Title of Project: Developing Adjunctive Agents to Enhance Prostate Cancer Therapy

Principal Investigators and Departments: Dr. John G Baust and Dr. Robert G VanBuskirk, Department of Biological Sciences/Institute for Biomedical Technology (IBT)

Funding Requested: $33,480

Project involves: (check all that apply):
- Human Subjects
- Biosafety/rDNA
- Radiation Safety
- Stem Cells
- Vertebrate Animals
- Hazardous Waste
- Select Agents
- None of the above

Project Abstract (200 words or less):

Cryotherapy is an effective means of ablating cancer tissue. Our group has spent a decade identifying and analyzing the molecular targets of cryosensitizers – agents when applied with freezing increase killing efficacy. These agents are now actively being tested in pre-clinical trials on prostate cancer at Duke Medical. Many of the cryosensitizers identified include chemotherapeutic compounds such as 5-FU, taxol, and others that are known to be toxic and, as such, can generate side-effects in patients. In the last few years our team has identified two bioactive food components, resveratrol and Vitamin D3, that are known for their nutritional value yet that when used as cryoadjuvants can (1) be as lethal as 5-FU, and taxol and (2) have minimal innate toxicity. Both compounds appear to have defined molecular targets. While promising as non-toxic, bioactive food components that can be used effectively in a cytotoxic program, their mechanisms of action must be determined before they are incorporated into a clinical protocol. This study proposes to (1) determine the molecular basis underlying these unique bi-functional behaviors and (2) identify more effective derivative compounds. Once accomplished, this project should lead to more effective and safe destruction of prostate cancer independent of the cancer phenotype.
Title: Developing Adjunctive Agents to Enhance Prostate Cancer Therapy

Project Narrative
Describe the proposed hypothesis-developing research, and how it represents an innovation to an important problem in prostate cancer. Explain how the planned research will be no greater than minimal risk, exempt under 32 CFR 219.101 (B) or eligible for expedited review.

Numerous ablative therapies are currently in use for the treatment of prostate cancer (PCa). While applied with curative intent, these monotherapies (radiation, chemotherapy and radical surgery) provide only modest success in disease suppression but not cure, as cancer cell mutation often enables resistance to therapy. This outcome, despite a half century of research, yields only modest improvements in patient’s durable response to diverse treatment strategies.

Radical prostatectomy has long been the standard of care, but this treatment has significant morbidity. Cryoablation (CA) treatment due to its growth over the past two decades is now recognized as an acceptable alternative to radical excision. However CA, too, has morbidity related to the freezing of extraprostatic tissues during treatment. The need to enhance the efficacy of CA in the treatment of neoplasms has led to the use of adjunctive therapeutic agents which have been diverse in nature. In general, the goal of adjunctive therapy is to enhance the destructive activity of freezing at the border of the frozen zone where the sub-freezing temperatures are between -40°C and -0.5°C. The intended effect of adjuvants is to cause cell death by apoptosis. Many challenges remain in the selection, dose, and use of the appropriate agents. Adjunctive chemotherapy with selected drugs, such as 5-Fluorouracil or Imiquimod, enhances cell injury when used for skin cancers. Trans-arterial chemoembolization, as an adjunct to CA, has proven useful in the management of large metastatic live cancers.

For the past decade we have focused on enhancing the therapeutic power of low temperature by understanding the molecular consequences of a freeze insult to cancer. We have identified and tested a number of compounds, cryoadjuvants or cryosensitizers, which improve the efficacy of CA when used in vitro on a variety of cancers. Most of these cryoadjuvants such as cisplatin, 5-FU and taxol among others, are highly toxic and non-selective resulting in a host of side effects when used alone. Two years ago we launched an extensive program to search for naturally occurring compounds that might serve as cryoadjuvants with the following characteristics: (1) demonstrate little to no toxicity in the absence of freezing and (2) are highly effective sensitizing agents when used in a CA paradigm. Our research group has identified resveratrol and vitamin D3 as agents that fulfill these criteria. The overall innovation behind this project is (1) the development of a unique Cryoablation-Sensitization small molecule (2) that is an injectable and can be applied prior to a freezing episode such that (3) it more effectively kills the target tumor tissue at -0.5°C to -40°C but (4) does not harm neighboring normal tissue at >0°C. Our hypothesis, based on preliminary data, is that the freezing insult and associate stresses experienced by a PCa cell will in combination with an appropriate small molecule adjuvant enhance the activation of multiple cell death cascades thereby providing a curative potential.

To provide the basic sciences foundation for this likely therapeutic option we propose to: A. Determine the role of apoptosis and the unfolded protein response (UPR) in cancer cell death associated with resveratrol and vitamin D cryosensitization. B. Identify the cell stress response pathways (cytotoxic and protective) that are selectively activated by cryosensitization and ablation. C. Examine the specific response of normal and cancerous cells to cryosensitization. D. Evaluate analogs of resveratrol and vitamin D as cancer specific cytotoxic agents when applied alone or in combination with cryoablation.
**Experimental Outline**

**Cell Culture:** Human prostate (PC3) cancer cells will be obtained from ATCC (Manassas, VA) and maintained at 37°C, 5% CO₂/95% air in basal media (Caisson Labs) in Falcon T-flasks. Cells will be sub-cultured every 5-7 days and media exchanged every three days. **Freeze Protocol:** Cells will be plated into 96 well strip-well plates, cultured for 24 hours and then given fresh media 30 minutes before freezing. Samples will be frozen to -10, -15, -20 and -30°C for 10 minutes. After sample cooling to -2°C, ice formation will be initiated then samples held at the target temperature. Samples will then be thawed at room temperature and returned to culture for recovery. **Cryosensitization:** Stock solutions of agents will be prepared as per SOP and agents will be used at 1, 10, 100 and 500 µM – the effective range identified. Agents will be added either 1 day or immediately prior to freezing. Samples will be frozen with or without cryoadjuvants. Agents will be applied at sublethal doses as part of the overall goal of eliminating toxic side effects. Controls will consist of diluents vehicle and cryoadjuvant non-frozen exposure. **Viability Assessment:** Cell viability will be determined using alamarBlue™, a metabolic activity indicator (Invitrogen), and Calcein-AM (Invitrogen), a membrane integrity dye. Briefly, after a 24-hour recovery period samples will be removed from the incubator, the medium decanted, 100 µl of the alamarBlue™ or Calcein-AM solution added to each well and incubated for 60 min (+ 1 min) at 37°C in the dark. Cell viability will be determined using a fluorescent plate reader (Tecan SpectroFluor plus). Following assessment, the alamarBlue™ will be removed, growth medium added, and plates returned to culture for continued recovery. **Flowcytometry:** Samples will be collected at 1, 4, 8, and 24 hours post-thaw. Modes of cell death will be assessed using the Vybrant Apoptosis Assay (Invitrogen) via triple staining with propidium iodide (necrosis), YO-PRO-1 (apoptosis), and Hoechst (live). Briefly, fluorescent probes will be applied, incubated for 20 min then analyzed using a Guava Easycyte flow cytometer and to quantitate the sub-populations. **Protein Analysis:** Samples will be collected at 1, 4, 8, and 24 hours post-thaw and proteins extracted using RIPA buffer as per SOP. Samples will be quantified via Bio-Rad BCA assay. 25ug protein will be aliquoted, volume adjusted, 2X loading buffer added then heated at 95°C. Samples will be resolved on a discontinuous SDS-PAGE gel, and transferred to PVDF membranes by semi-dry electrophoretic transfer (Trans-Blot SD Semi-Dry Transfer Cell, Bio-Rad). Membranes will be blocked in 5% BSA (EMD Chemicals), washed, incubated in 1° antibodies overnight at 4°C, washed, placed into 2° antibody-HRP linked, washed, developed using chemiluminescent detection (OptiBlaze, G-Biosciences) and visualized using a FujiFilm LAS-3000 imaging system. **Data Analysis:** Viability and Flowcytometry: Fluorescence units will be converted to percent based upon non-treated controls (37°C). SD (±) and ANOVA calculations will be used to determine statistical significance. A minimum of 4 repeats will be performed with an intra-experimental n=8 yielding a total N ≥ 32 for each experiment. **Western Blotting:** Blots will be digitized and analyzed using densitometry. Samples will be normalized to Tubulin and compared to matched time point controls. Data will be converted into graphical form to determine increases and decreases in product concentration over time. Statistical significance will be determined using single factor ANOVA. A minimum of 4 repeats will be performed for each condition.

This research proposed will be no greater than minimal risk as there is no use of human subjects, and the human cell line will be obtained from a commercially available source (ATCC). The cell line and applicable reagents will be handled by trained personnel.

**Describe the project’s originality, significance, quality and future impact.**

This study will be the first to investigate prostate cancer treatment that relies on a small molecule adjuvant. This is of significant clinical importance due to the current difficulties in treating PCa without complicating co-morbidities. The work proposed here will be led by a team with an extensive history of
successful research in the field. The future impact of this study may lead to the clinic in a relatively short period as the initial study relies on the use of natural products.

Describe PI and Co-investigator contributions to the project. Demonstrate the team’s experience in prostate cancer research (grant funding, publications, conference presentations, etc.) Discuss the student’s participation on the project.

Dr. John G Baust (PI) and Dr. Robert G Van Buskirk (Co-investigator) have decades of experience in PCa research. Dr. Baust has been active in PCa research for over twenty-five years and has served as a member of the American Urological Association Best Practices Panel on Cryotherapy of Localized Prostate Cancer. Dr. Van Buskirk has experience in PCa tissue engineering, including helping to develop two models (MatTek and CPSI). Drs. Baust and VanBuskirk have co-authored over 20 original research articles, review articles, and book chapters in the field of PCa. Dr. Baust routinely presents on PCa research at scientific conferences, including the Society for Cryobiology, American Urological Association, and Society for Thermal Medicine annual meetings. Dr. Baust is the Editor-in-Chief of the journal Technology in Cancer Research and Treatment.

Anthony Robilotto, MS, a Doctoral Candidate, will participate by utilizing his laboratory experience to perform the experiments necessary for the proposed studies. He has authored original research papers and given numerous presentations at scientific conferences on the topic of PCa, focusing on adjuvant treatments to cryotherapy to increase treatment efficacy. He has also developed a prostate cancer model and will work closely with, Drs Baust and Van Buskirk, to ensure proper progress.

Provide a brief statement of the benefits of this project, if funded, to the university. Specifically address the increase in fundability or visibility that would be achieved.

Peer reviewed publications; presentations at international conferences including the American Urological Association (AUA), the American College of Cryosurgery (ACC) and the annual Focal Therapy meetings will enhance Binghamton University’s reputation in the field of cancer research. Additionally, the proposed studies represent funding priorities within the DOD and NIH NCI (listed below).

Explain the project’s ability to attract future federal, state, philanthropic or private funding.

Extensive funding in PCa is available from diverse sources. The NIH's National Cancer Institute annually funds approximately $300-400 million annually for prostate cancer. We intend to apply for an R21 or R43 near the conclusion of this project. The American Cancer Society in 2015 funded 76 awards in PCa. We intend to explore ACS funding. The DOD’s Congressional Directed Medical Research Program funds eight PCa programs annually. We are eligible for four categories with funding levels between $750K - $2.5M. Private and philanthropic groups such as the Prostate Cancer Foundation, the American Association for Cancer Research, and nearly a dozen other groups provide grant support to basic research projects in PCa. We intend to seek funding from each of these organizations. Finally, Dr. Van Buskirk is chair of the NIH study section, “Cell and Molecular Biology,” and Dr. Baust maintain numerous clinical contact as such a collaboration network for project extension into clinical application exist.

Describe the plans for seeking external support for this project based upon this collaboration and include a listing of potential sponsors and timelines for proposal preparation.

As described, there are several potential sources of PCa funding. Further, we have a relationship with the DOD - U.S. Army to fund research in association with the U.S. Military Academy of an Institute for Molecular Research at West Point. We will seek to expand that relationship to include PCa research over the next six months. We intend to apply for a NIH NCI R21 grant in the spring 2016. We will approach private foundations to determine their emphasis in PCa research and apply as appropriate.


**Budget Justification**

The funds available will be used for the supplies needed to carry out the proposed research, including cells and all necessary culture reagents, antibodies and assays described. Additionally, we plan to use a portion of the funds for travel to the American Urologic Association North East Conference in Buffalo, NY. The remainder of the funds would be used for graduate student support, including tuition and stipend. A detailed budget is included below.

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BIOGRAPHICAL SKETCH

NAME
John G Baust, Ph.D.

POSITION TITLE
UNESCO Professor & Director

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
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<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
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<td>Inst. of Arctic Biology, Univ. of Alaska, Fairbanks, AK</td>
<td>Ph.D.</td>
<td>1967-70</td>
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A. Positions and Honors.

Positions and Employment
2011 - 2015 President, American College of Cryosurgery
2008 – UNESCO Professor
2000 - Director, Institute of Biomedical Technology, Professor, Dept. of Biological Sciences

Other Experience and Professional Memberships
1994 – 2003 Adjunct Professor of Neurosurgery, Department of Surgery, Medical College of Pennsylvania
1986 – 87 Professor and Chair, Department of Biological Sciences, State University of New York
1984 – 87 B.J. Luyet Distinguished Professor and Director, Institute of Low Temperature Biology, University of Houston

Honors
2013 Gold Medal Award. Intl. Society for Cryosurgery
2011 Fellow, American College of Cryosurgery
2009 Fellow, Society for Cryobiology
2009 Outstanding Alumnus, SUNY Fredonia
2008 UNESCO Professor
2002 SUNY Chancellor’s Award for Entrepreneurship

B. Selected peer-reviewed publications most relevant to current application
C. Book Chapters (16)
2. Image Guided Thermal Therapy.Presidential Symposium.Soc.Thermal Medicine, Minneapolis,MN.
NAME                  Van Buskirk, Robert G.
POSITION TITLE       Professor

EDUCATION/TRAINING   (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

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<tr>
<td>University of Vermont</td>
<td>B.A.</td>
<td>05/72</td>
<td>Zoology</td>
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<tr>
<td>University of Vermont</td>
<td>Masters</td>
<td>05/75</td>
<td>Cell Biology</td>
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<tr>
<td>Harvard University</td>
<td>Ph.D.</td>
<td>05/83</td>
<td>Cell Biology</td>
</tr>
<tr>
<td>Harvard University (Medical School)</td>
<td>Post-doc</td>
<td>1985</td>
<td>Biological Chemistry</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Employment
1983 - 2001  Visiting Faculty Member, Harvard University, Cambridge, MA
1986 - 1991  Asst. Professor, Dept. Biological Sciences, State University of New York at Binghamton (SUNY-Binghamton)
1996 - 1997  Interim Chair, Department of Biological Sciences
1997 - PRESENT Professor, Dept. Biological Sciences, SUNY-Binghamton
1998 - 2001  Director of Research, BioLife Solutions, Inc., Binghamton, NY
2000 - PRESENT  Assoc. Director, Institute of Biomedical Technology, SUNY-Binghamton
2000 - 2002  Chair, Dept. Biological Sciences, SUNY-Binghamton
2001 - 2004  Director, Advanced Biotechnologies Center, SUNY-Binghamton
2001 - 2002  Vice President R&D, BioLife Solutions, Inc., Binghamton, NY
2002 - 2004  Vice President of Business Development, BioLife Solutions Inc.
2004 - PRESENT Senior Vice President, Cell Preservation Services, Inc. (CPSI Biotech), Owego, NY

Selected Research Activity/Awards/Honors
- 2014 - PRESENT - SRARTUPNY Review Board
- 2012 - PRESENT - New York Academy of Sciences Blavatnik Review Board
- 2013 - Small Business Administration Tibbetts Award (Baust/Van Buskirk/Snyder of CPSI for excellence in NIH SBIR grantsmanship, entrepreneurship and economic development)
- 2006 - Small Business Administration Tibbetts Award (Baust/Van Buskirk of CPSI)
- 2002 - Chancellor’s and SUNY Research Foundation Entrepreneur Award (excellence in medical research)
- 1990 - Russell and Burch Award (for developing engineered human skin used worldwide)
- 1995 - PRESENT - Member of NSF, NIH (R01, R43/44, PO1), DoD grant review panels

Major Achievements:
- Awarded 80 grants and contracts (NSF, NIH, DoD, NYS, Private, Pharma)
- Chair, NIH IMSTJ 15 Study Section "Cell and Molecular Biology" 6/14 to current.
- Developed tissue engineered human epidermis (skin) now sold internationally by MatTek Corp. (Ashland, MA) and used by many US and international cosmetic and pharmaceutical companies as well as basic research scientists.
• Originated concept of and co-developed the CytoFluor 2300 fluorescent plate reader while a consultant for Millipore Corporation. First plate reading spectrofluorometer developed.

• Co-developed BioLife Solutions Inc’s (BLFS; Nasdaq) HTS-FRS storage solution now in approx. 150 cell therapy clinical trials. These and related solutions are used for the short term and long term storage of cells in a variety of applications.

• Past Vice President of Research/Business Development, BioLife Solutions, the first incubator biotechnology company on the SUNY Binghamton campus.

• Vice President of CPSI Biotech, the second incubator biotechnology company on the SUNY Binghamton campus.

• Past consultant for MatTek Corporation, US Army Medical Research Institute of Chemical Defense, Corning Corporation, Millipore Corporation and Cryomedical Sciences Inc.

• Authored CD-ROM as electronic companion for Cell and Molecular Biology. Author of two problem-solving text books in cell and molecular biology (Scientific American/W.H. Freeman; Cogito)

B. Selected Peer-Reviewed Publications and Patents (limited to 5)


Patents (limited to 5):


D. Active Research Support

**NYS DOH** New York State *Baust and Van Buskirk (PIs) 11/1/15 - 1/31/17*

*Prostate Cancer Hypothesis Development Grant* Goals: To develop innovative approaches to treating prostate cancer through two graduate fellowships. RVB role: co-PI

**DHP15-014** DoD *Van Buskirk (PI) 9/28/15 - 9/27/20*

*Optimal Rewarming Solutions for Cryopreserved Tissue Specimens* Goals: To test a variety of cell stress modulators as both pre-conditioning and post-conditioning agents as a means to improve vitrification of cells and tissues. RVB role: PI

**1R43CA195948-01A1** NIH/NCI *Baust (PI) 9/1/15 - 8/31/16*

*Development of a Minimally Invasive Surgical Device for the Treatment of Esophageal Cancer*
BIOGRAPHICAL SKETCH

NAME
Anthony T. Robilotto

POSITION TITLE
Doctoral Candidate

eRA COMMONS USER NAME (credential, e.g., agency login)

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>MM/YY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornell University, Ithaca, New York</td>
<td>B.S.</td>
<td>1999-2002</td>
<td>Biological Engineering</td>
</tr>
<tr>
<td>State University of New York at Binghamton</td>
<td>M.S.</td>
<td>2002-2005</td>
<td>Biology – Cell/Molecular</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Positions and Employment

2001 – 2002 Teachers Assistant, Dept. Biological and Environmental Engineering, Cornell University
2005 – present Research Scientist and Engineer, CPSI Biotech
2008 – present Doctoral Candidate, Binghamton University

Other Experience and Professional Memberships

2002-present Member, Society for Cryobiology

Honors

2002 Alpha Epsilon Agricultural, Food, and Biological Engineering Honors Society
2002 Graduate Research Assistanceship
2003 Society for Cryobiology Travel Award

B. Selected Peer-Reviewed Publications

Recent publications of importance to the field (in chronological order)


**Select Patents** (Note: Select Issued and Pending Patents Listed)


