Title of Project: Targeting tumor stroma to detect and treat prostate cancer

Principal Investigators and Departments: Dr. Susan Bane/Chemistry; Dr. Brian Callahan/Chemistry

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):

Despite earlier detection and improved therapies, prostate cancer remains a leading cause of cancer-related death among men in the United States. Castration resistant prostate cancer (CRPC) represents the most dangerous and presently incurable stage of the disease. Our goal is to develop a molecular prevention strategy that blocks prostate cancer from progressing to CRPC. Using a novel class of bioconjugates (protein-small molecule fusions), we aim to detect and eliminate an oncogenic tumor-stroma communication pathway, called hedgehog signaling. Tumor-stroma communication via hedgehog proteins represents a key factor driving progression to CRPC. This collaborative proposal integrates Dr. Callahan’s experience in the area of protein engineering and hedgehog signaling with Dr. Bane’s expertise in bioconjugation chemistry and fluorescence spectroscopy. Both Callahan and Bane have active research programs in the area of cancer research and a track record of external funding.
PRÉCIS: In 1889 Paget likened the relationship between a cancerous cell and its surrounding microenvironment to that of a seed and soil (Paget). While the cell may subsist in isolation, malignant growth requires sustenance—oxygen, growth factors, hormones etc. Benign cells surrounding the tumor, the so-called stroma (or soil), supply this nourishment. After languishing for more than 100 years, Paget’s idea has reemerged as a guiding principle to understand tumorigenesis and metastasis (Fidler 2003; Langley and Fidler 2011), while also spurring improved therapies. “Stromal therapy” can provide a powerful adjuvant to conventional drugs (Junttila and de Sauvage 2013), leading to regressions that are more pronounced and remissions that are more durable (Zhang and Liu 2013). Thus, poisoning the soil does suppress the seed. Here we seek to fulfill the promise of stromal therapy for prostate cancer.

INTRODUCTION: Bidirectional communication between the tumor and surrounding stroma is key to the seed-soil hypothesis, and hence malignancy. Secreted signaling proteins provide the general means for this interaction. In prostate cancer, the primary drivers are proteins in the hedgehog (Hh) family (Gupta et al. 2010; Chen et al. 2011; Domenech et al. 2012; Levina et al. 2012; Bansal et al. 2015). Tumor cells synthesize and secrete Hh proteins; stromal cells sense Hhs through specific receptors, then respond by releasing tumorigenic signals. Reciprocal signals sent by the stroma include protein growth factors and in the case of prostate cancer, androgenic steroids (Shaw and Bushman 2007; Chen et al. 2009; Gonnissen et al. 2013) (FIG. 1). Persistent tumor-stroma signaling via Hh proteins can increase tumor vasculature, suppress immune response, and promote chemoresistance (Sims-Mourtada et al. 2007; Olive et al. 2009). These effects may explain the accelerated growth of prostate tumor xenografts that overexpress Hh proteins, as well as the suppressed growth of primary prostate tumors by anti-Hh antibodies (Sanchez et al. 2004; Shaw et al. 2009).

HYPOTHESIS: We propose that disrupting oncogenic tumor-stroma communication can repress and even reverse prostate cancer, thereby averting the most lethal, castration-resistant stage of the disease.

INNOVATION: By focusing on the transformed cell only, conventional chemotherapy for prostate cancer ignores the important oncogenic influence of surrounding stroma (Sluka and Davis 2013). Here we pursue this cellular compartment directly with a panel of novel, stroma-targeting bioconjugates.

RATIONALE FOR EXPERIMENTAL APPROACH: We enlist Hedgehog (Hh) proteins as vehicles for targeted delivery of imaging (Aim 1) and therapeutic (Aim 2) cargo to prostate stromal cells (FIG. 2). Our approach exploits the strong, specific recognition of Hh by its cell
surface receptor (Fuse et al. 1999); the presence on stromal cells of the Hh surface receptor (Theunissen and de Sauvage 2009; Zhang et al. 2009); cellular uptake of the Hh-receptor complex by endocytosis (Incardona et al. 2000); and the correlation between Hh signaling and prostate cancer stage (Gleason score) (Azoulay et al. 2008; Kim et al. 2011).

**SPECIFIC AIMS** for this collaborative 1-year project are as follows:

1. **Detect tumorigenic stroma with Hh-fluorophore conjugates.** We will generate and test a novel class of Hh-fluorophore conjugates as molecular probes to image prostate stroma cells expressing the Hh receptor. Native Hh proteins are chemically modified by lipids at their N- and C-terminal amino acids during biosynthesis. We will replace Hh’s lipids with fluorophores using conjugation techniques developed in the Bane (RB-487) and Callahan (RB-479) labs. Those Hh-fluorophore conjugates will be evaluated as imaging agents to detect the cell-surface Hh receptor on prostate stromal cells.

**Approach:** WPMY-1 cells and RWPE-1 cells are human derived prostate stromal lines, available from the ATCC, that respond to Hh proteins. We will use our Hh-fluorophore conjugates to detect receptor binding and endocytosis by both prostate stromal cell lines. As a positive control, we will use an established Hh-receptor expressing C3H10T1/2 cells (Kinto et al. 1997; Pathi et al. 2001). To establish selectivity, incubations will be carried out in the presence of Hh blocking antibody, 5E1, which prevents Hh binding by its surface receptor (Maun et al. 2010). Both N-terminal and C-terminal Hh-fluorophore conjugates will be tested. We expect that the Hh-fluorophore conjugates will be internalized by the cell and trafficked into lysosomes, which we have experience detecting. Our experience with protein bioconjugation (Banerjee et al. 2010; Mukherjee and Bane 2013), fluorescence microscopy (Shanker et al. 2011; Mukherjee et al. 2015), and hedgehog signaling (Callahan and Wang 2015; Owen et al. 2015; Xie et al. 2015), prepares us for this work.

2. **Eliminate reactive stroma with novel Hh-drug conjugates.** Using a Trojan-horse approach, we propose targeting tumorigenic stroma using Hh-conjugates equipped with the cytotoxic agent, monomethyl auristatin E (MMAE). This FDA-approved chemotherapeutic (Ansell 2014), while potent (EC50, 1-5 nM), is limited by cell-killing action that is indiscriminate. MMAE inhibits tubulin polymerization, leading to cell cycle arrest and apoptosis (Francisco et al. 2003; Maderna et al. 2014). One appealing solution is to tether the drug to a targeting agent — such as an antibody or a protein ligand that is bound by a cell specific receptor (Beck and Reichert 2014).
Bioconjugates of this type represent a fast growing sector in cancer therapy (Srinivasarao et al. 2015). An antibody-MMAE conjugate (Adcetris™) is in clinical use to treat Hodgkin’s lymphoma (Doronina et al. 2003; Ma et al. 2006). Using similar chemistry to generate the Hh-fluorophore conjugates (above), we will link MMAE to the N- and C- terminal amino acids of human Hh protein as potential stroma-directed toxins.

**Approach.** We expect that the Hh-MMAE conjugates will prove toxic to cells expressing the Hh receptor. Moreover, the dose of Hh-MMAE that results in ½ maximal killing (EC$_{50}$) will roughly equal the Hh-receptor affinity (K$_d$ ~10$^{-9}$ M). To evaluate cytotoxicity, conjugates will be added to cells (10$^6$) at concentrations ranging from 10$^{-9}$ to 10$^{-6}$ M. After 72 h, toxicity will assessed using LIVE/DEAD Assay (Thermo). As a control, we will use Hh protein that is not conjugated over the same range of concentrations. If toxicity of the Hh-MMAE conjugates depends on interaction with Hh cell surface receptor, its cytocidal effect should be alleviated by the Hh-specific antibody 5E1. We plan carry out cytotoxicity studies using a single dose of Hh-MMAE, calculated EC$_{50}$ value, and increasing concentration of 5E1. We expect to see a decrease in toxicity with escalating concentrations of blocking antibody. As a control, we will carry out a similar experiment except using MMAE that is not conjugated to Hh. 5E1 is not expected to influence the toxicity of free MMAE.

**Summary/Outlook Statement.** Through these aims, we explore a novel stromal targeting approach designed to improve our understanding of prostate cancer and enhance disease treatment. The proposal capitalizes on the strengths of the two investigators, who have track records of external funding in cancer research. Results from the work will provide preliminary data essential to a competitive proposal to the NIH, the DoD, and the Prostate Cancer Foundation. Finally, and more broadly, we point out that the approach described here may find application beyond prostate cancer, as tumor-stroma communication via Hh proteins has been linked to malignant growth of pancreatic and breast tissue.

**PROJECT’S ORIGINALITY, SIGNIFICANCE, QUALITY AND FUTURE IMPACT:** We are pursuing a largely overlooked aspect of prostate cancer development and progression, using cutting edge experimental techniques developed at BU by the two principal investigators, described in their recent invention disclosures (Bane, RB-487; Callahan, RB-479).

**DESCRIBE PI AND CO-INVESTIGATOR CONTRIBUTIONS; STUDENT’S PARTICIPATION ON THE PROJECT:** Callahan and Bane assume equal responsibility for managing the project – from designing experiments, interpreting results, responsible expenditures of funds, preparing manuscripts and training of graduate students. If funded, the project will support summer fellowships for two students.

**BRIEF STATEMENT OF THE BENEFITS OF THIS PROJECT, IF FUNDED, TO THE UNIVERSITY:** This project seeks to develop and apply new agents to study and treat prostate cancer. Studies with direct therapeutic intent in the area of cancer help raise the University’s profile as a research institution.

**PLANS FOR SEEKING EXTERNAL SUPPORT:** The PIs plan to incorporate results from this project into an NIH application for the October 2016 cycle.

**MINIMAL RISK:** Research proposed herein poses less than minimal risk. No animals and no infectious agents are involved.
REFERENCES


**Budget**

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<tr>
<td>Small Equipment</td>
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<td>Two Graduate Research Assistants, Summer 2016</td>
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<td>Supplies</td>
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<td>Equipment User Fees</td>
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<td><strong>TOTAL</strong></td>
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**Budget Justification**

**Small Equipment.** Requested in the budget in support of this project is a Li-Cor C-DiGit® Blot Scanner for chemiluminescence detection of western blots. WPMY-1 cells and RWPE-1 cells are human derived prostate stromal lines, available from the ATCC, that respond to Hh proteins; however expression of the Hh surface receptor has not been tested explicitly. We will carry out the overdue experiments of assessing Hh receptor expression in these prostate stromal lines using antibodies specific for the Hh receptor. The low abundance of receptor protein in these samples requires an instrument with greater sensitivity than we currently possess.

**Graduate Research Assistants, Summer 2016.** Support is requested for two graduate students to participate in this project during the summer of 2016. A stipend of $4500 (+$720 fringe benefits) is requested for each student. The primary responsibility of one of the students will be chemical synthesis of probes and conjugation reagents, preparing and characterizing the Hh-conjugates. The primary responsibility of the second student will be cell studies, fluorescent imaging and cytotoxicity studies. The students are presently supported as teaching assistants during the academic year.

**Materials and Supplies.** Funds are requested for the purchase of chemical and laboratory supplies, including culture reagents for mammalian cells, buffers and reagents for protein purification, use and storage, antibodies, organic reagents for chemical synthesis and spectroscopic analysis, chromatography solvents and supports, plus miscellaneous supplies and disposables.

**Equipment User Fees.** Funds are requested for the use of shared instrumentation in the chemistry department (NMR spectrometer) and Innovative Technologies Complex (mass spectrometers, FACS, confocal microscope), and user and analysis fees at the Biotechnology Resource Center at the Cornell Institute of Biotechnology.
Susan L. Bane

a. Professional Preparation.

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<th>Institution</th>
<th>Major</th>
<th>Degree</th>
<th>Year</th>
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<tr>
<td>Davidson College, Davidson, NC</td>
<td>Chemistry</td>
<td>BS</td>
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<td>Vanderbilt University, Nashville, TN</td>
<td>Biochemistry</td>
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b. Appointments

2003-Present  Director, Biochemistry Program, Binghamton University, Binghamton, NY
2001-Present  Professor of Chemistry, Binghamton University, Binghamton, NY
1992-2001    Associate Professor of Chemistry, Binghamton University, Binghamton, NY
1985-1992    Assistant Professor of Chemistry, Binghamton University, Binghamton, NY
1983-1985    Postdoctoral Scientist, Bioorganic Chemistry, University of Virginia, Charlottesville VA

c. Products

PRODUCTS MOST CLOSELY RELATED


OTHER SIGNIFICANT PUBLICATIONS


d. Synergistic activities.

1. A great deal of my effort has gone toward training graduate and undergraduate students to do research. Virtually all of the research in my lab is performed by graduate and undergraduate students. Many of my former graduate students have done postdoctoral work at high level institutions and have permanent positions in academic, industrial and governmental organizations. Most of my undergraduate research students go to graduate school or medical school, or are employed in a research-related business or agency. Eight of my former graduate and undergraduate research students are university faculty members. I encourage and assist my undergraduate research students to present their work at professional meetings. Most recently one of my students was coauthor on two posters at the annual meeting of the American Society for Cell Biology, and he was invited to present one of them at a special undergraduate research session.

2. Recently initiated at Binghamton University is “The Freshman Research Immersion” (FRI) program, which provides first-year students with a year-long authentic research experience in sciences and engineering. I am one of the four faculty members leading the newly established Biomedical Chemistry research streams. This year’s students will do research related to our oxidative stress projects (see Free Radical Biol Med publication).

3. For more than 10 years I have been director of the undergraduate Biochemistry program at Binghamton. This is an interdepartmental major that has grown from about 70 majors in 2003 to approximately 190 students today. I serve as primary academic advisor for all the majors. In 2004 I initiated the Biochemistry Student Advisory Board (which became the student run Biochemistry club) and the Biochemistry Alumni Newsletter, which is written and produced entirely by undergraduate students. I have served as search chair for the University’s recent expansion of biochemistry research, successfully recruiting three new assistant professors in the last three years.

4. I have been involved in writing and evaluating questions for the Graduate Record Exam in Chemistry for more than 15 years. For six years I served on the Committee of Examiners, which consists of eight faculty members who assemble and evaluate the examination.

e. Collaborators & other affiliations.

1. Collaborators. (total of 9)

   Brian Callahan (Binghamton University), Dan Sackett (NIADDK, NIH), David G. I. Kingston (Virginia Polytechnical University), James P. Snyder (Emory University), Robert Coates (University of Illinois), Barbara Poliks (Binghamton University), Susannah Gal (Binghamton University), Omar M. Aly (Menia University, Egypt), Rajiv R Ratan (Burke-Cornell Medical Research Institute)

2. Graduate and Postgraduate advisors. (total of 2)

   J David Puett University of Georgia (retired)
   Timothy Macdonald University of Virginia (postdoctoral)

3. Thesis advising. Current graduate students (total of 4): Takian Chio, Samantha Greco, Han Yu, Saptarchi Ghosh. Former graduate students, 2010-2015 (Current location) (total of 5): Zhen Lei (MS; Ph D program, SUNY-Stony Brook), Kamila Mukherjee (Ph D: postdoctoral associate, Department of Nephrology, Massachusetts General Hospital/Harvard Medical School), Maura Loew (Ph D: scientific writing consultant), Abhijit Banerjee (Ph D; Senior Scientist, Thermax, Ldt), Özlem Dilek (Ph D., Asst Prof, Istanbul Kemerburgaz University Medical School). Cumulative total graduate students: 41; postdoctoral: 5.
Brian P. Callahan

a. Professional Preparation.

<table>
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<th>Institution</th>
<th>Major</th>
<th>Degree</th>
<th>Year</th>
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<td>State University of New York, Cortland</td>
<td>Biology</td>
<td>B.S.</td>
<td>1993</td>
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<tr>
<td>University of North Carolina, Chapel Hill</td>
<td>Biochemistry</td>
<td>Ph.D.</td>
<td>2005</td>
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b. Appointments.

2012-Present  Assistant Professor, Department of Chemistry, State University of New York, Binghamton
2011-2012    Postdoctoral Fellow, Department of Physics, Tufts University, Medford, MA
2006-2010    Postdoctoral Fellow, Wadsworth Center New York State Department of Health, Albany, NY
2005-2006    Postdoctoral Fellow, University of North Carolina, Chapel Hill

c. Products.

PRODUCTS MOST CLOSELY RELATED

OTHER SIGNIFICANT PRODUCTS

d. Synergistic activities.

1. Since beginning my independent career in 2012 at Binghamton, I have served as the advisor to three PhD students and to a postdoctoral scientist in my lab. For the last two years, I have also served on the graduate program committee, a group of Chemistry faculty at Binghamton charged with improving the Chemistry PhD program through recruiting qualified graduate students to the PhD and developing assessment tools.
2. I have mentored 10 undergraduate students at Binghamton and two high school students through research projects in Biochemistry. Currently my group includes 1 male and 4 female undergraduates.
To date, two published papers, and another nearing submission have undergraduates as coauthors. In the last two years, I have given three lectures at local middle and high schools, in addition to serving as a judge for a grade school science fair.

3. I am a referee for Bioorganic Chemistry, Pharmaceutical Bioprocessing, Biochemistry, BBA - Proteins and Proteomics

e. Collaborators & other affiliations.

1. Collaborators (total of five)

Dr. Susan Bane  Binghamton University
Dr. Nilesh Banavali  Wadworth Center, NYS-DOH
Dr. Michelle Arkin  University of California San Francisco
Prof. Ralph Buttyan  Vancouver Prostate Institute
Prof. Peter Tessier  Rensselaer Polytechnic Institute
Prof. Chunyu Wang  Rensselaer Polytechnic Institute

2. Graduate and post-graduate advisors (total of three)

Prof. Richard Wolfenden  University of North Carolina
Prof. Keith Derbyshire  Wadsworth Center, Albany
Prof. Marlene Belfort  University at Albany

3. Thesis advising and postgraduate scholar sponsor (total of four)

Graduate: Timothy Owen, Binghamton University (2012-present); Simon Waihenya (2015-present); Sarah Otieno (2015-present)
Postdoc: Dr. George Ngoje, Binghamton University (2014-present)