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TITLE: Making Aggressive Prostate Cancer Quiescent by Abrogating Cholesterol Esterification

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14. ABSTRACT Our overall objective in the current application is to establish the viability of a new strategy of treating late stage PCa through therapeutic targeting of cholesterol metabolism in vivo, using combination of cutting edge spectroscopic imaging and other technologies, including biochemistry assays and preclinical testing. The innovation of this study is that it targets altered cholesterol metabolism, an understudied field of cancer research. Our central hypothesis is that abrogating cholesterol esterification events will result in an effective strategy for treating late stage PCa. This hypothesis will be tested by first validating the presence of altered cholesterol metabolism in human prostate cancer patient specimens. We will then evaluate the therapeutic benefit of CE depletion in appropriate animal models of PCa, and elucidate pathways linking cholesterol metabolism with cancer aggressiveness. An interdisciplinary research team has been assembled, with expertise in spectroscopic imaging & nanomedicine (Dr. J. X. Cheng, PI), biochemistry (Dr. X. Liu, co-PI), and prostate cancer biology (Dr. T. Ratliff, co-PI).					
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Lay Abstract:

Since the introduction of prostate specific antigen screening, prostate cancer has become the most widely diagnosed non-skin cancer in men in the United States (220,800 cases estimated in 2015). While often diagnosed in clinically localized stages, PCa remains the second leading cause of cancer-related mortality in American men with over 27,540 projected deaths in 2015. For men with advanced prostate cancer, androgen deprivation therapy in the form of bilateral orchiectomy or pharmacologic castration is an accepted standard therapy. Despite initial disease control, androgen deprivation therapy alone is non-curative and the subsequent development of castration-resistant prostate cancer (CRPC) occurs in the lifespan of almost all men who do not succumb to non-cancer deaths. For men with metastatic CRPC, docetaxel was approved in 2004 as the first-line cytotoxic chemotherapy owing to a modest increase in overall survival compared to mitoxantrone. Since 2010, there has been a tremendous increase in treatment options available for metastatic CRPC patients, including novel anti-androgen therapy with abiraterone and others. Nevertheless, the effectiveness of current therapies is palliative with an improvement in overall survival of 2-5 months compared to placebo. Therefore, a critical need exists to develop novel therapeutic strategies for advanced prostate cancer.

Cancer cells adopt metabolic pathways that differ from their normal counterparts by high rates of glycolysis and biosynthesis of essential macromolecules to fuel rapid growth. Among dysregulated metabolic pathways, altered lipid metabolism is increasingly recognized as a signature of cancer cells. Enabled by label-free coherent Raman scattering microscopy, our laboratory has performed the first quantitative analysis of lipogenesis at single cell level in human patient cancerous tissues. Our imaging data revealed an aberrant cholesterol ester accumulation in high-grade prostate cancer and metastases, but not in normal prostate or prostatitis. Cholesterol is an essential biomolecule that plays important roles in the maintenance of membrane structure, signal transduction, and provision of precursor to hormone synthesis. While cholesterol accumulation is known to be a hallmark of atherosclerosis, its exact role in cancer progression remains elusive. Our unexpected finding of cholesterol ester accumulation in advanced human prostate cancer triggered us to ask whether such cholesterol ester accumulation could become a potential target for prostate cancer treatment. Our pilot study has indeed showed that pharmacological inhibition of cholesterol ester accumulation significantly suppressed prostate cancer aggressiveness without affecting normal cell viability. Based on these appealing data, we hypothesize that abrogating cholesterol ester accumulation will result in an effective strategy for treating advanced prostate cancer. This hypothesis will be tested through two specific aims. First, we will develop a clinically viable strategy of cholesterol depletion and evaluate its therapeutic effect on tumor growth in appropriate animal models of prostate cancer. Second, to understand how such treatment strategy benefits prostate cancer, we will elucidate the mechanism by which cholesterol ester accumulation contributes to prostate cancer aggressiveness.

At the completion of this project, it is our expectation that we will have provided strong evidences to support the concept that inhibition of cholesterol ester accumulation is a viable and potentially attractive therapeutic intervention strategy to treat advanced prostate cancer. Notably, several small molecule inhibitors of cholesterol accumulation, e.g. avasimibe, have gone through clinical trials to treat atherosclerosis but failed due to the lack of effectiveness. Our proposed study will demonstrate a novel use of existing drugs to treat advanced prostate cancer, and it is anticipated that preclinical studies and/or clinical trials will follow shortly after the completion of this project. Ultimately, the adoption of such strategy will substantially improve the clinical outcome for metastatic prostate cancer patients that are resistant to hormone therapy. Our deeper mechanistic study will contribute to the understanding of dysregulated cholesterol metabolism in advanced prostate cancer, which in turn provides the biological foundation of targeting cholesterol accumulation for treatment of metastatic prostate cancer.

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1. INTRODUCTION:

Our *overall objective in the current application* is to establish the viability of a new strategy of treating late stage PCa through therapeutic targeting of cholesterol metabolism *in vivo*, using combination of cutting edge spectroscopic imaging and other technologies, including biochemistry assays and preclinical testing. *The innovation of this study* is that it targets altered cholesterol metabolism, an understudied field of cancer research. Our *central hypothesis* is that abrogating cholesterol esterification will result in an effective strategy for treating late stage PCa. This hypothesis will be tested by first validating the presence of altered cholesterol metabolism in human prostate cancer patient specimens. We will then evaluate the therapeutic benefit of CE depletion in appropriate animal models of PCa, and elucidate pathways linking cholesterol metabolism with cancer aggressiveness. An interdisciplinary research team has been assembled, with expertise in spectroscopic imaging & nanomedicine (Dr. J. X. Cheng, PI), biochemistry (Dr. X. Liu, co-PI), and prostate cancer biology (Dr. T. Ratliff, co-PI).

2. KEYWORDS:

Prostate cancer, lipid droplet, metabolism, cholesterol, cholesteryl ester, Raman spectroscopy

3. ACCOMPLISHMENTS:

a. What were the major goals of the project?

The major goals of this project are (1) Develop a clinically viable cholesteryl ester depletion strategy to suppress the proliferation of late-stage prostate cancer *in vivo*; (2) Determine the relative contribution of altered cholesterol metabolism to prostate cancer aggressiveness.

- i. *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

b. What was accomplished under these goals?

During year 1 of this project, we have submitted the animal protocol to Purdue University Animal Care Committee (PACUC) and to DoD Animal Care and Use Review Office for review. The protocol has been approved by PACUC as well as by the DoD Animal care and Use Review Office. Upon this approval, we have performed the following experiments as we proposed:

First, we have determined the maximum tolerated dosage (MTD) of avasimibe by tail vein injection of various doses (40, 20, 15, 10, 6 mg/kg) to Balb/c mouse (n=6 for each dose). Avasimibe was loaded in avasimin with 10% loading efficacy. Phosphate buffer (PBS) injection was used as a control. **Finding:** We did not observe weight loss in any of these doses. The body

weight data of mice treated with 15, 10, 6 mg/kg avasimibe was shown in **Figure 1** below. These data indicate that avasimibe is a relatively non-toxic drug.

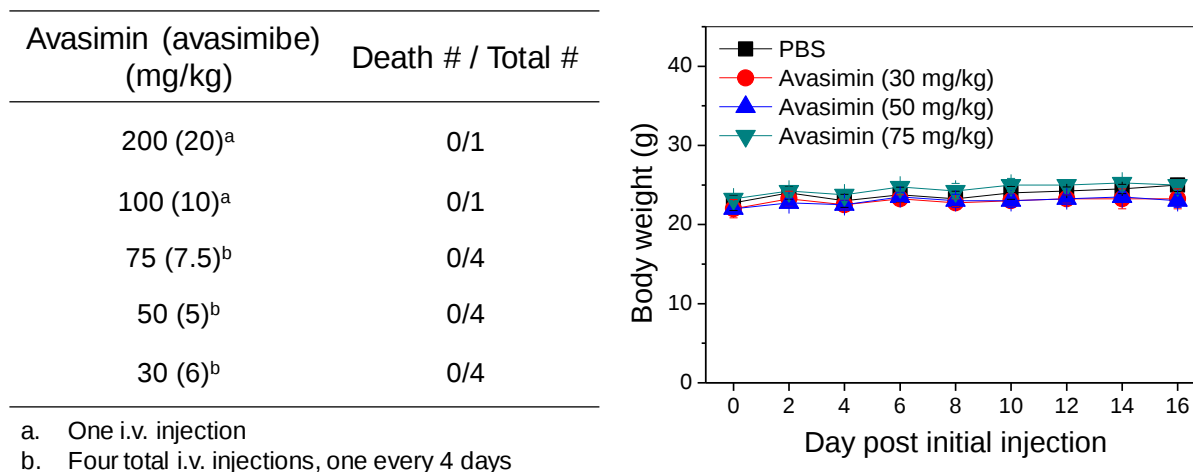


Figure 1. Characterization of in vivo safety of high-doses avasimibe.

Second, we have determined the blood-bioavailability upon intravenous injections of the avasimibe/albumin formulation at the dose of 75mg/kg (containing 7.5 mg/kg avasimibe) to Balb/c mouse (n=3). The blood concentration of avasimibe at 7 different time points (2 min, 15 min, 30 min, 60 min, 2 h, 3 h, and 6 h post injection) was measured by LC-MS at Purdue Bindley Bioscience Facility. Data are shown in **Figure 2**. **Finding:** Area under the curve (AUC) of Avasimibe/ albumin formulation by intravenous administration was 136.36 μ g.h/mL. As control, Avasimibe was administered orally at two different doses, 15mg/kg and 100mg/kg. The AUC for the 15mg/kg, and 100mg/kg oral doses was 14.92 μ g.h/mL, and 49.9 μ g.h/mL respectively. These results are an indication that avasimibe/albumin formulation significantly increases avasimibe bioavailability.

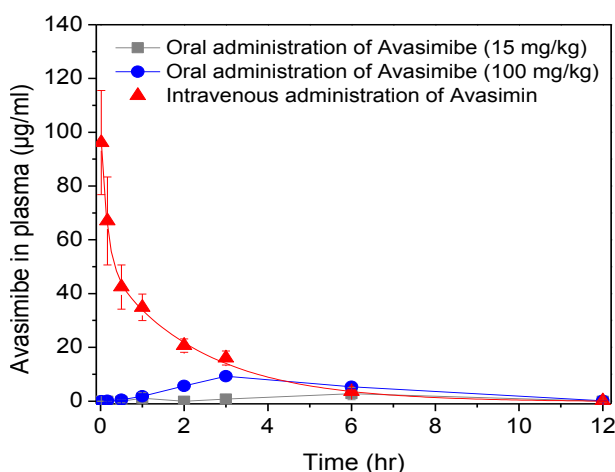


Figure 2. Blood bioavailability of avasimibe following intravenous administration of avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or oral administration of avasimibe (15 mg/kg) (n=3). The data were fitted with two-compartment model ($y = A_1 e^{(-x/t_1)} + A_2 e^{(-x/t_2)} + y_0$).

Third, we determined the bio-distribution of avasimibe in a prostate cancer xenograft mouse model (n=3, nude mice). To establish the human prostate cancer xenograft mouse model, PC-3 cells were used. The concentration of avasimibe was measured by LC-MS analysis of tissue homogenates. **Finding:** After intravenous administration of avasimibe/ albumin formulation, the concentration of avasimibe in the tumor was determined to be 34 μ M, which is more than 4-fold higher than the IC₅₀ of the drug in PC3 cells (7.3 μ M). In contrast, there was no drug detected in tumor after oral administration of avasimibe, whereas most of the orally administered drug was detected in the liver, intestines and feces.

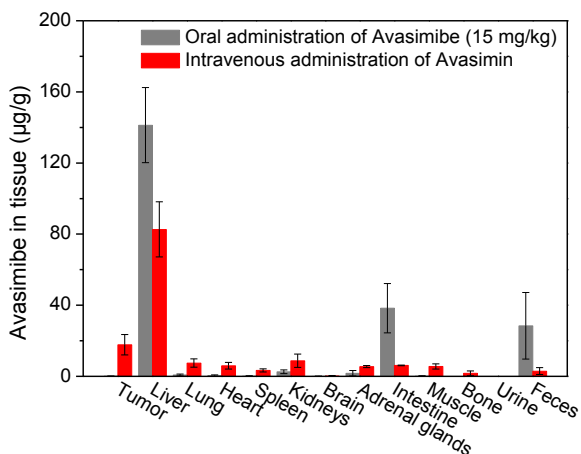


Figure 3. Tissue distribution of avasimibe at 2 hr after intravenous injection of avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or oral administration of avasimibe (15 mg/kg) (n=3 for each group). Data are expressed as mean $\pm$ SD.

Fourth, we determined the anti-cancer effect of the avasimibe/albumin formulation in prostate cancer xenograft mouse model. As control, PBS was intravenously injected at the same interval. Tumor volume and body weight were monitored daily. Survival rate was determined over a 10-week period. **Finding:** When compared to the control group, avasimibe/ albumin formulation significantly reduced tumor growth (**Figure 4 left column** and **Figure 5**) with no obvious body weight change (**Figure 6**). An increase in survival rate was also evident with the avasimibe/albumin treatment (**Figure 4 right column**).

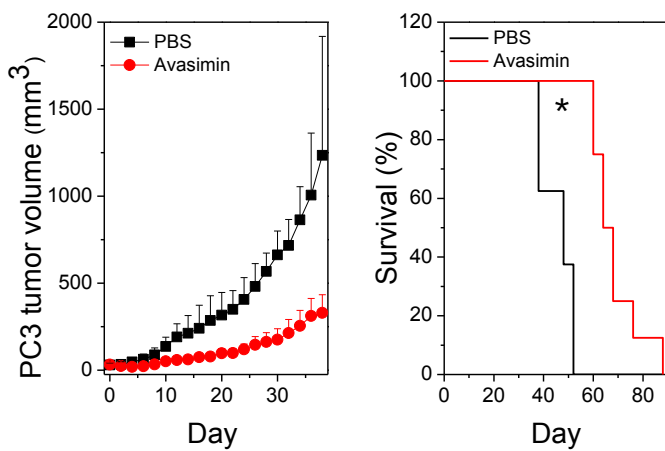


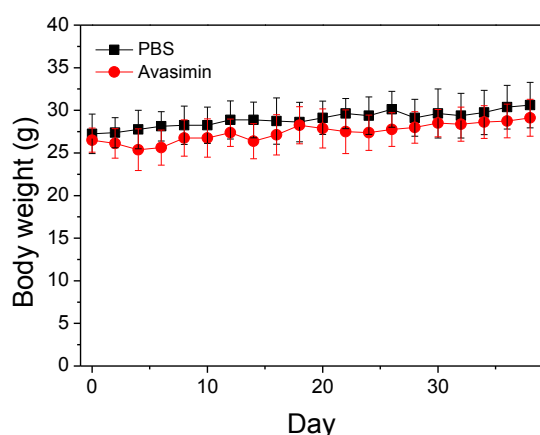
Figure 4. Anti-tumor effects of avasimin for PC3 tumor xenograft. Tumor volume (left) and survival rate (right) of PC3 tumor xenograft mice (n=8 for each group). The mice received avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or PBS by intravenous injection daily for the first 5 days, following by intravenous injection once every 4 days. Day was counted after post initial treatment. * $P < 0.005$ (log-rank test).

PC3 tumor xenograft mice at day 30



Figure 5. Comparison of tumor volume between PBS-treated and avasimin-treated groups. Photographs of PC3 tumor xenograft mice (representatively, n=3) at 30 days post treatment.

a PC3 tumor xenograft mice



b HCT116 tumor xenograft mice

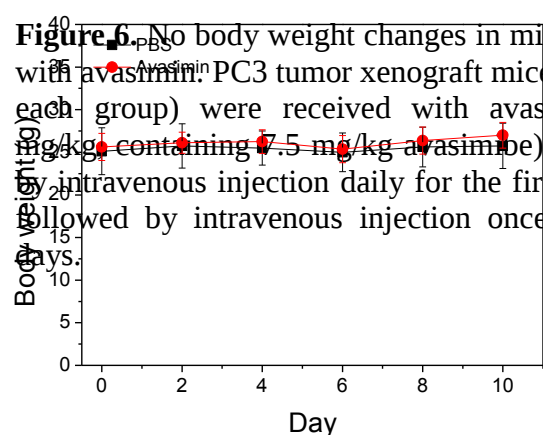


Figure 6. No body weight changes in mice treated with avasimin. PC3 tumor xenograft mice (n=8 for each group) were received with avasimin (75 mg/kg containing 7.5 mg/kg avasimin) and PBS by intravenous injection daily for the first 5 days, followed by intravenous injection once every 4 days.

c. What opportunities for training and professional development has the project provided?

Nothing to report.

d. How were the results disseminated to communities of interest?

The results were disseminated to communities of interest through a few invited presentations:

Aug 2015, "Spectroscopic imaging of living systems: emerging platform for biology and medicine", Eli Lilly, Indianapolis

May 26-28, 2015, Targeting Cancer Metabolism Conference, "Spectroscopic imaging of cancer metabolism at single cell level", Boston, MA

April 2, 2015, Purdue Center for Cancer Research, "In vivo spectroscopic imaging: emerging platform for biology and medicine", Purdue University.

e. What do you plan to do during the next reporting period to accomplish the goals?

During the next funding period (year 2), we will continue to finish the in vivo testing in a xenograft mouse model, including evaluating the extent of organ toxicity, determining the rate of apoptosis in prostate cancer tissues in control and treated mice, and measuring the cholesterol ester level and arachidonic acid levels in the harvested tumor tissue. Moreover, we will start to dissect the role of altered cholesterol metabolism in prostate cancer aggressiveness. In particular, we will aim to establish a correlation between cholesterol ester accumulation and the potential of tumor cell migration/invasion in vitro.

4. **IMPACT:**

- a. **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report.

- b. **What was the impact on other disciplines?**

Nothing to report.

- c. **What was the impact on technology transfer?**

Nothing to report.

- d. **What was the impact on society beyond science and technology?**

Nothing to report.

5. **CHANGES/PROBLEMS:**

- a. **Changes in approach and reasons for change**

Nothing to report.

- b. **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

- c. **Changes that had a significant impact on expenditures**

Nothing to report.

- d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

e. **Significant changes in use or care of human subjects**

Nothing to report.

f. **Significant changes in use or care of vertebrate animals.**

Nothing to report.

g. **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. **PRODUCTS:**

a. **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

i. **Journal publications.**

Seung-Young Lee, Junjie Li, Jien-Nee Tai, Timothy L. Ratliff, Kinam Park, Ji-Xin Cheng*,
“Avasimibe encapsulated in human serum albumin blocks cholesterol esterification for selective cancer treatment”, ACS Nano, 2015, 3: 2420-2432.

Acknowledgement of DoD support (yes).

ii. **Books or other non-periodical, one-time publications.**

Nothing to report.

iii. **Other publications, conference papers, and presentations.**

Nothing to report.

b. **Website(s) or other Internet site(s)**

Nothing to report.

c. **Technologies or techniques**

Nothing to report.

d. **Inventions, patent applications, and/or licenses**

Based on the albumin formulation of avasimibe, a non-provisional patent was filed through Purdue University, filing date: Sept 10, 2015, application No. 14/850,941

“Cholesteryl Ester-Depleting Nanomedicine for Nontoxic Cancer Chemotherapy”, PRF 66947

- e. **Other Products**
Nothing to report.

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- a. **What individuals have worked on the project?**

Name: Ji-Xin Cheng

Project role: PI

Research Identifier: NA

Nearest person month worked: 1 month

Contribution to project: Dr. Cheng guided the development of an injectable formulation of avasimibe and in vivo testing of the anti-cancer efficacy of this new formulation.

Name: Seung-Young Lee

Project role: graduate student

Research Identifier: NA

Nearest person month worked: 8 months

Contribution to project: Mr. Lee developed an injectable formulation of avasimibe and performed the *in vivo* testing of the anti-cancer efficacy of this new formulation.

Name: Jien-Nee Tai

Project role: undergraduate student

Research Identifier: NA

Nearest person month worked: 1 month

Contribution to project: Ms. Tai helped Mr. Lee in developing an injectable formulation of avasimibe and performing the *in vivo* testing of the anti-cancer efficacy of this new formulation.

Name: Junjie Li

Project role: graduate student

Research Identifier: NA

Nearest person month worked: 1 month

Contribution to project: Mr. Li helped Mr. Lee in performing the *in vivo* testing of the anti-cancer efficacy of this new formulation.

- b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- c. **What other organizations were involved as partners?**

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**

a. **COLLABORATIVE AWARDS: NA**

b. **QUAD CHARTS: NA**

9. **APPENDICES: NA**