effect. In a phase II clinical trial of hormone refractory prostate cancer with oral calcitriol and dex, we observed a 50% reduction in prostate specific antigen (PSA) in 31% of patients with no hypercalcemia and a modulation of VDR from peripheral blood monocytes. We propose to determine the mechanisms of calcitriol and dex/mp interaction by the following specific aims: 1) To determine the mechanisms involved in glucocorticoid effects on tumor cells by examining: a) the role of steroid binding to the glucocorticoid receptor (GR); b) the differential role of RXR; and c) whether glucocorticoid effects require calcitriol binding to the VDR, modulate binding to the VDRE, and/or tumor model systems by determining: a) the effect on receptor (VDR/GR) and non-receptor (MAPK) mediated activities, and b) the role of RXR; and 3) To evaluate oral calcitriol and dex in hormone refractory prostate cancer through conduct of a phase I trial to determine: a) the maximum tolerated dose (MTD), toxicities and calcitriol pharmacokinetics, b) the clinical and PSA response, and 4) modulation of peripheral blood monocyte VDR, RXR and MAPK activity by calcitriol and calcitriol/dex.

D. Carl Porter

1. Polyamine Analog Regulation of Cell Cycle and Apoptosis (2R01CA022153-28 **)**: Polyamines are well recognized for their critical role in supporting cell proliferation-a role that has been preclinically validated as a worthy anticancer target. During the past granting period, we have described the signaling, effectors and survival responses elicited by polyamine inhibitors or analogs in arrest-prone MALME-3M melanoma cells and in apoptosis-prone SK-MEL-28 cells. In the case of analogs, potent induction of the catabolic enzyme, spermidine/spermine N1-acetyltransferase (SSAT), emerged as the key initiator of metabolic and signaling events that culminate in apoptosis or cell cycle arrest. That linkage was confirmed by SSAT-targeted RNAi, making DENSPM the only polyamine analog having a defined mechanism of growth inhibition. In pursuit of downstream metabolic events, we have genomically identified and biochemically characterized (a) an analog-inducible spermine oxidase, (b) an analog-inducible polyamine oxidase, and (c) a second SSAT. Taken together, these various findings present converging opportunities for investigating how analog induction of polyamine catabolism relates to apoptosis and cell cycle arrest and for devising mechanism-based strategies for exploiting these findings towards a therapeutic advantage. The following Specific Aims pursue these significant new leads in the same two melanoma cells with the goal of determining how inducible polyamine catabolic systems mechanistically interface with recently defined pathways of cell cycle arrest and apoptosis. Aim 1 will determine the relative contribution of new polyamine catabolic enzymes in analog-induced apoptosis of SK-MEL-28 cells; Aim 2 will identify downstream signaling molecules linking polyamine catabolism to apoptosis in analog treated SK-MEL-28 cells; Aim 3 will determine the relative contribution of p53 and new polyamine catabolic enzymes in analog-induced cell cycle arrest of MALME-3M cells; Aim 4 will identify downstream signaling molecules linking polyamine catabolism to apoptosis in analog treated MALME-3M cells, and Aim 5 will validate the role of polyamine catabolic enzymes in mediating or contributing to DENSPM antitumor activity.

2. Antiproliferative Potential of Polyamine Catabolism (5R01CA076428-06): The ability of polyamine-directed agents to achieve meaningful and selective antitumor activity is indicated by preclinical efficacy studies and the recent progression of several agents to clinical trial. Fundamental findings linking polyamine biosynthesis to oncogenic dysregulation of the cell cycle and apoptosis lend understanding to these developments. As an alternative to strategies targeting polyamine biosynthesis, we submit that activation of pathways involved in polyamine catabolism or export out of the cell will have useful antiproliferative consequences. This assertion is predicated on the fact that conditional overexpression of the polyamine-acetylating enzyme, spermidine/spermine Nl-acetyltransferase (SSAT), depletes intracellular polyamine pools

and inhibits cell growth. A recently identified novel mammalian spermine oxidase (SMO) has similar potential. Since both enzymes are now known to be inducible, strategies can be devised to exploit their ability to differentially contribute to polyamine pool depletion--SMO, by polyamine catabolism and SSAT, by facilitating polyamine export out of cells. The following Aims will genetically and pharmacologically evaluate the therapeutic potential of this approach. Aim 1 will complete biochemical characterization of SMO and examine another recently discovered polyamine oxidase (PAO) with respect to substrate specificity, intracellular function and analog inducibility. Aim 2 will examine the effects of conditional SMO or PAO over-expression on polyamine homeostasis and cell growth. Aim 3 will develop transgenic mice that systemically overexpress SMO and PAO to determine the physiological and pharmacological significance of these genes. Aim 4 will genetically evaluate the antitumor and/or tumor preventive potential of activated polyamine catabolism by cross-breeding SMO and SSAT transgenics with mice that are predisposed to intestinal neoplasia (APCmin). Guided by findings in Aim 4, Aim 5 will discover and develop small molecule modulators of polyamine catabolic enzymes as potential anticancer agents. In addition to evaluating a novel anticancer approach, the proposed studies have implications for improving the use of existing polyamine-directed therapies and for understanding Dolvamine homeostasis and its response to pharmacological perturbations.

E. Donald Trump

1. Vitamin D in Prostate Cancer: Tumor Vascular Effects (5R01CA095045-03): We have demonstrated that the most active vitamin D moiety 1,25 dihydroxycholecalciferol (calcitriol) has significant antiproliferative activity in vivo and in vitro. Calcitriol induces G0/G1 arrest, modulates p27/p21, induces apoptosis and enhances the antitumor activity of cisplatin, carboplatin, paclitaxel and docetaxel. In vitro and in vivo, dexamethasone (dex) potentiates calcitriol-mediated antitumor activity and vitamin D receptor (VDR) ligand binding in vitro and in vivo and enhances VDR protein expression. Calcitriol/dex also suppress activated mitogen-activated protein kinase (MAPK), Ert 1/2) activity and phosphorylated Akt (P-Akt). In a phase II clinical trial of hormone refractory prostate cancer with high dose oral calcitriol and dex, we observed a 50 percent reduction in serum prostate specific antigen (PSA) in 28 percent of patients with no hypercalcemia; modulation of VDR and other potential markers of calcitriol activity in peripheral blood monocytes. We have preliminary data indicating that calcitriol and dex induces apoptosis, modulates cell cycle, decreases P-Erk 1/2 and P-Ark and significantly up-regulated VDR expression of tumor-derived endothelial cells (TDEC) as compared to endothelial cells isolated from normal tissues (aortic). These studies suggest that the mechanism(s) of calcitriol/dex antitumor activity may involve effects on intratumor vasculature. We propose to examine this hypothesis by the following Specific Aims: 1) to determine the mechanisms involved in calcitriol/dex effects in TDEC isolated from prostate tumors as compared to normal by examining: a) effects on apoptosis and cell cycle; b) effects on invasiveness, motility, and angiogenesis; and c) whether activities require calcitriol binding to the VDR; 2) To determine the prostate tumor models the role of TDEC in calcitriol/dex antitumor effects by determining: a) effects on the vasculature within the tumor; b) effects on TDEC isolated from animals treated with calcitriol; and c) whether a relationship exists between modulation of effects on TDEC and antitumor effects and 3) To evaluate oral calcitriol (12mg QDx3) and/or dex (4mg QDx4) administered weekly x 4 to patients with localized prostate cancer immediately prior to prostatectomy to determine: a) effects on tumor endothelial cells; b) effects of cell cycle status, apoptosis markers and prostatic intraepithelial neoplasia (PIN) and c) prostate specific antigen (PSA) response.

F. David Goodrich

1. Requirements for Normal Rb Function In Vivo (5R01CA070292-08): Mutational inactivation of the retinoblastoma tumor suppressor gene (Rb) causes retinoblastoma and contributes to most human cancers. Rb is also required for normal embryonic development in the mouse. The long-term goals of our lab are to characterize the mechanisms utilized to effect normal Rb function, to ascertain how these mechanisms are regulated, and to determine the contribution these mechanisms make to normal function in vivo. In vitro, Rb

can regulate several major cellular processes, including the cell cycle, differentiation, and apoptosis. Since naturally occurring and synthetic mutations have been discovered that affect one of these Rb-dependent functions but not others, they are likely mediated by independent mechanisms. Mutational analysis also points out that these Rb-mediated molecular mechanisms can be regulated independently by phosphorylation at different subsets of serine or threonine residues. These observations suggest that normal Rb function in vivo may require the execution and proper coordination of distinct molecular mechanisms. It is currently unclear what contribution each of these molecular mechanisms, or their proper coordination by site-specific phosphorylation, make to normal embryonic development or Rb dependent tumor suppression. We hypothesize that normal Rb function in vivo will be partially compromised by mutations that affect a single Rb-mediated mechanism, or by mutations that affect normal site-specific phosphorylation. Through phenotypic analysis of such mutant alleles in vivo, the contribution of distinct Rb mediated molecular mechanisms to embryonic development and tumor suppression can be ascertained. We propose the following aims to test the hypothesis: 1) Identify mutant alleles specifically deficient in regulation of E2F 1-dependent transcription or normal site-specific phosphorylation; 2) Characterize the molecular defect in selected Rb mutant alleles; 3) Create and characterize mouse Rb mutant alleles analogous to selected human mutant alleles identified in aims 1-2; 4) Generate mice containing selected mutant Rb alleles; 5) Confirm the molecular deficiency of select mutant alleles in vivo; 6) Characterize the phenotype of mice containing mutant Rb alleles.

G. Christine Ambrosone

1. Pharmacogenetics: Breast Cancer Treatment and Prognosis (5R01CA095222-02): The proposed study uses data and specimens from a Southwest Oncology Group (SWOG) breast cancer clinical trial of CAF or CMF to investigate inherited factors that may impact prognosis. Disease-free survival following adjuvant therapy, as well as treatment-related toxicity, are likely predicted by a number patient and tumor characteristics. Pharmacogenetics, inherited differences in metabolism of chemotherapeutic agents and variability in capabilities to protect ceils (tumor and normal) from treatment-generated reactive oxygen species (ROS), may be of particular importance, but has been understudied, to date. For example, cyclophosphamide undergoes hepatic activation by CYP3A4 and metabolites may be detoxified by conjugation with glutathione, catalyzed by glutathione S-transferases P1 and A1. We hypothesize that determination of ultimate cytotoxic metabolites by these variants will impact treatment efficacy. Furthermore, variability in enzymes that protect the mitochondria (MnSOD, GPX1, CAT), key to controlling cancer cell death, and those in the secondary line of defense against products of oxidative stress (GSTM1, GSTT1), may also impact disease-free survival after treatment for breast cancer. These hypotheses are supported by our pilot data, in which women with GSTP1 and GSTA1 variants encoding lower enzyme activity and those with gene deletions in GSTM1 and GSTT1 had significantly less risk of recurrence and death after treatment for breast cancer. Lesser protection of cells from cytotoxic and reactive species could also result in more severe toxicity. We propose analysis of systemic, inherited DNA derived from normal archived tissue for polymorphisms in CYP3A4, GSTP1, GSTA1, MnSOD, GPX1, CAT, GSTM1 and GSTT1 among a subset of women who participated in a SWOG clinical trial in relation to recurrence and toxicity. Eligibility criteria included T1-3a node-negative invasive adenocarcinomas of the breast with tumors < 2cm and positive for estrogen and progesterone receptors. Based on flow cytometry results (S-phase), women were assigned to a higher risk group (n=528) and were randomized to either CMF or CAF, + or - tamoxifen. The lower risk group received no adjuvant treatment. DNA from these women will be genotyped and results merged with the SWOG patient database. Kaplan-Meier survival function, log rank tests for survival differences and Cox proportional hazards models will be used to determine the impact of genetic variability on disease-free survival, adjusting for other prognostic factors. Regression analysis will be used for associations between genotypes and toxicity endpoints. To evaluate if genetic polymorphisms have an association with survival that is independent of treatment, analyses will be conducted among women who received no adjuvant therapy. Findings from this study may be useful in prediction of which patients will respond to treatment for breast cancer and which will not, with an effort towards individualized therapy. Individualized dosing according to genotype could contribute to increased efficacy and decreased toxicity among patients being treated for cancer.

2. Race & Risk Factors for Early/Aggressive Breast Cancer (1R01CA100598-01A1): Breast cancer in African-American women occurs at an earlier age than in Caucasian women and is more likely to have aggressive features associated with poorer prognosis, regardless of age at diagnosis. The mechanisms underlying these differences are as yet unknown. Although factors related to socioeconomic status (SES) are likely to play a role in racial differences in stage at diagnosis and disease-free survival, SES factors are unlikely to fully explain the earlier age at diagnosis or the more aggressive nature of breast cancer in African-American women. Most recognized risk factors for breast cancer involve reproductive or hormonal characteristics. Importantly, there are notable differences between African-Americans and Caucasians in distribution of polymorphisms in genes that govern steroid biosynthesis and metabolism, in endogenous hormone profiles, and in menstrual and childbearing histories. These risk factor differences could impact breast cancer in African-American and Caucasian women, although the potential role of SES factors cannot be discounted. To our knowledge, there have been no studies specifically designed and with adequate power to address these questions. Building upon a funded case-control study of bio-behavioral risk factors for breast cancer in African-American women, we propose to investigate determinants of early age at onset and high grade, ER negative disease among African-Americans and Caucasians and to evaluate the proportion of disparities in aggressive disease that can be accounted for by differential distribution of genetic and hormonally-related risk factors in both racial groups. We intend to enroll 1200 Caucasian cases and 1200 controls and to enroll an additional 800 African-American women. We will over-enroll women under age 50 so that we will have equal proportions of younger and older women in both racial groups. Combined with data from the ongoing study, the proposed study will include 1200 African-American women with breast cancer and 1200 controls and an equal number of Caucasians. We hypothesize that factors associated with higher exposures to circulating estrogens, as well as the timing of those exposures, including early age at menarche, early and multiple pregnancies, energy balance and body size, as well as polymorphisms that result in higher active hormone levels will be associated with early age at diagnosis and high-grade, ER negative breast cancer, and that these factors will be more prevalent among African-American than Caucasian women. We will also examine the role of socio-economic factors, including screening behaviors and access to care, in the higher prevalence of high-grade, ER- tumors among African-American women. Results from this study will provide much-needed answers as to why African-American women are more likely than Caucasians to be diagnosed with more aggressive disease at an earlier age.

3. Lung cancer molecular markers by sex: intergroup study (1R01CA106815-01): We propose to conduct a case-series study in men and women with lung cancer, investigating molecular markers of susceptibility, biologically effective dose, and tumor tissue alterations. These markers will be evaluated in relation to exposure data on active and passive smoke exposure, occupational exposures and reproductive and hormonal factors, in order to elucidate reasons for the increase in lung cancer, particularly adenocarcinoma, in both smoking and non-smoking women. To optimize our capabilities to enroll nonsmokers with lung cancer, we will work with the Lung Committee investigators of the Southwest Oncology Group and collaborating members of the NCI lung intergroup to identify large numbers of patients with lung cancer, enrolling 120 never-smoking women with lung cancer and an equal number of males, as well as 200 each of ever smoking females and males. We have two primary hypotheses: that women are at higher risk due to susceptibility to tobacco-smoke carcinogens, based on metabolic variability. We propose that markers of the interactions of environmental and genetic risk factors for lung cancer (DNA adducts), as well as genetic alterations in tumor tissue (p53 and kras mutations), will reflect differences in exposures and susceptibility between men and women, smokers and non-smokers, and will elucidate the disproportionate variability in risk by gender and smoking status. Our second hypothesis is that steroid hormones, which appear to play a role in lung carcinogeneis, will have a greater effect in women than in men and in never smokers rather than smokers. We predict that tumor HER2 amplification and estrogen receptors will vary by gender and smoking status, particularly in relation to environmental and genetic (SNPs in carcinogen and steroid hormone metabolism pathways) risk factors. Biomarkers of exposure, susceptibility, and effect will be assayed in the blood and tumor tissue of these women men with lung cancer and evaluated in relation to questionnaire data to test the proposed hypotheses. Results from this study, the largest to date

to evaluate biomarkers of susceptibility and effect, will likely elucidate reasons for the rise in lung cancer, particularly adenocarcinomas, in smoking and non-smoking women.

II. Neurodegenerative/Neuroscience- Investigating the disease mechanisms of several neurological disorders, including Alzheimer's, MS, Epilepsy, Krabbe's and Parkinson's disease. Major themes include demyelination, amyloid plaque formation, and loss of cellular function.

A. John Cowell

1. Molecular Analysis of Epilepsy Predisposition (5R01NS046706-02): Epilepsy is a complex disorder that affects over 15 million Americans. Despite the considerable clinical heterogeneity in the epilepsy phenotype it has been clearly established that predisposition to epilepsy can result from inherited mutations in specific genes. The epilepsy genes which were first described encoded voltage-gated sodium channels, SCN1A, SCN2A and SCN1B and a GABA receptor subunit, GABRG2. It is thought that the epilepsies associated with these genes result from enhanced excitability of neuronal membranes resulting from prolonged depolarization of the neuronal membranes. Recently it has been demonstrated, however, that another type of gene is involved in focal epilepsy of the temporal lobe. This is the LGI1 gene which was mutated in a small series of families showing linkage to chromosome region 10q24. The LGI1 gene is a putative transmembrane protein with homologies to a Drosophila gene which is involved in axon guidance during development of the brain. It is hypothesized that epilepsy in these cases results from abnormal brain development and provides an opportunity to investigate the function of this gene and its role in normal development as well as epilepsy. The P.l.'s group was responsible for the original cloning of the LGI1 gene which has also been shown to be involved in the progression of brain tumors. The phenotype involved in loss of LGI1 function in glial cells is an increased ability to migrate and invade local brain tissue which, again may be related to signals which ensure the orderly distribution of cells within the brain. We now propose to use a combination of cell and molecular approaches to characterize the function of this gene in order to improve our understanding of the events which give rise to epilepsy and possibly identify ways to treat it.

B. Zhen Yan

1. The Role of Cholinergic Functions in Alzheimer's Disease (5R01AG021923-02): Alzheimer's disease (AD) is a devastating neurodegenerative disorder that afflicts millions of people worldwide. Two major features of AD are: (1) degeneration of basal forebrain cholinergic neurons and ensuing deficient cholinergic functions in cortex and hippocampus; (2) extracellular protein aggregates containing beta-amyloid peptides (Abeta) in these cholinergic target areas. So far, the most effective therapeutic strategy in AD treatment is to enhance cholinergic transmission. Neuromodulatory functions of the cholinergic system are mainly mediated by muscarinic receptors (mAChRs). It has long been recognized that mAChRs are crucial for the control of high-level cognitive processes. Drugs that activate mAChRs are helpful in ameliorating cognitive deficits of AD. Despite the discovery of correlation between cholinergic hypofunction and AD, the cellular and molecular mechanisms underlying the function and dysfunction of mAChRs in normal cognition and dementia remain elusive. The long-term goals of this project are to understand (1) how muscarinic signaling is involved in the regulation of neuronal activity and synaptic transmission in frontal cortex, which is critical for learning and memory under normal condition; and (2) how this regulation is altered in animal models that simulate cognitive and memory impairments associated with AD. Recent evidence indicates that GABAergic inhibition in frontal cortex plays an important role in "working memory" by controlling the timing of neuronal activities during cognitive operations. We hypothesize that the GABAA receptor channel is potentially a key cellular substrate for muscarinic signaling in cognition and memory, and disruption of its regulation by mAChRs in AD might contribute to the cognitive impairment. Transgenic mice overexpressing a mutant gene for beta-amyloid precursor protein (APP) show behavioral and histopathological abnormalities resembling AD, and therefore will be used as an AD model in our experiments. Emerging evidence suggests that Abeta plays pleiotropic roles in the regulation of cholinergic functions in cortex. We hypothesize that the muscarinic modulation of GABAA receptor function is lost in AD models due to the interference of Abeta

on muscarinic signaling. Combined electrophysiological, pharmacological, biochemical and molecular analyses will be used to test these hypotheses. This research would shed some light on how the two prominent features of AD (cholinergic hypofunction and Abeta accumulation) may be linked to cause cognitive impairments. Such knowledge should offer important insights into the cellular and molecular basis of AD and the development of new pharmacological agents in the treatment of this disease.

2. Neuromodulator Actions in Prefrontal Cortical Neurons (5R01MH063128-03): Dysfunction of the mesocortical neurotransmitter systems has been implicated in many neurological and mental disorders, including schizophrenia, depression and anxiety. Increasing evidence has suggested that many of these diseases result from altered serotonergic pathways and their dysregulation of neuronal activity in prefrontal cortex (PFC), a critical brain region controlling cognition and emotion. Many novel antipsychotic agents, antidepressants and anxiolytics act on serotonin receptors in PFC neurons. Understanding molecular and cellular mechanisms underlying the actions of serotonin in PFC is crucial for designing more effective therapeutic strategies in the treatment of brain disorders. Despite considerable progress on understanding the significance of serotonin at the system level, how serotonin regulates cellular excitability and synaptic function of PFC neurons remains unclear. It is our long-term goal to understand the molecular and cellular mechanisms of serotonin signaling in prefrontal cortex. Serotonin exerts its actions mainly through a complex set of G protein-coupled receptors. PFC is composed of glutamatergic pyramidal neurons and GABAergic interneurons. AMPA-type glutamate receptors and GABAA receptors, both of which contain intrinsic ion channels, are major players mediating excitatory and inhibitory synaptic transmission respectively in PFC. It is my working hypothesis that GABAA receptor channels and AMPA receptor channels are two major targets of the serotonin system. Serotonin plays a pivotal role in the regulation of PFC functions by suppressing GABAergic inhibitory activity and potentiating counterbalancing glutamatergic neurotransmission in PFC neurons through different 5-HT receptor-mediated signaling cascades. To characterize the signal transduction mechanisms underlying the serotonergic modulation of these channels in PFC neurons, a combination of electrophysiological, pharmacological and molecular approaches will be used. The coordinated expression and subcellular localization of serotonin receptors in PFC neurons will be determined using single-cell mRNA profiling and immunocytochemistry methods. The effect of serotonin on the biophysical properties of GABAA and AMPA receptor channels will be studied using patch-clamp analyses of PFC neurons. The intracellular signaling pathways utilized by serotonin to modulate these channels will be revealed using pharmacological tools, molecular and biochemical assays. This study will provide important insights into the molecular and cellular mechanisms underlying serotonergic regulation of cellular activity and synaptic transmission in PFC neurons. Knowledge gained from this work would contribute to the development of novel therapeutic strategies for many neurological and mental disorders.

3. Neuromodulator Actions in Prefrontal Cortical Neurons (5R01MH063128-04): Dysfunction of the mesocortical neurotransmitter systems has been implicated in many neurological and mental disorders, including schizophrenia, depression and anxiety. Increasing evidence has suggested that many of these diseases result from altered serotonergic pathways and their dysregulation of neuronal activity in prefrontal cortex (PFC), a critical brain region controlling cognition and emotion. Many novel antipsychotic agents, antidepressants and anxiolytics act on serotonin receptors in PFC neurons. Understanding molecular and cellular mechanisms underlying the actions of serotonin in PFC is crucial for designing more effective therapeutic strategies in the treatment of brain disorders. Despite considerable progress on understanding the significance of serotonin at the system level, how serotonin regulates cellular excitability and synaptic function of PFC neurons remains unclear. It is our long-term goal to understand the molecular and cellular mechanisms of serotonin signaling in prefrontal cortex. Serotonin exerts its actions mainly through a complex set of G protein-coupled receptors. PFC is composed of glutamatergic pyramidal neurons and GABAergic interneurons. AMPA-type glutamate receptors and GABAA receptors, both of which contain intrinsic ion channels, are major players mediating excitatory and inhibitory synaptic transmission respectively in PFC. It is my working hypothesis that GABAA receptor channels and AMPA receptor channels are two major targets of the serotonin system. Serotonin plays a pivotal role in the regulation of PFC functions by suppressing GABAergic inhibitory activity and potentiating counterbalancing glutamatergic neurotransmission in PFC

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4. Functions of Dopamine D4 Receptors in Prefrontal Cortex (1R01NS048911-01): Prefrontal cortex (PFC) is a brain region critically involved in the control of high-level "executive" processes including information storage, complex reasoning, novelty seeking and emotional control. Functions of PFC neurons are strongly influenced by dopaminergic inputs. Aberrant dopaminergic actions have been implicated in PFC abnormalities associated with mental disorders such as schizophrenia. Several lines of evidence suggest that the dopamine D4 receptor, which is highly enriched in PFC neurons, plays an important and unique role in regulating PFC functions. However, little is known about the cellular mechanisms and functional consequences of D4 receptor-mediated signaling in PFC circuits. The long-term goal of this project is to understand the physiological functions of D4 receptors in PFC neurons. Particularly, we would like to elucidate how D4 signaling is involved in the regulation of synaptic transmission and plasticity in PFC, which is critical for cognitive and emotional processes. We hypothesize that D4 receptors exert an important impact on PFC functions by regulating the multifunctional Ca2+/calmodulin -dependent protein kinase II (CaMKII) and the downstream NMDA and AMPA type glutamate receptor channels. In agreement with this hypothesis, we have found that D4 receptors exert a dynamic regulation of CaMKII activity and translocation, and modulate NMDA receptor-mediated ionic and synaptic currents in a CaMKII-dependent manner. Moreover, AMPA receptor-mediated transmission and AMPA receptor internalization are also strongly regulated by D4 receptor activation. Combined electrophysiological, pharmacological, biochemical and molecular analyses will be used to extend these studies. This proposal will investigate novel mechanisms for D4 receptors to modulate PFC neuronal activity. Elucidation of these mechanisms would provide a framework for understanding how the dopaminergic and glutamatergic systems may be mechanistically linked. Such knowledge would not only offer important insights into functional roles of D4 receptors in normal PFC network, but also shed light on the development of new pharmacological agents for the treatment of many diseases associated with dopaminergic dysfunction in PFC.

C. Jian Feng

1. Parkin--In vivo Function and Role in Parkinson's Disease (5R01NS041722-04): Parkinson's Disease (PD) is one of the most frequent neurodegenerative disorders. It is an extrapyramidal movement disorder characterized by the progressive loss of dopamine (DA) neurons in substantia nigra (SN). Recent progress in linkage studies on patients with familial PD has led to the identification of several genes implicated in this disease. One of these genes, parkin, is linked to Autosomal Recessive-Juvenile Parkinson's Disease (AR-JP). Deletions, truncations, and point mutations of parkin in AR-JP patients are correlated with their PD symptoms. However, it is not clear whether the loss of function for this gene directly causes PD, and if so, how does it occur? We propose to answer these questions by generating the parkin knockout mice. They will be used to study whether the deletion of parkin directly leads to selective loss of nigral DA neurons, and PD-like symptoms in mice. Since parkin is widely expressed in many tissues, and yet the progressive cell death is restricted to DA neurons in SN, we hypothesize that parkin may interact with specific proteins in these neurons to sustain their survival. To test this hypothesis, we propose to identify proteins that interact with parkin by using the yeast two-hybrid system. Once these proteins are identified, we will investigate their roles,

in association with parkin, in the survival of nigral DA neurons. The specific goal of this project is to understand the in vivo function of parkin and its role in the etiology of AR-JP. As AR-JP and the sporadic form of PD share many similar clinical symptoms and pathological hallmarks (e.g. death of nigral DA neurons), knowledge gained from the study of parkin may shed some light on the potentially common mechanism for PD. It is our long-term objective to use this mouse genetic model to elucidate the molecular and cellular processes that lead to the progressive and selective death of nigral dopaminergic neurons and locomotor dysfunction in PD. This animal model would also be a valuable tool for the development of more effective therapeutic strategies for PD patients.

D. Stephen Rudin

1. Microradiographic Imager for Neurovascular Intervention (5R01NS038746-03): The overall goal is to build micro-radiographic, small field-of-view imagers for detecting small vessels such as perforators and features such as stent wires which may be crucial to present and future cerebro- vascular interventions but which may not be normally visualized with present day systems. The new cameras will be used with catheter-based minimally invasive procedures to provide very high resolution (10 line pairs per mm) digital images of a region of interest (ROI) around the interventional site. The ROI micro-angiographic imagers will be based on a high resolution x-ray absorbing phosphor viewed through a slight fiberoptic taper by a large area silicon chip charge coupled device (CCD) light sensor. A series of increasingly automated insertion mechanisms and a computer workstation will be developed to allow the camera to be positioned and used with standard angiographic imaging equipment. Once built and characterized physically, the micro- angiographic ROI cameras will be evaluated in terms of the role such improved imaging capability might have during neuro-vascular interventions where devices such as stents and coils or materials such as glue are placed so as to treat vessel stenoses, aneurysms and arteriovenous malformations. Extensive phantom and animal studies to test the applicability to specific interventional tasks will occur simultaneously with gradual application to human procedures and evaluation by clinical interventionalists. The effect that the additional high resolution images from the new cameras may have on each decision made by the interventionalist during the course of the procedure will be analyzed to determine whether the procedure was altered and improved. Use for both single-plane and bi-plane micro- angiography and for providing data for 3D rendered vascular models will be investigated.

2. Radiographic guidance of new cerebral aneurysm stent (1R01NS043924-01A2): The limitations of radiographic visualization and the lack of consideration of the influence of stentt deployment on details of blood flow have limited stentt design to forms which are uniform and circularly symmetric. Yet some of the most important potential applications of stents such as in the treatment of aneurysms are inherently non-uniform and non-symmetric in nature. In this project, the design of innovative asymmetric stents and their deployment enabled by guidance with new high resolution x-ray image detectors are investigated. In particular, the limitations and accuracy in localizing the rotational orientation as well as the longitudinal distance of small asymmetric devices delivered by catheters within the vasculature under radiographic guidance are explored for the first time. This new capability for accurate localization will allow the implementation of new devices such as asymmetric, variable porosity stentts for treatment of cerebral aneurysms by modifying aneurysm blood flow characteristics. To optimize the new stentt design, flow reduction in aneurysms needed to induce thrombosis will be explored. Also details of flow and flow modification will be investigated using advanced theoretical and experimental methods. It is expected that this new capability should enable more rapid treatment of aneurysms with less chance of hemorrhage and decreased possibility for recurrence compared with existing procedures and additionally readily enable treatment of wide-necked and giant cerebral aneurysms for the first time.

3. Microradiographic Guidance of Flow Modifying Stents (5R01EB002873-020): The aim of this project is to build a new very high spatial resolution, rapid frame rate region of interest (ROI) x-ray detector system, the micro-angiographic fluoroscope (MAF) and to use it for guiding and evaluating new stents used for flow modification in the treatment of intracranial aneurysms. The detector due to its large adjustable gain will be

capable of acquiring 30 fps at both fluoroscopic exposures in the fractional microR and higher range as well as at angiographic exposures in the mR range. The detector will have adjustable pixel size and be capable of pixel sizes less than 50 mu/m and will have high-contrast spatial resolution greater than 10 lp/mm while operating at typical angiographic kVp's. The MAF will have a viewing field of at least 5 cm diameter sufficient for viewing small regions where interventions and in particular flow modifying interventions are occurring. The MAF will be compatible with existing commercial angiographic x-ray sources and be mounted in such a way as to be interposed between the x-ray image intensifier, X/I, of the commercial system and the phantom, animal, or patient so that the MAF can be used when its high resolution ROI capabilities are needed, usually at critical times during interventional procedures. Additionally, the MAF will be used for cone-beam rotational microangiography and micro-computed tomography (CT) for determining vessel lumens needed for accurate flow assessment. Also, the new imaging capability for accurate localization will allow the development and implementation of new devices: asymmetric, variable porosity stents for treatment of cerebral aneurysms by modifying aneurysm blood flow characteristics. To optimize the new stent design, flow reduction in aneurysms needed to induce thrombosis will be explored. Also details of flow and flow modification will be investigated using advanced theoretical and experimental methods. Finally clinical application of the high-resolution microfluoroscopic detector will be used to evaluate the use of stents for flow modification in the cerebrovasculature of human patients.

E. Kenneth Hoffman

1. Image-guided treatment planning for neuro-interventions (5R01EB002916-02): Endovascular neurologic interventions are increasing in frequency and complexity. Devices are being placed above the Circle of Willis. Currently, interventionalists are judging accessibility via the proximal vessels using only subjective estimations from 2D angiograms. As a result, complication rates during procedures are as high as 10%. Calculation of the tortuosity of vessels can be performed from data extracted from computed tomography angiography (CTA), magnetic resonance angiography (MRA), and biplane or rotational angiograms, but the intervention a list also needs feedback of catheter position relative to the vasculature during the procedure. In addition, models are needed to estimate patient risk prior to placement. The goal of the proposed research is safer neuro-endovascular procedures, achieved by developing a system that can provide the interventionalist with information on vessel tortuosity, accessibility, device position, and potential complications during the procedure prior to and during device placement. Therefore, we propose to determine the hitherto unavailable quantities of curvature of, tortuosity of, and accessibility via the carotid, vertebral, and basilar arteries. Moreover, we will develop innovative, new techniques for estimating position and orientation of catheters, angioplasty balloons, and stents relative to vessel walls (proximity) and fusing this information with the vascular information for use during the procedure. With this information, we will develop models relating curvature, tortuosity, and proximity to complications so that the interventionalist can make more informed decisions regarding patient treatment involving interventional devices prior to and during attempts at placement, and thereby, reduce patient mortality and morbidity. With curvature and tortuosity available during interventional neurovascular procedures, coupled with relative 3D catheter positions and calculated probabilities of complications, we expect to improve access decisions as well as interventional device decisions. The impact of the proposed research is expected to lead to a substantial reduction of mortality and morbidity associated with neurovascular procedures.

2. On-Line 3D Vasculature from Biplane Angiograms (5R01HL052567-07): *The goal of the proposed research is to develop automated methods for accurate and reliable* quantitative vascular measurements (vessel diameters, cross sections, and distances) in clinical settings. The developed technology will not only provide the cardiologist with immediate quantitative information as well as the means for longitudinal patient assessment, but may also be the basis for more extensive and cross-modality patient evaluations. Under the previously funded research, we developed a system for accurate and reproducible determination of 3D vascular tree centerlines from biplane image pairs within 5 minutes of initiation of analysis. In this continuation proposal, we will not only improve the centerline accuracy, but we will also develop new techniques (a) for fully automatic centerline determination, (b) for lumen reconstruction using breakthroughs developed in our lab, and (c) for

alignment and comparison with IVUS data with speeds sufficient to provide clinically critical information during interventions. The studies will extend beyond evaluations of the accuracy and reliability of the measured quantities to include evaluations of impact of these data on clinical decisions during catheterization procedures in pig models and in patients. With the increasing complexity of cardiovascular interventions and the need for accurate characterization of the vessel lumen in clinical studies for cross modafity and longitudinal comparisons, we see this research as one of the critical next steps in vascular analysis. As a result, we are convinced that the proposed research will result in more accurate and more comprehensive vascular data, safer angiographic studies, better longitudinal tracking of patients, quantitative evaluations of progression and regression of stenosis, and subsequent outcome analysis.

F. Steven Pruitt

1. Neural Stem Cell Survival and Function in Aging Mice (5R01AG020946-03): The subventricular zone (SVZ) of the lateral ventricles and the hippocampus of the mammalian brain contain populations of multipotent neural stem cells (NSCs). These cells continually divide, asynchronously, resulting in a replacement stem cell and a proliferative progenitor. Following additional division and migration, proliferative progenitors differentiate towards multiple neural lineages. It has been hypothesized that NSCs participate in neurogenesis throughout the life of the organism and contribute to maintaining the health of the brain and/or its functions, including memory. It is possible that NSCs undergo age- related changes that may lead to dysfunction or contribute to pathologies including Parkinson's or Alzheimer's diseases. The objectives of this proposal are to define changes in the presence, cell cycle parameters and function of the NSC and proliferative progenitor populations during aging in the brain using the mouse as a model. Where possible, such changes will also be assessed in human samples. Aim 1: A double nucleotide analog labeling method, and immunological detection of the DNA replication licensing factor MCM2 will be utilized to define replication competent long-term label retaining cells (putative stem cells) and proliferative progenitors within the SVZ of mice of different ages. Cell cycle parameters for each population of cells will be defined from these data. This set of experiments will define changes in the stem cell and proliferative progenitor concentrations and cell cycling times as a function of age and serve as a baseline for additional studies. Additionally, we will test the utility of expression of the DNA polymerase subunit proliferating cell nuclear antigen (PCNA) as a surrogate for nucleotide analog incorporation in defining the proliferative progenitor population. Finally, changes in MCM2 and PCNA expression in the SVZ and hippocampal regions of human brain sections will be assayed, immunologically, as a function of age. Aim 2: Using methods described above to monitor NSC and proliferative progenitor parameters, we will determine if changes in the properties of these cells during aging are cell autonomous or dependent on the environment of the aging brain using NSC transplantation techniques. Aim 3: Transgenic mouse models will be constructed in which the MCM2 gene is marked by incorporation of either enhanced green fluorescent protein (EGFP) or a tamoxifin inducible Cre-estrogen receptor fusion. These models will allow: 1) determination of whether all quiescent neural stem cells express MCM2 or, alternatively, if a sub-population MCM2 negative cells exists and 2) the fate of NSC populations to be followed, respectively. The ability to monitor the fates of neural stem cell progeny in vivo will allow us to determine if age- related changes in the differentiation potentials of these cells occur.

2. Genetic Analysis of Pax3 induction (5R01HD036416-05): The ability of cells to receive and interpret cues directing them to express genes appropriate for their location is central to the process of embryonic development. Most cells of the embryo must respond to cues in both dorso-ventral (DV) and anterior-posterior (AP) axes. We have developed a unique set of reagents that make analysis of expression from the Pax3 gene in the mouse a particularly tractable system for understanding how DV and AP positional information is integrated at the level of a single gene in establishing the central body plan of mammals. Pax3 is an effector gene which functions in translating DV positional information into differentiation of discrete cell lineages within the neural tube and somites. Its initial expression domain marks what will ultimately be the dorsal pole of the embryo. Four specific aims will be accomplished towards understanding the mechanisms that restrict Pax3 expression to this domain. First, in the mouse, we hypothesize that Bmp4

inhibits Pax3 expression in a posterior-ventral domain and integration of this inhibitory signal with stimulatory signals, required for dorsal localization, establish the appropriate pattern of expression. This role for Bmp4 is different from that in the chick and implies a difference in the fundamental process of DV patterning between these two vertebrates. These results will be carefully examined using both embryo culture and transgenic analysis. Aims 2 and 3 will define the molecular basis by which Bmp4 signals are interpreted on the Pax3 promoter, and integrated with other signaling inputs, at the level of cis-acting DNA sequence elements. Our previous work has localized sequence elements required for Pax3 expression in the neural tube to 1.6 kbp of DNA sequence, and we have now refined the required elements to four discrete sites. The roles of these elements in vivo will be determined. Finally, proteins that bind the elements sequence specifically have been isolated. Aim 4 will identify these proteins and generate cDNAs and Abs to allow us to define their functional roles. These studies will move our understanding of how DV pattern is established, and overlaid by AP patterning mechanisms, to localize Pax3 expression in mammals to a new level of resolution.

III. Cardiovascular/cardiothoracic- Mechanisms by which inflammatory processes associated with clinical aspiration events progress to pneumonitis and ventilator-associated pneumonias; sudden cardiac failure due to arrythmias.

A. John Canty

1. Chronic Adaptations of Myocardial Ischemia (5R01HL055324-07): Viable, chronically dysfunctional myocardium progresses from chronically stunned (with normal resting flow) to hibernating myocardium (with reduced resting flow) as the physiological significance of a coronary stenosis supplying a dysfunctional region increases to the point that subendocardial vasodilator reserve is exhausted. At the cell and molecular level, the transition to hibernating myocardium is accompanied by a regional downregulation of sarcoplasmic reticulum calcium uptake proteins, apoptosis-induced myocyte loss with myocyte hypertrophy, and an attenuated contractile response to beta-adrenergic stimulation in the absence of infarction. Although contractile reserve is attenuated, hibernating myocardium is surprisingly protected against the development of metabolic evidence of ischemia. Based upon these findings our central hypothesis is that hibernating myocardium represents an adaptive myocardial response that arises from synergistic mechanisms acting to minimize a regional metabolic imbalance between a limited regional oxygen supply and demand. These adaptations serve to limit metabolic evidence of ischemia and minimize superimposed acute myocardial stunning. We will test this by completing four specific aims. Aim l will determine whether contractile function and metabolism differ following sudden vs. gradual increases in myocardial oxygen consumption and define whether the blunted metabolic response of hibernating myocardium is related to flow limitation or an intrinsic property of the myocardium. Aim 2 will determine whether hibernating myocardium is endogenously preconditioned against acute myocardial stunning produced by demand-induced ischemia via chronic induction of iNOS and determine whether it can be blocked by selectively inhibiting COX-2 or the mitochondrial K+ATP channel. Aim 3 will determine if repetitive episodes of ischemia lead to regional reductions in presynaptic neuronal norepinephrine uptake that contribute to the transition from chronically stunned to hibernating myocardium. Aim 4 will determine whether chronic alterations in beta-adrenergic receptor function and adenylyl cyclase activity are responsible for the blunted contractile response characteristic of hibernating myocardium. The results of the study are likely to lead to novel information that will improve our mechanistic understanding of chronic adaptations to myocardial ischemia and the physiological and biochemical factors responsible for modulating contractile reserve in the setting of a chronic coronary stenosis.

2. Apoptosis and Contractility in Ischemic Cardiomyopathy (5R01HL061610-04): Ischemic cardiomyopathy is the most common etiological cause of heart failure but the factors responsible for initiating decompensated LV dysfunction are unknown. Although considerable work has focused on irreversible injury following infarction, many patients have symptoms of heart failure in association with viable dysfunctional or "hibernating" myocardium. Pathological studies support the notion that the degree of dysfunction frequently exceeds the amount of structural fibrosis identified at postmortem exam. Preliminary studies by the applicant

have reproduced the physiological features of hibernating myocardium in pigs with a chronic LAD stenosis. While this occurs with normal LV function and without infarction, there is increased regional myocyte apoptosis, a 30 percent loss of myocytes and compensatory myocyte hypertrophy after a period of 3 months. At the molecular level, there is a regional downregulation of SR calcium uptake proteins. These changes, arising from reversible ischemia (i.e. angina pectoris) and with normal global LV function, are identical to the abnormalities found in end-stage heart failure. Thus, the overall hypothesis of this application is that myocyte apoptosis and SR dysfunction arise in areas with chronically reduced coronary flow reserve and are early rather than late events in the pathogenesis of ischemic cardiomyopathy. Aim 1 will define the role of apoptosis mediated myocyte loss and LV remodeling from reversible ischemia in hibernating myocardium. A 2-vessel stenosis model that progresses to global LV dysfunction with LV dilatation and increased LV filling pressure will be used to determine how diastolic stretch and the size of the dysfunctional region modulates apoptosis and LV remodeling. Aim 2 will identify the temporal progression of apoptosis in ischemic and normal regions in relation to the expression of the pro- and anti-apoptotic proteins Bax and Bcl-2 which will be quantified in vivo on a regional basis. Aim 3 will define the extent that apoptosis mediated myocyte loss and altered SR protein expression affects the reversibility of function in hibernating myocardium after surgical revascularization and after stimulating angiogenesis with basic fibroblast growth factor (FGF-5). Aim 4 will determine whether apoptosis and altered SR protein expression can be prevented pharmacologically with beta blockade, by stimulating angiogenesis prior to the development of myocyte loss and by overexpressing Bcl-2 in vivo. This integrative approach should provide a better understanding of the events that lead to the progression of ischemic LV dysfunction at a time when therapeutic interventions such as revascularization and in vivo gene transfer can be used to interrupt the progressive myocyte loss, contractile dysfunction and irreversible structural fibrosis.

3. Hibernating Myocardium and Sudden Cardiac Death (1R01HL076252-01): Currently available electrophysiological approaches are limited in their ability to identify the majority of patients with CAD and LV dysfunction that succumb to sudden cardiac death (SCD). Over half of the patients developing SCD are not inducible at EP testing underscoring the need for new ways to identify substrates leading to arrhythmic death. Viable chronically dysfunctional or hibernating myocardium, not amenable to revascularization, appears to be a major risk factor for subsequent cardiac death and is present in up to 60 percent of patients with ischemic cardiomyopathy. Revascularization improves survival but most patients with hibernating myocardium are managed medically due to prohibitive procedural risks or technical limitations. Cause specific mortality data is limited but appears to be arrhythmic rather than from fatal MI or progressive CHF. In support of this, swine with hibernating myocardium develop SCD from VT/VF in the absence of MI or CHF. Potential triggers of lethal arrhythmias include regional reductions in SR calcium uptake and release proteins, myocyte hypertrophy and altered sympathetic innervation. The central hypothesis of this proposal is that the presence of hibernating myocardium as opposed to scar identifies a large subset of patients with ischemic cardiomyopathy that are at high risk for SCD. We further hypothesize that this risk is related to inhomogeneity in sympathetic innervation arising from chronic repetitive ischemia. We propose a prospective observational study that will enroll 360 patients with CAD, Class I-III CHF and an EF <= 35 percent. Using positron emission tomography (PET), the prevalence and amount ofhibemating myocardium will be quantified in patients that are not candidates for coronary revascularization. Aim 1 will determine whether imaging the mismatch between viability (preserved (18)F-2-deoxyglucose) and reduced resting flow (13)N-ammonia can predict an increased risk of SCD (or ICD discharge for VT/VF as a surrogate end-point) in hibernating myocardium. Aim 2 will image norepinephrine uptake using (11)C-hydroxyephedrine to determine whether inhomogeneity in myocardial sympathetic innervation predicts SCD risk better than viability testing. Aim 3 will identify whether the substrate identified by PET is stable after an aborted SCD event by evaluating temporal changes in function, viability and sympathetic innervation in patients with an ICD. Our long-term objective is to develop better approaches to identify patients with CAD who are most likely to benefit from primary prevention of SCD with placement of an ICD.

B. Thomas Russo

1. Bacterial Modulation of Lung Inflammatory Response (5R01HL069763-04): Gram-negative bacilli (GNB) are pathogens that are capable of causing severe, life-threatening pneumonia. More than 60 percent of nosocomial pneumonias are caused by GNB and associated mortality rates are often >50 percent. Over the last 10-15 years, there has been little improvement in outcome from this infection. As a result, this syndrome continues to cause significant morbidity and mortality and strongly contributes to the economic burden of our national health care system. The successful use of immune intervention in the treatment or modulation of infections has marked the beginning of a new era in the management of infectious diseases. There exists a delicate balance between an efficacious and injurious host defense response. An understanding of the host response in GNB pneumonia and how bacterial components affect this response will, in turn, lead to the development of rapid diagnostic tests that will enable the clinician to effectively utilize a variety of biologic modulators. It is also necessary to understand the relative role of bacterial components versus host factors in mediating damage to the lungs prior to therapeutic manipulations on which little is known. This information will enable us to appreciate the relative risk benefit ratio of altering the host response. Further, a more precise clarification of which host components are damaged is also needed. This knowledge may identify independent therapeutic interventions. Their global hypothesis is that surface components of GNB and/or secreted proteins differentially alter host antibacterial defenses and directly, and/or indirectly (by inflammatory mechanisms) promote lung injury. Preliminary data supports this hypothesis. These responses will have significant implications when attempting therapeutic immune interventions. The goals of this proposal are to determine the mechanisms by which the bacterial capsule and 0-specific antigen modulate neutrophil recruitment into the lungs in a diametrical manner and extend our evaluations on the relative roles of bacterial factors (e.g. hemolysin) and bacterially induced host response elements in directly mediating the pathogenesis of lung injury.

C. Paul Knight

1. Host response to post-operative pneumonia (5R01AI046534-04): Anesthesia/surgery predisposes the patient to develop nosocomial pneumonia by mechanisms that are not completely defined. The presence of a viral respiratory tract infection (RTI) during anesthesia/surgery further complicates the host antibacterial response. Evidence from our laboratory has demonstrated anesthesia/surgery induces changes in cytokine response (e.g., TNFalpha, MIP-2, IFNgamma), leukocyte recruitment, and lung injury to influenza RTI. These responses are also critical to innate host defenses against bacterial pathogens. Our focus is to examine cellular mechanisms during a viral RTI that predispose the host to a post-surgical bacterial pneumonia. We hypothesize that anesthesia/surgery will change host responses differently during distinct periods in the course of a viral RTI by altering expression of pro- and antiinflammatory cytokines, thereby decreasing antibacterial defenses. Aim #1 will assess the effects of anesthesia/surgery during influenza on bacterial clearance, inflammatory cell influx, and cytokine expression an Escherichia coli challenge. We predict that laparotomy during influenza will promote the relative expression of MCP-1 and IL-10 over TNFalpha, MIP-2, and IFNgamma. Aim #2: will assess ex vivo the combined effect of laparotomy and influenza on a) LPS stimulated aMphi cytokine expression and phagocytic activity, and b) the ability of in vitro antiMCP-1, antiIL-10, or IFNgamma administration to improve M dysfunction. We postulate that laparotomy during influenza will alter aMphi regulatory functions and decrease effector functions as a result of selective enhancement of expression anti-compared to proinflammatory cytokines. Finally, in Aim #3, we will examine the contribution of endogenous cytokines in the suppression of antibacterial defenses following laparotomy during influenza by selective cytokine manipulations. Bacterial clearance, inflammatory cell influx, and cytokine levels will be assessed. We anticipate that neutralization of IL-10 or MCP-1, administration of IFNgamma, or increased TNFalphaexpression will improve antibacterial host defenses following laparotomy during physical signs of influenza. These studies will examine mechanisms that lead to alterations in bacterial clearance post-surgically following a viral RTI, assess the pathogenesis of post-surgical pneumonia in general, and suggest immune adjuvant strategies to prevent this complication.

2. Pathogenesis of Aspiration Pneumonia (5R01HL048889-07): Gastric aspiration is a major risk factor for the development of ARDS, and also places the patient in jeopardy of secondary bacterial pneumonia. The

major objective is to examine cellular mechanisms that increase the risks of these diseases. Although aspiration-induced host changes are poorly understood, this laboratory has demonstrated a likely role for TNF-alpha, IL-1-beta, MIP-2, MCP-1, and toxic inflammatory chemical mediators including proteinases, oxidants, and nitric oxide (NO) in the pathogenesis of the secondary lung injuries. The applicants hypothesize that increased susceptibility results from acid-induced, cytokine-mediated "priming" of leukocytes. They have demonstrated that "priming" enhances release of oxidants, NO, and proteinases following an additional sustained insult. They predict that oxidants and NO can subsequently augment cytokine progression and accelerate the shift to monocytic recruitment, thereby increasing lung injury and mortality. Aims #1 and #2 will focus on mechanism(s) involved in "priming" and Aim #3 will evaluate the effects of "priming" on susceptibility of the host to bacterial infection. Aim #1 will assess: a) recruitment and turnover of the MO population following acid (ACID), small non-acidified particle (SNAP), or combined acid small particulate (CASP) aspiration and b) in vivo TNFalpha and/or IL-1beta, or MCP-1 neutralization on the ability to reverse alterations in ex vivo MO expression of cytokines, iNOS, and MO toxic products. The investigators predict that CASP aspiration will augment the progression of the cytokine response to a more rapid expression of MCP-1 and MO influx with enhanced effector activity. Aim #2 will examine the role of superoxide, H2O2 and/or NO to the CASP-induced synergistic lung injury and increased mortality by examining the proinflammatory cytokine cascade and leukocyte influx following: a) selective inhibition of superoxide, H2O2, or NO generation, before or following CASP aspiration, or b) hyperoxia-induced generation of oxidants and/or NO following ACID aspiration. They postulate that increased H2O2 and NO generation will augment the progression of proinflammatory cytokines and accelerate the appearance of MCP-1-mediated monocyte recruitment. Finally, in Aim #3 they will examine cytokine expression, lung inflammatory cell influx, bacterial clearance, lung injury, and mortality following gastric aspiration. The investigators anticipate that aspiration will increase the lung injury to bacterial challenge by initially enhancing the inflammatory injury, but eventually impairing TNFalpha, IL-1beta, and/or MIP-2 production, thereby decreasing bacterial clearance. These studies may uncover mechanisms on which to base therapeutic strategies.

D. Frederick Sachs

1. Mechanical Transduction by Cardiocytes (2R01HL054887-10): All cells have mechanosensitive ion channels MSCs). Their physiological roles in non-specialized sensory tissues, such as the heart, are unknown, but new tools have become available. A peptide isolated from tarantula venom specifically blocks a subset of mechanosensitive ion channels, including channels found in heart, kidney, glia and smooth muscle. This proposal addresses the fundamental biophysics of mechanosensitive channel gating, and the molecular properties of the channel viewed through the structure of peptide blockers. The work has direct clinical relevance to stretch induced cardiac arrhythmias, muscular dystrophy and glial tumors. The peptides have been shown to block atrial fibrillation, inhibit stretch induced Ca+2 uptake in dystrophic muscle and stretch-induced secretion of growth factors from glia. Most MSCs open with increases in membrane tension, but mechanisms of activation and inactivation are not clear. In eukaryotes, tension is distributed across the complex mechanical structure of the membrane cortex. This includes the heterogeneous lipid bilayer with transmembrane proteins, the cytoskeleton and the extracellular matrix. Measuring the mechanics of pure bilayers, and natural and simplified cell membranes will show the stresses are distributed. This will reveal new ways channels can be modulated. The major technique is patch clamping. Patch capacitance is a sensitive measure of membrane mechanics. The capacitance changes of defined lipid vesicles under stress will help calibrate the meaning of capacitance changes in natural membranes. Cytoskeleton depleted patches will help define how tension is shared. Current peptide blockers have relatively low affinity, and unknown sites of interaction with target channels. Using recombinant protein expression to exhaustively mutate the peptides we will attempt to find peptides with 1) significantly higher affinity, 2) different affinities for MSCs in different cell types. This knowledge will help pave the way for channel isolation by affinity chromatography and rational drug design.

E. Robert Genco

1. Periodontal Intervention For Cardiac (5U01DE013940-03): This proposal is to develop the clinical trial organization for studies of periodontal treatment in the secondary prevention of cardiovascular events and to develop the institutional teams to accomplish the research. With the appropriate infrastructure and procedures developed, a future definitive randomized clinical trial of the relationship between periodontal infection and cardiovascular disease can be successfully carried out. There is growing evidence of a strong relationship between infection and atherosclerosis as well as a specific link between periodontal infection and heart disease. Previous studies have established possible effective treatments of periodontal disease. These treatments may lead to a diminution of subsequent occurrence of myocardial events in persons at high risk for cardiovascular disease. A clinical trial process to demonstrate the feasibility of a periodontal intervention trial in heart patients requires the establishment of a new team of cardiologists, periodontists, epidemiologists, infectious disease specialists, biostatisticians, research nurses in periodontics and cardiology, and data managers. In addition, such a study requires a large number of institutional investigative centers. In contrast to previous heart disease studies, the primary treatment in the new trial process would be directed toward infectious agents with specific attention to periodontal sites. We propose a multi-center Planning and Pilot Study involving 5 Investigational Centers at the University of Buffalo, University of North Carolina, Boston University, Kaiser Permanente/Oregon Health Science University, University of Maryland, and a Coordinating Center at the University of North Carolina. This study will be supported by Central Microbiology and Cytokine Labs, and a Biologic Specimen Bank. The establishment of the investigative machinery to determine the relationships and treatments linking infection, periodontal disease and heart disease requires a careful, cost-effective approach. This planning proposal outlines a process designed to develop and demonstrate the procedures for such studies, to conduct a pilot randomized clinical trial to test the feasibility of these procedures, and to select and refine a periodontal intervention. We propose that this planning and pilot study will ultimately lead to a definitive randomized clinical trial of the relationship between periodontal infection and cardiovascular disease, and may be a vanguard study for such a trial.

F. Maurizio Trevisan

1.

G. Frank Scannapieco

1.

H. Richard Donahue

1.

IV. Pathogenesis & Biodefense- Investigation of virulence factors for bacteria and viruses important in human disease and in bio-warfare. Novel vaccines and anti-microbial agents will be developed based on structure-function understanding of these factors.

A. Anthony Campagnari

1. Analysis of M Catarrhalis Receptors as Vaccine Antigens (2R01HL054887-10): Moraxella catarrhalis, a human mucosal pathogen, is a prominent cause of otitis media in young children and lower respiratory tract infections in adults with C.O.P.D. The significant financial burden on the health care system in this country, has stimulated research studies aimed at identifying possible vaccine components expressed on the bacterial surface. Despite these efforts, a vaccine target remains elusive. Recent studies have focused on the components of the bacterial outer membrane, as these structures would most likely be available for interaction with the host immune response. However, it is clear that very little is known about the host

immune respons3e to infections with M. catarrhalis. We have reported studies demonstrating that M. catarrhalis can obtain iron from human transferrin and lactoferrin for in vitro growth, an the absence of any detectable siderophore specific proteins on the bacterial surface. We hypothesize that these proteins, named OMP B1, which binds human transferrin and LBP, which binds human lactoferrin, are likely similar to the iron-repressible, receptor proteins expressed by various Gram- negative pathogens. Using monoclonal antibodies we have shown that OMB B1 and LBP are conserved on the surface of all M. catarrhalis strains tested to date. In addition, we have shown that children recovering from otitis media have IgG antibodies to both proteins in their convalescent sera. We hypothesize that OMP B1 and LBP contain conserved, surface exposed epitopes which are expressed in vivo and are immunogenic in children. We further hypothesize that OMP BL and LBP are expressed in vivo in response to iron-limitation, thus making these proteins potential targets of the human immune response. The two broad goals of this proposal are to determine the specific structural arrangement of OMP B1 and the LBP and to determine if these proteins have potential value as vaccine components by analyzing the human immune response to each protein in children with otitis media. Our hypothesis will be tested by the following specific aims: (1) To develop and characterize monoclonal antibodies (MABs) directed to different epitopes of a specific transferrin receptor and a lactoferrin receptor. (2) To delineate membrane topography of each receptor and define antigenic domains. (3) To analyze and characterize the human antibody response, elicited in vivo, to each specific receptor.

2. Genetics & Biology of M. catarrhalis LOS in Otitis Media (5R01DC005837-03): Moraxella catarrhalis is a Gram-negative human pathogen, which predominantly causes middle ear infections and sinusitis in infants and children, and lower respiratory tract infections in adults. This organism is the third leading cause of otitis media and it is estimated that approximately 50% of children will become colonized by this bacterium in the first 6 months of life. Recurrent acute otitis media infections have also become prevalent resulting in potential hearing loss and subsequent developmental and learning problems as these children reach school age. Middle ear infections are very common and it is estimated that 80% of children under the age of 3 will experience at least one episode. There are also significant health care costs associated with treatment for otitis media and it has been determined that M. catarrhalis is responsible for approximately 3 to 4 million physician office visits annually. This estimate is considered conservative now that over 90% of M. catarrhalis clinical isolates produce b-lactamase. Together, these data have stimulated research efforts aimed at identifying specific virulence factors involved in colonization and infection. One prominent bacterial surface component implicated as a potential virulence factor, is the lipooligosaccharide (LOS). Structural studies show that M. catarrhalis LOS is similar to the LOS of other Gram-negative human pathogens, including Neisseris meningitidis, Neisseria gonorrhoeae and Haemophilus influenzae. More importantly, these common LOS structures shared by M. catarrhalis and these prominent human mucosal pathogens have been implicated as virulence factors. Although M. catarrhalis express these LOS epitopes, there have been minimal studies describing the role of LOS in pathogenesis. In addition, there is currently no information available regarding the assembly and expression of this major surface glycolipid. In this proposal we will perform a comprehensive analysis of the genetics and biology of M. catarrhalis LOS. These studies will be instrumental to our understanding of the role of LOS in the pathogenesis of otitis media and should provide insight into new strategies designed to prevent this disease. We will test our hypotheses by the following specific aims: (1) Isolate and characterize of the genes involved in the biosynthesis and assembly of M. catarrhalis LOS. (2)Perform biologic studies to define the role for M. catarrhalis LOS in otitis media.

B. Timothy Murphy

1. Development of a Vaccine for Moraxella Catarrhalis (5R01AI028304-15): Moraxella (Branhamella) catarrhalis is a common and important human respiratory tract pathogen. It is the third most common bacterial cause of otitis media in children after Streptococcus pneumoniae and non- typeable Haemophilus influenzae. M. catarrhalis is also an important cause of lower respiratory tract infection in adults with chronic obstructive pulmonary disease (COPD). In view of the morbidity and cost associated with infections caused by M. catarrhalis, there is significant interest in developing vaccines to prevent these infections. During the previous funding Period, two outer membrane proteins (OMP CD and OMP E) with are potential vaccine

antigens have been identified and initially characterized. The goal of the proposed studies is to more rigorous study OMP CD and E as potential vaccine antigens. Our hypothesis is that immunization with OMP CD and E will generate an immune response which will protect from infection cased by all strains of M. catarrhalis. Emphasis is placed on studying the human immune response because M. catarrhalis is an exclusively human pathogen. The proposal has four specific aims. In Aim 1, the degree of sequence conservation of OMP E among strains of M. catarrhalis will be characterized. In Aim 2, potentially protective (bactericidal) epitopes on OMP on CD and OMP E will be identified. In Aim 3, the human immune response to OMP CD and E and potentially protective determinants on OMPs CD and E will be studied in epidemiologically well defined samples from children and adults. Finally, in Aim 4, to study the human immune response to immunization with OMP CD and E, SCID mice reconstituted with human peripheral blood lymphocytes will be immunized and the immune responses will be characterized with regard to a) isotype and subclass distribution of antibodies, b) the establishment of a memory response, and c) the bactericidal activity of the antibodies. The proposed studies will generate information on the human immune response to OMP CD and E. This information will be important in assessing the feasibility of proceeding to the testing of OMP CD and E as vaccine antigens in humans to prevent infections caused by M. catarrhalis.

2. Development of a vaccine for nontypeable H influenzae (5R01AI019641-20): Nontypeable Haemophilus influenzae (NTHI) is an important cause of otitis media in children and lower respiratory tract infection in adults with chronic obstructive pulmonary disease (COPD). A vaccine to prevent otitis media in children and lower respiratory tract infections in adults with COPD would reduce mortality (in case of COPD) and morbidity, improve quality of life and reduce health care costs. The development of such a vaccine is one of the goals of the work proposed. The antigenic structure of two important outer membrane proteins of NTHI (P6 and P2) will be elucidated and their potential as vaccine antigens will be assessed. P6 has several characteristics suggesting that it will be an effective vaccine antigen. In Specific Aim 1, the structure of P6 as it exists in the outer membrane will be elucidated and the human immune response following immunization with P6 will be characterized with emphasis on identifying protective epitopes recognized by human antibodies. P2 is the major protein in the outer membrane. Antibodies to conserved regions of P2 are highly bactericidal for multiple strains of NTHI, indicating that these conserved regions may form the basis of an effective vaccine. In Specific Aim 2, the sequence heterogeneity of two relatively conserved surface loops will be determined, antibodies to conserved loops will be developed and characterized, the stability of P2 in the human respiratory tract will be assessed, and broadly cross-reactive human bactericidal antibodies will be studied for binding to conserved loops of P2. The proposed work will advance the area of vaccine development for NTHI by elucidating the antigenic structure of two important surface proteins and rigorously assessing their potential as vaccine antigens with emphasis on elucidating protective immune responses in humans.

3. Recurrent otitis media and COPD: immunity and vaccines (5P01AI046422-04): Otitis media leads to enormous morbidity and to direct annual healthcare costs estimated to be $3 billion in the U.S. Chronic obstructive pulmonary disease (COPD) is the fourth most common cause of death in the U.S. Bacterial infection in COPD causes substantial morbidity and mortality. Two of the three most common bacterial pathogens to cause otitis media and respiratory tract infections in COPD are non-typeable Haemophilus influenzae (NTHI) and Moraxella catarrhalis. The development of vaccines to prevent otitis media and respiratory tract infections in adults with COPD would have important impact in reducing mortality, preventing morbidity and reducing healthcare costs in these settings. This program project proposes studies which will elucidate the immune response to specific antigens of NTHI and M. catarrhalis. Our hypothesis is that the pattern of respiratory tract infections due to NTHI and M. catarrhalis in children and adults depends on the immune response to specific surface antigens of the organisms. The aims of the project will be accomplished through the efforts of a multi-disciplinary research team which will collaborate to carry out three interrelated projects. Project 1 will characterized the role of human T cell responses to protein P6, a promising vaccine antigen will be determined through studies with carefully defined samples from humans. Project 3 will focus on antigenic characterization of the lipooligosaccharide (LOS) of M. catarrhalis, the role of NOS as an adhesin and the human immune response to determinants on the LOS molecule. An

Administrative/Statistical Core will coordinate the program and provide statistical expertise to each of the projects. Since NTHI and M. catarrhalis are exclusively human pathogens, a strong emphasis is placed on elucidation of the human immune response. The proposed studies will advance the field of vaccine development to prevent otitis media and respiratory tract infections in COPD.

V. Drug Discovery and Delivery- Biologically relevant compounds that can be linked to effects on specific gene expression patterns, creating new drug targets. Included in this program are two new start-up companies.

A. Huw Davies

1. Neurobiological Investigations using Pharmaceutical Applications of Catalytic Asymmetric Reactions (5R01DA015225-03): The central goal of this project is to synthesize novel methylphenidate analogs and evaluate their potential as medications for the treatment of cocaine addiction and as precursors to novel neuroimaging agents. Even though a reasonably wide range of methylphenidate analogs have been explored in the past, a major shortcoming with the previous studies in this area was the limitations associated with the traditional method used for the synthesis of these analogs. Having developed a very powerful two-step asymmetric synthesis of threo-methylphenidate, this enabling technology will be used for the synthesis of a wide array of methylphenidate analogs. The specific synthetic targets of the project are the following: naphthyl analogs of threo- and erythro-methylphenidate, heterocyclic analogs of threo-methylphenidate, oxa and carba analogs of threo-methylphenidate, piperidine ring-modified analogs, and polycyclic derivatives. All compounds will be initially screened for their binding to the dopamine, serotonin and norepinephrine transporters. Classes of compounds that display interesting biological properties will be extended into a more extensive series. These new targets are being used for several neurological problems, including medications for treatment of cocaine addiction and Ritalin analogs that allows exploration of long acting and short acting dopamine reuptake inhibitors for attention deficit disorders. Further in vitro and in vivo evaluation of promising compounds will be conducted. Initial studies will also be carried out to determine if useful PET radioligands can be made from any potent and selective analogs that are discovered during these studies.

2. Neurochemistry of Novel Tropanes (NSF): The vinylcarbenoid chemistry leads to the direct syntheses of many compounds of biological interest. Particularly useful has been a short scheme we have developed for the synthesis of tropanes. These rather rigid compounds are extremely potent and selective in their interactions at the central nervous system. Using this chemistry we have developed compounds that interact with the dopamine neurotransmitter, as potential medications for cocaine addiction. Additionally, other compounds that interact with the serotonin neurotransmitter have generated great interest as potential antidepressants. Many of the compounds that we prepare are valuable tools to study neurochemical disorders. Consequently, we are involved in a number of multidisciplinary projects with research groups in Physiology, Pharmacology and Radiology.

B. William Jusko

1. Corticosteroid Pharmoacokinetics and Pharmacodynamics (5R01GM024211-28): Major factors determining corticosteroid receptor binding and pharmacodynamics will be measured and improved mathematical models for quantitating the pharmacokinetics and pharmacodynamics (PK/PD) of corticosteroids will be sought. These important drugs exert their hormonal, immunosuppressive, and anti-inflammatory effects by diffusion into cells, reversible binding to cytosolic receptors, and then either have direct effects on biochemical processes or produce inhibition or stimulation of gene/mRNA-mediated synthesis of diverse effector proteins or enzymes. Realistic and comprehensive PK/PD models of corticosteroid action are feasible which permit more mechanistic insights into drug, dosage, and interaction factors which determine their effects. One specific aim is to extend our current gene-mediated models of steroid PK/PD in rats (measuring steroid disposition, hepatic receptors and mRNA, and hepatic TAT mRNA and TAT enzyme) to account for chronic dose effects, which are often highly deleterious. The second

aim is to generalize our "fourth-generation" receptor/gene PK/PD model for methylprednisolone to other steroids based on advanced QSAR principles and the expectation that alterations in pharmacokinetics and receptor binding will account for differences in responses. The third aim will be to evolve improved methods for assessing drug interactions for indirect response processes with focus on joint effects of prednisolone and COX-2 inhibitors. The fourth aim seeks to utilize quantitative gene array techniques to compare types and time patterns of suppression and enhanced expression of diverse genes altered by corticosteroids. These studies will improve the pharmacologic rationale of corticosteroid therapy as well as continue the generation of important pharmacodynamic models which apply to drugs causing effects by complex and indirect mechanisms.

2. Mathematical Models in Pharmacodynamics (5R01GM057980-06): The major factors determining drug responses are the input and disposition rates controlling pharmacokinetics, drug distribution to the site of action (biophase), the mechanism of drug action in altering mediator or receptor level, and transduction processes. May improvements in quantitating pharmacologic responses came from our recognition that diverse pharmacodynamic effects can be characterized using a family of four basic (and extended) indirect response models. These (and most) models require analysis using differential equations which usually cannot be fully integrated. This project seeks to characterize and quantify the properties of drugs acting by diverse mechanisms. Specific aims include development of a compendium with comparison of the array of relevant mechanism-based PK/PD models, further development of indirect response models with a precursor compartment including the occurrence of feedback alterations; evolution of extended indirect lifespan models for application to natural cell responses including effects such as bone marrow stimulation and/or cytotoxicity; and the detailed characterization of non-linear and time- dependent transduction models which may be applied to numerous membrane receptor (e.g., G-protein) mediated responses. Advanced methods of calculus and stimulations will be employed to seek exact or approximate solutions or behaviors for these models to identify how the onset, extent, return, duration, AUC, and steady-stage of responses are controlled, to recovery parameters more easily from experimental data, and to discriminate among diverse models available to describe typical data, and to determine how to optimize drug dosing regimens. These efforts will yield insights, methods, and resources for understanding and quantitating the time course of drug responses as related to major mechanisms of action.

C. Steve Pruitt

1. Development of a high throughput gene trap vector (5R01DA014773-03): The long-term objective towards which the current proposed work will contribute is the establishment of a random sequence tagged insertion library in C57/B6 embryonic stem cells from which cells carrying individual, known, integration events could be widely distribution to the scientific community. Four specific aims are proposed towards this objective. First, we will refine a high throughput sequence tag acquisition technology, which we have invented, for use in an ES cell gene-trapping vector in an automated format. Second, we will generate an ES cell gene trap vector carrying the sequence elements required for the sequence tag acquisition technology and recombinogenic sequence elements (FRT sites) that will allow the sequence at a given integration site to be modified. As part of this aim, vectors that allow "delivery" of alternative sequence elements to the integration site will be constructed and tested. Third, we will establish a repository of early passage C57BLI6JSvEvTac ES cell lines that retain the ability to contribute to the germ line in chimeric mice even after many passages. Additionally, methods for the automated isolation of ES cell clones will be tested. Finally, a demonstration library of 5,000 sequence tag indexed ES cell clones will be generated. The methodologies and reagents developed here will be fully sufficient to allow the rapid establishment of a random sequence tag insertion library of 100,000 or more events in which the location of each insertion sites is known.

2. Comprehensive Protein Interaction Mapping in Mammals (5R21GM068856-02): Knowledge of the interactions occurring between specific proteins can provide important links to their functions. Attempts to develop an understanding of the interactions that occur on a genome wide scale for model organisms, yeast

and nematodes, are ongoing. Similar information form mammalian systems is highly desirable. However, the approximately 10 fold greater complexity of the mammalian genome leads to an exponential increase in the difficulty of assessing all of the potential interactions. The present proposal will develop methodologies that enhance the efficiency with which techniques that have been successful in establishing genome wide protein interaction maps in model organisms can be applied to mammalian systems. The specific technique that will be modified here is the traditional yeast two-hybrid system. Once the methodologies are established, they will be sufficiently powerful to allow a complete protein interaction map to be established for all of the 1 x 10(10) potential interactions that are possible in a mammalian cell within less than 3 months using a single high throughput capillary sequencer. Based on the utility of similar protein interaction maps in model organisms, this work can be expected to provide an enormous wealth of insight into the function of mammalian cells.

D. Robert Straubinger

1. Drug carrier of blood/tumor/brain permeability (1R01CA107570-01)**:** Drug carriers can alter pharmacology and confer novel mechanisms of action upon agents having properties that compliment the characteristics of the carrier. Doxorubicin encapsulated within long-circulating liposomes by means of a "remote loading" method (L-DXR) represents the first in a new class of anticancer agents, and was recently approved by the FDA. However, its full spectrum of action and mechanisms remain to be defined. Previously we demonstrated substantial extension of lifespan for rats bearing an orthotopically-implanted drug-resistant brain tumor when administered L-DXR, but not free DXR. We have recently observed that repetitive doses of L-DXR (but not equivalent doses of free DXR) increase tumor vascular permeability and mediate vascular barrier breakdown. Normal vasculature appears to be unaffected. This effect, unprecedented in the literature, has important clinical and basic implications. The objectives of this proposal are (a) to understand the mechanistic basis by which this drug carrier system confers upon DXR the property of compromising vascular permeability, and (b) to determine the functional consequences of this effect. In particular, we will (c) explore the potential to enhance therapy through rational application of tumor vascular barrier breakdown, not only to optimize tumor deposition and therapeutic effect of L-DXR itself, but also to promote the therapeutic effects of other agents. Selective tumor vascular barrier breakdown could improve the penetration and effects of gene-carrier systems, and could increase the sensitivity of tumors to drugs having complimentary mechanisms of action, such as antiangiogenic agents. Vascular permeability changes resulting from repetitive L-DXR treatment will be investigated using a series of permeability probes and immunohistological approaches. Free DXR will be used as a control. Functional Magnetic Resonance (fMR) imaging will enable dynamic observation of tumor perfusion and vascular permeability changes. Effects of the vascular permeability compromise on the activity of potentially complementary agents will be investigated using TNP-470 and paclitaxel; the former is a well-characterized antiangiogenic agent, while the latter is an active chemotherapeutic agent having some antiangiogenic properties. The effect of vascular permeability changes on delivery by other macromolecular carriers will be probed using viral vectors carrying model genes.