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Neoadjuvant Therapy for Ovarian Cancer Using BioglycogenTM Nanoparticles SBIR Grant Proposal

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An honors thesis presented to the College of Nanoscale Science & Engineering,
University at Albany, State University of New York
in partial fulfillment of the requirements for graduation with Honors in Nanoscale Science
and
graduation from the Honors College

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Research Advisor: Nadine Hempel, Ph.D

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Project Summary

Technical Abstract

This Small Business Innovation Research Phase I project, presented by Talamo Inc., is to test an ovarian cancer therapeutic delivery system, comprised of a nanoscale biocompatible vesicle that carries a bioactive small interfering RNA molecule (siRNA), in vivio in mice. The siRNA has shown to reduce the expression of an enzyme (Sod2) that is highly expressed in multiple ovarian cancer types. The reduced expression of Sod2 will allow the tumor to become increasingly susceptible to chemotherapy agents, while simultaneously diminishing tumor progression. Research has proved that the nanoscale vesicle is non-toxic and in combination with siRNA effectively reduced Sod2 expression, in vitro, in ovarian cancer cells.

Based upon these findings Talamo, Inc. plans to move forward to in vivo mice testing. It is necessary to prove that the nanoscale vesicle system is effective in inhibiting Sod2 expression and is also non-toxic in mice. The funds from phase I of this Small Business Innovation Research (SBIR) grant will allow Talamo, Inc. to proceed with in vivo mice testing. The anticipated results are that the mice given the therapy will have smaller tumors and lower Sod2 levels than the control group.

Commercialization Abstract

This neoadjuvant therapy has great commercial potential. Ovarian cancer is the deadliest of the gynecologic cancers. There will be 14,030 deaths in 2013 from ovarian cancer and ranks as the 10th most common cancer among women. This therapy would be administered to a patient as soon as she was diagnosed with ovarian cancer. Immediate therapy would slow the tumor progression and increase sensitivity to chemotherapy agents. This would allow physicians more time to proactively treat or to remove the tumors. Thus, the survival rates of patients with clear cell carcinoma will increase.

Talamo Inc. has established conditional collaboration with Bristol-Myers Squibb, if the nanoscale delivery system is proven to be non-toxic and effective at inhibiting Sod2 in mice. Thus, it is imperative for Talamo, Inc. to be awarded this SBIR Phase I grant in order to fund in vivo mice testing. If the results from the in vivo mice testing prove favorable, Bristol-Myers Squibb will fund Phase 1 FDA clinical trials in conjunction with the $1,000,000 SBIR Phase II award. Once this therapy has been brought to market, after passing Phase 1-3 FDA clinical trials, Talamo Inc. will use its profits to fund future R&D for nanoscale delivery wesicles to be used to treat other cancers that also exhibit high levels of Sod2.

Key Words

Nanotechnology, Nanoparticle, Ovarian Cancer, Drug Delivery Systems, Drug Targeting, Neoadjuvant Therapy, Superoxide Dismutase-2, Bioglycogen, Taxol.

Research Topic Biotech and drug delivery

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Table of Contents

Project Description:

1. Identification and Significance of Opportunity

Talamo Inc., has created a nanoparticle drug delivery system that in combination with current chemotherapy treatments will be able to better treat clear cell carcinoma ovarian cancer. After more research, we would like to use this nanoscale delivery system to treat other cell types. Our proposed therapy will result in slowed tumor progression and increased sensitivity to chemotherapy agents. By slowing the tumor progression, physicians will have additional time to successfully eradicate or remove the tumor. The tumor's increased sensitivity of chemotherapy agents will make the chemotherapy treatments more efficient and will result in lower necessary dosages of chemotherapy. This novel treatment will result in decreasing the mortality rate of women afflicted with ovarian cancer, while simultaneously allowing these women to have a less painful treatment process.

2. Background and Phase I Technical Objectives

A. Ovarian Cancer Basics:

Ovarian cancer forms in the tissues of the ovary in women. It accounts for approximately three percent of cancers in women. While the 10^{th} most common cancer among women, ovarian cancer is the fifth leading cause of cancer-related death among women, and is the deadliest of gynecologic cancers. A woman's lifetime risk of developing ovarian cancer is 1 in 72 and a woman's lifetime risk of dying from ovarian cancer is 1 in 95. The relative five-year survival rate is 44%, survival rates vary depending on stage of diagnosis. [1] It is estimated that approximately $5.1 billion is spent in the United States each year on ovarian cancer treatment. [2]

In 2013, in the United States, there will be 22,240 new cases of ovarian cancer and 14,030 ovarian cancer related deaths. [2] Since the 1980s, the incidence rates have been declining due to modern chemotherapy technologies and surgery, however ovarian cancer still has the highest mortality rate of all cancers of the female reproductive system. (See Figure 1) This high mortality count is a result of a lack of early symptoms and of effective ovarian cancer screening tests. Thus, ovarian cancer is often diagnosed at an advanced stage. Only 15% of ovarian cancer patients are diagnosed early.

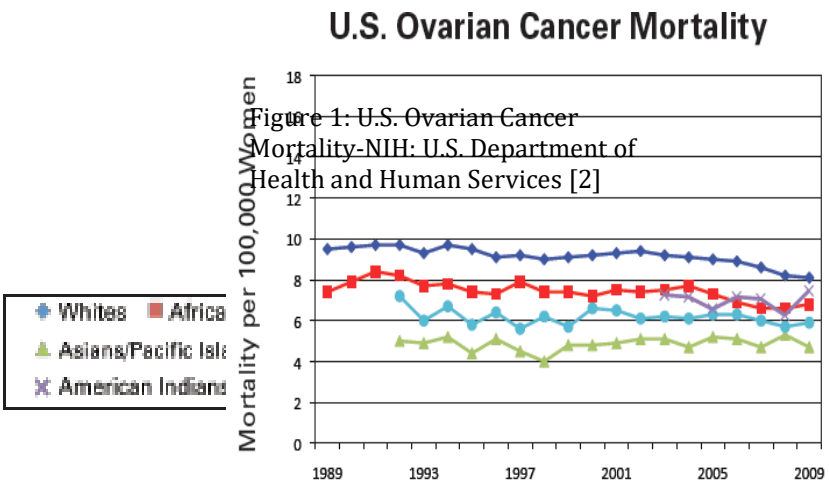


Figure 1: U.S. Ovarian Cancer Mortality-NIH: U.S. Department of Health and Human Services [2]

The National Cancer Institute's (NCI) investment in ovarian cancer research increased from $96.9 million in 2007 to $110.8 million in 2011. (See Figure 2) In addition to this funding, NCI supported $22 million in ovarian cancer research in 2009 and 2010 using funding from the American Recovery and Reinvestment Act (ARRA). [2] The federal government has recognized that ovarian cancer is an important area of scientific research to invest in. Thus, we believe that our company has a product with great market value, significance, and innovation.

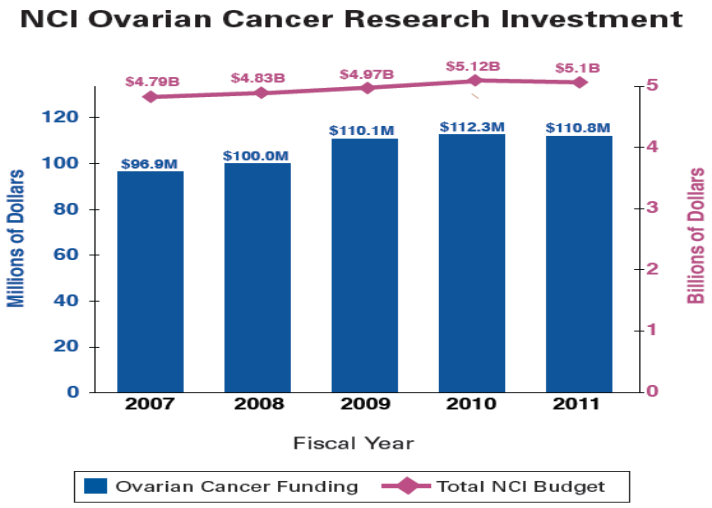


Figure 2: NCI Ovarian Cancer Research Investment-NIH: U.S. Department of Health and Human Services [2]

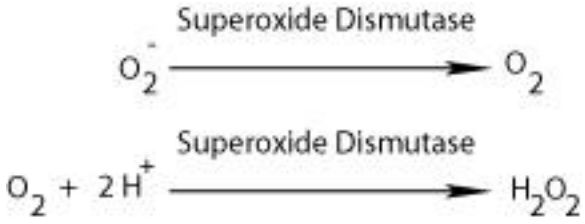
B. Superoxide Dismutase-2

The most common form of ovarian cancer arises from the cells covering the surface of the ovary, known as epithelial carcinoma. There are five major types of epithelial carcinoma: carcinoma-serous, mucinous, endometrioid, clear cell and undifferentiated. [3] These types are then further divided into grades, depending on

how virulent they appear. Of these five, clear cell carcinoma and undifferentiated carcinoma have the poorest prognosis.

Mitochondrial antioxidant enzyme superoxide dismutase-2 (Sod2) is an enzyme in humans that is a member of the iron/manganese superoxide dismutase family. An antioxidant is a molecule that inhibits the oxidation of other molecules. [4] This enzyme binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen. (See Figure 3)

Figure 3: Reaction of Superoxide Dismutase (Sod2)



Mutations in this enzyme have been associated with idiopathic cardiomyopathy (IDC), premature aging, sporadic motor neuron disease, and cancer. [4] Sod2 overexpression in many instances enhances the metastatic phenotype that is reversed by efficient H_2O_2 scavenging. [5-6] Increased levels of Sod2 expression have been observed in ovarian cancer clear cell carcinoma when compared to the other 4 types of ovarian cancer. (See Figure 4) ES2 and TOV21G are two cell lines of clear cell carcinoma ovarian cancer that have higher levels of Sod2 than other ovarian cancer cells lines. (See Figure 5) All initial in vitro testing, performed by Talamo Inc., used these two cells lines. These increased levels of Sod2 expression have been linked to tumor progression and chemoresistance.

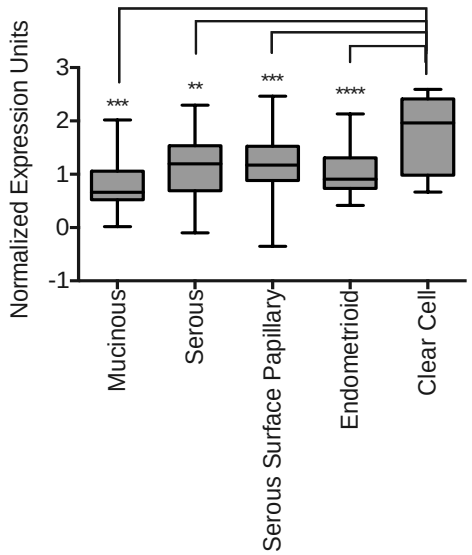


Figure 4: Comparison of Sod2 levels between the five major types of ovarian cancer

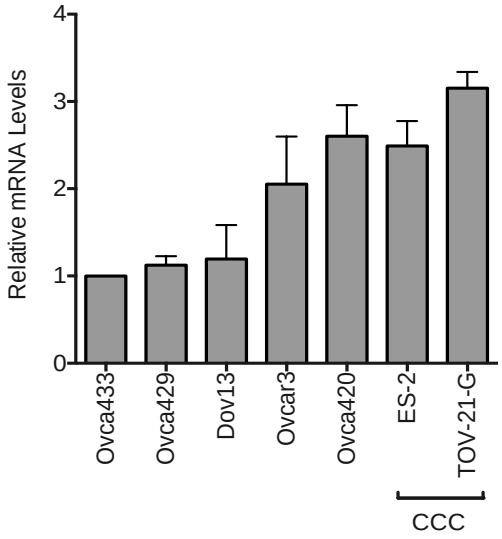


Figure 5: Comparison of Sod2 levels between different cell lines of ovarian cancer. CCC stands for clear cell carcinoma.

In order to slow the progression of tumor cells and to increase sensitivity to chemotherapy agents it will be necessary to focus on the down-regulation of Sod2 in ovarian cancer. The down-regulation of Sod2 will occur by transfection of small interfering RNA, siRNA, into ovarian cancer cells. siRNA is a class of double-stranded RNA molecules, 20-25 base pairs in length. siRNA's main function is to play a role in RNA interference (RNAi) pathway, where it interferes with the expression of specific genes, in our experiments the knockdown of Sod2. [7]

It was important to determine if the siRNA we used was inhibiting Sod2 expression or if our transfection agent was responsible for this inhibition. We compared three different siRNA types: scramble control, Sod2 siRNA #1, and Sod2 siRNA #2 in a protein expression gel experiment. We also added a mock control in order to prove that our transfection agent was not responsible for the Sod2 inhibition. When comparing the top row of the gel, the two bands under the siRNA #1 and siRNA #2 are significantly smaller than the other two bands. The smaller band size shows that the Sod2 expression has decreased. (See Figure 6)

We then imaged the effects of siRNA on spheroids. Multicellular spheroids are composed of tumor cells growing in 3-dimensional structure stimulating the growth and microenvironmental conditions of real tumors. Based on these images, Sod2 siRNA #1 and Sod2 siRNA #2 performed a greater knockdown (reduction) of Sod2 than the mock and scramble controls. (See Figure 7) From these image the effects of the different siRNAs on the microscale was compared. As we expected siRNA #1 and siRNA #2 broke down the spheroids while the mock and scramble controls had significantly less impact on the spheroids. The break down of these spheroids signifies a decrease in Sod2 expression. Thus, all of our experiments will use Sod2 siRNA #1 and Sod2 siRNA #2 due to their ability to break down the spheroids.

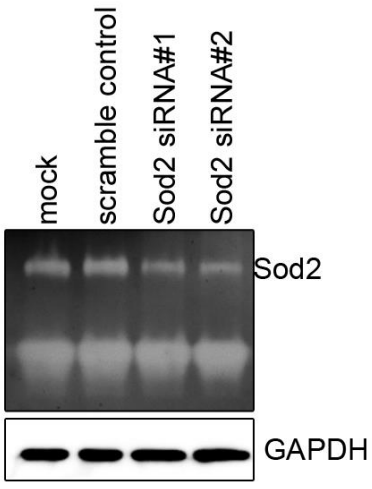


Figure 6: ES2 Sod2 Knockdown in protein expression gel

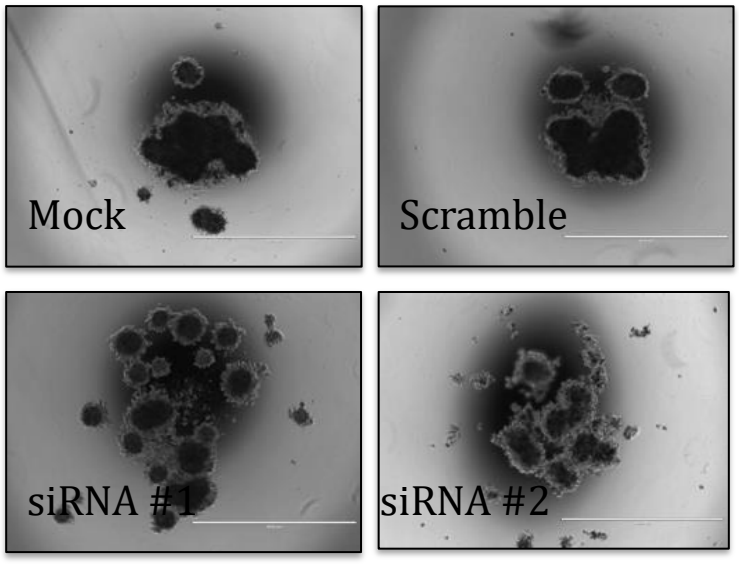


Figure 7: Break down of spheroids-Images taken by an AMG Evos Fl microscope in phase contrast.

Transfected molecules	Purpose
siRNA #1	Experimental
siRNA #2	Experimental
Scramble Control	siRNA intersects with a number of other pathways, so nonspecific effects can be triggered
Mock Control	Absence of siRNA

Table 1: Transfected molecules-Break down of Spheroids

After our initial observations of Sod2 expression in clear cell carcinoma, we begin to create a non-toxic nanoparticle, a vehicle that would be able to transport siRNA directly to the cancerous tumor. [8] We tested the toxicity by means of an MTT Assay of three potential vehicles: Phytoglycogen, Cluster Dextrin®, and Bioglycogen™. Our results proved that Bioglycogen™ was the least toxic to ES2 and TOV21G cells and proved to be the ideal therapeutic nanocarrier (data not shown).

C. Nanoparticle Delivery System-Bioglycogen™

Nanoparticles have many potential benefits for drug delivery to targeted ovarian cancer cells. This study tested the ability of a nanoparticle to transport a complex molecular cargo, siRNA, to the clear cell carcinoma ovarian cancer. Most nanoscale vehicles have been derived from biological, organic or inorganic origins in an attempt to address a wide variety of biological mechanisms and targets. [9]

Bioglycogen™ is an α-D glucan dendrimer, branched glucose. It resembles natural phytoglycogen, which is extracted from corn. [10-11] It has low cytotoxicity, which is essential for a drug delivery vehicle. Since it is a derivative of glucose humans naturally have enzymes for its degradation. This is important so that the vehicle can be taken up by the cell and the siRNA can be released and allowed to inhibit the Sod2. [9, 12] The FDA has already approved of Bioglycogen™ for food usage, so it should be easy to bring this neoadjuvant therapy to the market. Bioglycogen™ is commercially available in large quantities and is more economical than many other available dendrites. Bioglycogen™ is approximately $750/kg.

In order for us to attach the siRNA to our Bioglycogen™ nanoparticle it is necessary to modify our particle. [9] We have created two different modification methods: oxidation and charge conjugation.

The purpose of our oxidation modification is to create aldehyde groups that will then be able to bind to the siRNA. [13] We oxidize our particle for 6 hours using sodium periodate, $NaIO_4$. (Figure 7) The solution was then purified by dialysis against distilled water, dH_2O. Finally, the sample was freeze-dried on a lypholizer. The aldehydes groups would then be bonded to an aminated siRNA. The oxidation was confirmed by using Fourier Transform Infrared Spectrometry (FTIR) and

Dynamic Light Scattering (DLS). This modified nanoparticle was proven to be taken up in ovarian cancer cells by fluorescence microscopy (data not shown).

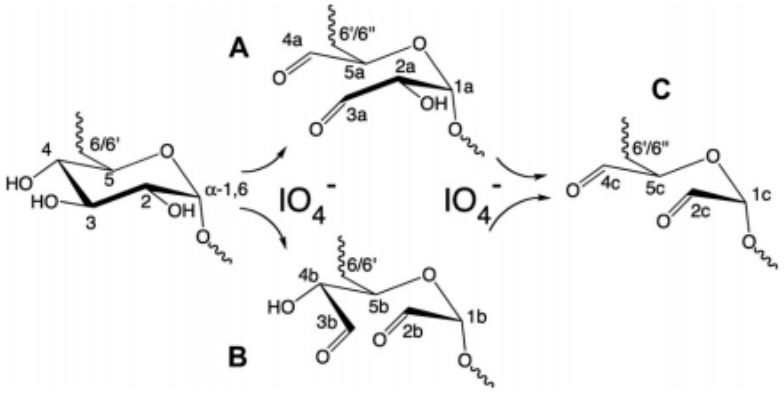


Figure 8: Oxidation of glucose polymer with sodium periodate, $NaIO_4$. [13]

The purpose of our charge conjugation modification is to introduce positive charge to the particle that will then be able to react with the siRNA. The Bioglycogen™ particle was reacted overnight with glycidyltrimethylammonium chloride (GTMA). (Figure 8) We then purified our solution by dialysis against distilled water, dH_2O. Finally, the sample was freeze-dried on lypholizer. The charge conjugation was confirmed by DLS. This modified nanoparticle was proven to be taken up in ovarian cancer cells by fluorescence microscopy (data not shown).

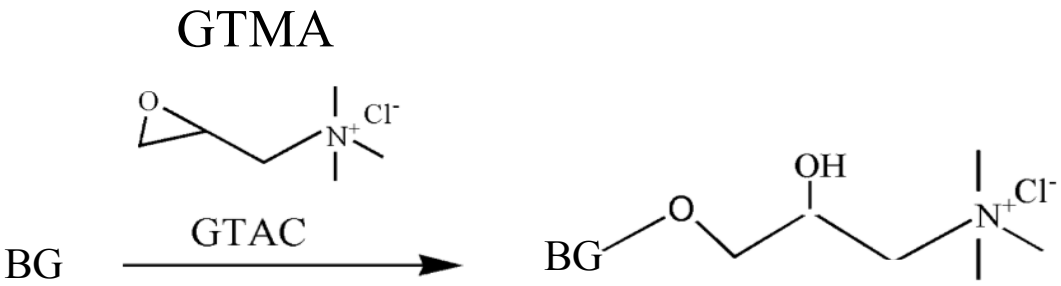


Figure 9: Conjugation of Bioglycogen™ with glycidyltrimethylammonium chloride (GTMA)

Our previous studies have shown that our modified Bioglycogen™ nanoparticles are more efficient and less toxic in delivering siRNA molecules than traditional RNAi transfection techniques such as Lipofectamine™ RNAiMAX (data not shown).

Modification Methods	Description
Oxidation	Introduce aldehyde to glucose units Reaction of aldehyde with aminated siRNA
Charge Conjugation	Introduce positive charge to particle Bind to siRNA with charge interaction

Table 2: Summary of nanoparticle modification methods

3. Phase 1 Research Plan:

Previous studies have demonstrated that our modified Bioglycogen™ nanoparticle is non-toxic and effective at inhibiting Sod2 expression in vitro in clear cell carcinoma ovarian cancer cells. Before we can proceed to FDA clinical trials in humans, we must first prove that our results in vitro are consistent with in vivo mice testing. We will be performing these in vivo mouse tests for a 6-month period. Since we are researching ovarian cancer, we must use female mice.

Task 1:

During the first two weeks we will inject the Bioglycogen™ modified nanoparticles, without siRNA, to healthy mice, via the intraperitoneal cavity, to observe their reactions to the particle. During the in vitro studies two different nanoparticle modifications were synthesized. Both of these modifications will be tested alone in the mice. In order to proceed to the next experiment, these mice must not exhibit any abnormal behavior. We will observe their behaviors such as eating/drinking patterns, weight loss, activity, and blood levels. As long as all of these criteria are met and the mice caregiver does not have any concerns, we can conclude that the particle is non-toxic to the mice.

Task 2:

After proving that the particle itself is non-toxic to the mice, we can proceed to test whether our particle will inhibit the Sod2 levels, resulting in slowed tumor progression and increased chemosensitivity, in the mice. First, we will intraperitoneally inoculate the mice with clear cell carcinoma cells. [14] Once the mice develop tumors, we will inoculate the mice with our neoadjuvant therapy intraperitoneally. Since not all of the mice will develop tumors we will use ten mice per group in our experiments.

We will start with 20 mice and injecting each mouse intraperitoneally with $1x10^7$ clear cell carcinoma ovarian cancer cells. [15] (The sample size of 20 was chosen based upon the equation for single-group experiments: $n = \frac{\log b}{\log p}$ In this equation β is the chosen power and p represents the proportion of the animals in the colony that are not infected [22]) After 14 days, we will randomize the mice into two groups of ten mice in each. Group 1 will begin treatment with the neoadjuvant therapy, given once a day, Monday through Friday for one week. The mice in group 2 and 3 will be treated exactly the same way as the mice in group 1, however group 2 will be treated with salt water instead of our neoadjuvant therapy and group 3 will be treated with our modified nanoparticle without siRNA.

Six weeks from the start of the experiment, the mice will be sacrificed in order to observe the tumors. The size of the tumors will be monitored and compared between both groups. We will also monitor the Sod2 levels in the tumors as well as the survival rates between groups. We expect that the tumors in group 2 and 3 will be larger and will demonstrate higher levels of Sod2 expression. If these expectations hold true then the original hypothesis was correct that this treatment would reduce the Sod2 levels, thus slowing down tumor progression.

This experiment will be repeated and modified multiple times over the 6-month period. Throughout the in vitro studies, two potential nanoparticle modifications were synthesized, GTMA and oxidation, and two potential strands of siRNA, siRNA #1 and siRNA #2 were tested. Thus, each modified nanoparticle will be tested with each siRNA. Depending on how the mice react to the treatment it may be necessary to modify the dosages of the initial clear cell carcinoma cancer cells, as well as the dosages of the therapeutic nanocarrier system administered to the mice.

Experiment	Injection	Total Duration
Experiment 1: Toxcity testing of modified Bioglycogen™ alone	GTMA Modification	2 week
	Oxidation Modification	2 week
Experiment 2: Testing of modified Bioglycogen™ and siRNA	GTMA + siRNA #1 Salt Water GTMA no siRNA	6 weeks
	GTMA + siRNA #2 Salt Water GTMA no siRNA	6 weeks
	Oxidation + siRNA #1 Salt Water Oxidation no siRNA	6 weeks
	Oxidation + siRNA #2 Salt Water Oxidation no siRNA	6 weeks

Table 3: Summary of Phase 1 in vivo Mice Testing

A statistically significant difference between the experimental and the control group will determine the success rate of the therapy. The tumors in group 1 should be significantly smaller and the Sod2 levels should be significantly lower. The p-levels for both of these should be greater than 0.05. The nanoparticle vehicle and siRNA combination that demonstrates the lowest levels of Sod2 and the smallest tumors will advance to Phase I FDA Clinical Trials. If the results prove to be consistent with expectations then Talamo Inc., will have fulfilled its end of the conditional collaboration with Bristol-Myers Squibb.

4. Commercial Potential

A. Chemotherapy Market:

The chemotherapy market is currently valued at $42 billion. Experts say that over the next ten years this market has staggering growth potential. It is forecasted to increase by 17% to $49 billion by 2020. [16] This is an ideal time for this therapy to enter the market. Our therapy will be marketed as a neoadjuvant therapy to current chemotherapy treatments that are currently on the market. A neoadjuvant therapy, in contrast to adjuvant therapy, is given before the main treatment. This Bioglycogen™ nanoparticle will be marketed as a neoadjuvant drug because it will allow the cancer cells to become more responsive to current chemotherapy agents. Ultimately, this will increase a patient's chances for survival and will hopefully diminish the pain and agony of ovarian cancer treatments.

There are many factors to account for when considering how much chemotherapy will cost the patient. It is important to consider: the types and doses of chemotherapy used, how long and how often chemotherapy is given, and where the chemotherapy can be administered. Our neoadjuvant therapy will result in decreased costs for the patient. The decreased costs are a result of a decrease in the size of the chemotherapy doses.

Talamo Inc.'s, CEO, Alex Talamo has spoken to several large pharmaceutical companies regarding potential collaborations. Several companies were interested in the in vitro results of our nanoparticle. These companies saw great potential in our non-toxic modified Bioglycogen™ nanoparticle to be a successful neoadjuvant therapy. However, each company we approached informed us that they would not collaborate with us until we performed in vivo testing in mice. Consequently, it is crucial to ensure that our nanoparticle will be non-toxic not only in vito, but also in live mice. Thus, it is imperative that we receive the $149,973 from this SBIR grant in order to proceed with our in vivo mice testing.

Of all the pharmaceutical companies we corresponded to, Bristol-Myers Squibb was the most receptive. Alex Talamo corresponded directly with Francis Cuss the Senior Vice President of R&D (since the time of correspondence Mr. Cuss has been appointed to Executive Vice President and Chief Scientific Officer, effective July 1st, 2013). Mr. Cuss is responsible for the discovery and exploratory development of all potential new medicines at Bristol-Myers Squibb. Bristol-Myers Squibb has a net worth of $39.90 billion (as of April 29th at 5:00 PM) He saw the uniqueness and innovation of our technology and believed that our neoadjuvant therapy would perform perfectly in combination with the company's leading chemotherapy drug Taxol®. Mr. Cuss has written Talamo Inc., a letter of support.

Taxol® (generic name: paclitaxel), a microtubule inhibitor, is one of the most widely used chemotherapy drugs in the world. It has been used to treat over a million cancer patients with ovarian, breast, and lung cancer. The mechanism of anticancer action of Taxol® involves mitotic arrest of the cells due to microtubule stabilization; eventually resulting in apopotosis, cell death. [17]

Taxol® gradually loses its effectiveness as tumors develop resistance to it during treatment. [17] The mechanism that allows tumors to become resistant to Taxol® is not completely understood, there is still ongoing research in this area. Research has shown that there are multitudes of survival signaling pathways, which activate the chemoresistance in the tumor. [17] Current studies suggest that altered intracellular calcium homeostasis may contribute to the Taxol-resistant phenotype.

Our neoadjuvant therapy, in theory, is a potential solution to the gradual resistance to Taxol®. Our nanoparticle therapy will be administered to a patient as soon as she is diagnosed with ovarian cancer; one of the keys to successful cancer treatment is immediate action. Our nanoparticle therapy will inhibit Sod2 expression, which will result in decreased tumor progression and increased sensitivity to chemotherapy agents. After our particle has been administered to the patient and they agree to undergo chemotherapy, they will be given Taxol® which, due to our therapy, will be more effective in treating the cancer.

In the pharmaceutical industry and in academia there are many scientists who are interested in creating a combinatory therapy with Taxol® and to discover how tumors build resistance to it. Currently, NFCR Fellow Susan Band Horwitz, Ph.D., at the Albert Einstein College of Medicine, is now exploring why tumor resistance to Taxol® occurs and how to make the drug work better. [18] She proposed a combinatory drug approach in which a second drug is used to inhibit the activated molecular pathway and make the tumor cells regain sensitivity to Taxol®. [19]

Dr. Horwitz's approach is very interesting and has great promise. However, her plan has one major drawback. She wishes to treat the tumor after it has already developed a resistance to Taxol®. In the world of cancer time is crucial. Dr. Horwitz's proposed method is reactive instead of proactive and may potentially cost a woman her life. Our approach is proactive and will permit a woman extra time to battle her ovarian cancer. Our method will hinder the growth of tumor progression and increase the sensitivity of Taxol® from the start of the treatment. It is also important to note that ovarian cancer is usually detected at an advanced stage due to a lack of early symptoms. Thus, our strategy of slowing down the tumor progression as soon as possible is a more efficient method to battling ovarian cancer, than attempting to force the tumor regain sensitivity to Taxol® after the tumor has already developed its protective molecular pathway(s).

B. Competitors:

We are in an interesting situation where our customers may also be our direct competitors. Our neoadjuvant therapy increases the effectiveness of current chemotherapy drugs, thus the dosing of chemotherapy drugs would be diminished. Companies who sell chemotherapy drugs, such as Bristol-Myers Squibb, would not make as much profit off of their original drug if they sold it in combination with

ours. Consequently, it would be in the pharmaceutical company's best interest, as well as ours, for a collaboration to occur.

Our indirect competitors will be companies who have products that treat the side effects of chemotherapy. There will be less of a need for these products once our product enters the market and increases the effectiveness of current chemotherapy drugs because the dosages of current chemotherapy drugs will be diminished. Decreasing the dosages of current chemotherapy drugs will result in less side effects and ultimately a decrease in the need to treat the side effects. Common side effects for chemotherapy are nausea and vomiting. The most common form of anti-nausea medication is a combination of dexamethasone and a serotonin blocker. These blockers stop serotonin from sending messages to the brain that trigger nausea. Serotonin blockers include dolasetron (Anzemet), granisetron (Kytril and others), ondansetron (Zofran and others), and palonosetron (Aloxi). [20]

C. Future Goals:

We project that after 6 months of in vivo testing in mice, our hypothesis will be correct and we will have statistically significant data portraying that our particle decreased Sod2 expression in the treated group, which resulted in smaller tumors. At that point we will begin our collaboration with Bristol-Myers Squibb to commence Phase I FDA clinical trials. The $1,000,000 award for phase II of this SBIR grant will also be used to proceed to Phase I FDA clinical trials.

Future Funding Sources
Phase II SBIR- $1,000,000
Federally Funded Phase III SBIR Awards
Bristol-Myers Squibb

Table 4: Future funding sources for Phase I FDA Clinical Trials

After bringing our neoadjuvant therapy to market, we hope to use the profits to fund R&D on other cancer cell lines in an attempt to observe which other cancers demonstrate high Sod2 levels. Screening of publicly available expression data from cancer microarrays indicated that Sod2 expression is consistently elevated in bladder cancer specimens. [21] Based upon these findings, it can be inferred that there may be other cancers that also demonstrate high Sod2 levels. Once we discover other cancers with high Sod2 levels, we wish to test how our neoadjuvant therapy would treat those cancers. After we have exhausted all of the cancer types that exhibit high Sod2 expression, we would like to use this nanoscale delivery technique to deliver other siRNAs to various cancers that exhibit additional enzymes which have been proven to increase tumor progression and increased chemoresistance.

D. Company Information

This company has emerged from a collaborative research initiative between four young, hard-working and enthusiastic scientists: Alex Talamo and Sarah Engelberth, This is a start-up company, which has substantial industrial potential. Research and development are all performed in-house, using our team of multidisciplinary staff. Our small size allows our company to focus its time and energy on the design, fabrication, and testing of our modified nanoparticle delivery system.

The business objective of Talamo Inc., is to bring non-toxic and extremely efficient therapeutic nanocarriers to the market, in order to treat cancer and reduce the suffering of humans worldwide. The company has strong R&D capabilities in nanoparticle fabrication and in vitro testing with clear cell carcinoma cells. Bristol-Myers Squibb has an interest in our drug delivery system. They have negotiated a conditional collaboration with our company.

Alex Talamo is currently a senior at the College of Nanoscale Science & Engineering-University at Albany. He will be graduating in May with a B.Sc(Honors) in Nanoscale Science with a concentration in Biology. He is the CEO of this company.

Sarah Engelberth is currently a Ph.D candidate at the College of Nanoscale Science & Engineering-University at Albany. She graduated from Ohio Northern University with a B.Sc(Honors) in Forensic Biology and Chemistry.

5. Consultants and Subawards/Subcontracts

Dr. Magnus Bergkvist is an Assistant Professor of Nanobioscience at the College of Nanoscale Science & Engineering-university at Albany. He obtained his B.Sc. in Chemical Engineering from Mälardalen University in Sweden in 1995 and his Ph.D in Surface Biotechnology from Uppsala University in Sweden in 2002. His research areas are: nanobiotechnology, bionanofabrication, biological structures, nanoparticle synthesis, surface chemistry, surface characterization, and atomic force microscopy. Dr. Bergkvist oversees the particle synthesis of this research. The creation and characterization of the particle was done in his lab using his instruments.

Dr. Nadine Hempel is an Assistant Professor of Nanobioscience at the College of Nanoscale Science & Engineering-University at Albany. She obtained her B.Sc.(Honors) in Pharmacology from the University of Queensland in Australia in 1999. She obtained her Ph.D. in Pharmacology from the School of Biomedical Sciences at the University of Queensland in Australia in 2004. She did her first Post-doctoral Fellowship at the Department of Hematology and Cancer Biology at Duke University Medical Center in 2007. She did her second Post-doctoral Fellowship at the Department of Immunology and Microbial Diseases at Albany Medical College in 2010. She was a Research Assistant Professor at the Department of Immunology and Microbial Diseases at Albany Medical College in 2011. She was a Senior Research Scientist at the College of Nanoscale Science & Engineering at the University at Albany in 2011. Her research areas include: cancer cell metastasis,

nanobiotechnology, tumor cell migration and invasion, reactive oxygen species and signal transduction, antioxidant enzymes, and tumor markers. Dr. Hempel oversees the biological aspects of this project. All cell-based experiments were done in her lab using her instruments.

Dr. Antigone McKenna is the Laboratory Animal Resources Director at the University at Albany. Dr. McKenna has a doctorate in veterinary medicine. He is the primary contact at the University at Albany's Laboratory Animal Resource center. Talamo, Inc. has already discussed our Phase I testing plans with Dr. McKenna. These discussions included concerns regarding inoculating the mice via the intraperitoneal cavity, how long the experiments would last, and general concerns regarding the treatment of the mice. He is in full support of our research efforts and has written us a letter of support.

6. Equivalent or Overlapping Proposals to Other Federal Agencies

Talamo, Inc. does not have any other equivalent or overlapping proposals to other federal agencies at this time.

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Biographical Sketches

Alex Talamo

Education:

Institution	Degree	Year	Field of study
University at Albany-College of	B.S. (Honors)	2013 (May)	Nanoscale Science with a concentration in

Nanoscale Science & Engineering			biology and minors in Mathematics and Greek & Roman Civilizations

Positions and Employment:
2011: College of Nanoscale Science & Engineering Summer Intern
2012-2013: NCAA Student Athlete Tutor

Honors: President's Award for Leadership, Purple & Gold Award-Emerging Student Leader, Chemistry Award for Advanced General Chemistry

Honor Societies: Phi Beta Kappa Honor Society, Omicron Delta Kappa National Leadership Honor Society, Presidential Honor Society, and the Honors College

Publications:
Rajan Kumar, Sheila Smith, James McNeilan, Michael Keeton, Joseph Sanders, Alexander Talamo, Christopher Bowman and Yubing Xie. Butterfly wing-inspired nanotechnology. In: The Nanobiotechnology Handbook (Y. Xie ed.). Boca Raton, FL, CRC Press (in print).

Budget and Budget Justification:

Sponsor: NSF SBIR		Title Neoadjuvant Therapy for Ovarian Cancer using Bioglycogen™ Nanoparticles			
Project Investigator: Dr. Nadine Hempel		Co- Project Investigator : Dr. Magnus Bergkvist			
Period of Time: 6 Months					
Year 1					
Salaries	**% Effort**	**Annual Salary**	**Total Cost**	**Requested Funding**	**Cost Share**
Faculty					
Dr. Nadine Hempel	15%	$100,000	$15,000	$15,000	$0
Dr. Magnus Bergkvist	17%	$83,018	$14,113	$14,113	$0
			$0	$0	$0
Total State Paid employees			**$29,113**	**$29,113**	**$0**
RF Paid Employess					
Alex Talamo	45%	$15,000.00	$6,750	$6,750	$0
			$0	$0	$0
Total RF Paid Employees			**$6,750**	**$6,750**	**$0**
Graduate Students	**FTE**				
Sarah Engelberth	**50%**	**$21,840.00**	$10,920.00	$10,920	$0
			$0.00	$0	$0
Total Graduate Students	50%		**$10,920**	**$10,920**	**$0**
Summer					
			$0	$0	$0
			$0	$0	$0
Total Summer Salaries	0%		**$0**	**$0**	**$0**
Total Salaries			$46,783	$46,783	$0

Fringe Benefits	Rate %	$ Base			
State Employees	50.81%	$29,113	$14,792	$14,792	$0
RF Employees *	45.00%	$6,750	$3,038	$3,038	$0
Graduate Students *	16.00%	$10,920	$1,747	$1,747	$0
Summer	17.00%	$0	$0	$0	$0
Total Fringe Benefits			**$19,577**	**$19,577**	**$0**
Total Salaries and Fringe Benefits			**$66,360**	**$66,360**	**$0**
Other Direct Costs - Attach Details for any item listed below					
Equipment			$0	$0	$0
*Tuition(Half Year)			$13,900	$13,900	$0
Travel			$320	$320	$0
Materials & Supplies			$18,257	$18,257	$0
Publications			$4,000	$4,000	$0
				$0	$0
				$0	$0
Total Other Direct Costs			**$36,477**	**$36,477**	**$0**
TOTAL DIRECT COSTS			**$102,837**	**$102,837**	**$0**
Facilities and Administrative Expense	Rate %	Base MTDC			
Does not incl:equip, install,tuit	53.00%	$88,937	**$47,137**	**$47,137**	**$0.00**
Total Estimated Project Cost			**$149,973**	**$149,973**	**$0**

Table 3: Budget Report for 6 month Phase 1 Testing

A. Personnel

Dr. Nadine Hempel will spend 15% of her time on the Bioglycogen™ nanoparticle neoadjuvant therapy testing. This corresponds to a total of $15,000.

Dr. Magnus Bergkvist will spend 17% of his time on the Bioglycogen™ nanoparticle neoadjuvant therapy testing. This corresponds to $14,113.

Sarah Engelberth will spend 50% of her time on the Bioglycogen™ nanoparticle neoadjuvant therapy testing. This corresponds to $10,920. Ms. Engelberth is also a full time Ph.D. student at the College of Nanoscale Science & Engineering. Her yearly tuition will be covered by this grant. This corresponds to $13,900.

Alex Talamo will spend 45% of his time on Bioglycogen™ nanoparticle neoadjuvant therapy testing. This corresponds to $6,750.

B. Fringe Benefits

Fringe benefits total $19,577. Dr. Nadine Hempel and Dr. Magnus Bergkvist will each receive $7,396. Alex Talamo will receive $3,038. Sarah Engelberth will receive $1,747. The total fringe benefits will cost $19,577.

C. Equipment

The company has already purchased all of the necessary equipment for the fabrication of the nanoparticles. Any supplemental instruments are readily accessible through other laboratories at the College of Nanoscale Science & Engineering or at the University at Albany's main campus.

D. Travel

After Phase I testing is complete Talamo Inc., will travel to New York, NY, from Albany, NY, to report our findings to Francis Cuss at the Bristol-Myers Squibb New York office. The price of a 1-way off peak Amtrak ticket is $40. Each member of our team will travel to New York and return to Albany. The total cost of travel is $320.

E. Materials and Supplies

1. In-vivo Mice Testing
   Our company will be using the facilities at the University at Albany for its in-vivo mice testing. The price (per cage, per day) at the uptown campus at the University at Albany for state or RF (Research Foundation) funded users is $0.55. There are 6 mice per cage. Over the six-month period, the Phase I testing will need roughly 400 mice, thus 68 cages will be purchased. The total cost of the mice over the six-month period will be $6,732.

2. Bioglycogen™
   Additional Bioglycogen™ will be needed in order to create new nanoparticles. One kilogram will be sufficient for these experiments. The cost of 1 kilogram is $750.

3. Glycidyltrimethylammonium Chloride (GTMA)
   For each kilogram of Bioglycogen™ our conjugation requires nine liters of GTMA. However, since there are two different modifications that will be synthesized from the 1 kg of Bioglycogen™, only 4.5 L of GTMA are required. The cost of 4.5 L of GTMA is $2,358.

4. siRNA
   For each kilogram of Bioglycogen™, 6 μmol of siRNA is required. The cost of siRNA #1 and siRNA #2 is $418/50 nmol. Our company will purchase 0.5 μmol of each siRNA. The cost of each siRNA is $4,180/0.5 μmol. The total cost of the siRNA is $8,360. Additional siRNA will be created in our laboratory using polymerase chain reaction (PCR).

5. Sodium Periodate
   For each kilogram of Bioglycogen™, 50g of sodium periodate is required. However, since there are two different modifications that will be synthesized

from the 1 kg of Bioglycogen™, only 25 g of sodium periodate will be purchased. The cost of 25 g of sodium periodate is $56.70.

F. Publications

The cost of publications, generally, varies from $1,000 to $5,000. The expectation of this work to be published is very high. Thus, publication costs are estimated at $4,000. This estimation allows us to publish one high profile journal or several publications in lower profile journals.

Current and Pending Support:

No similar proposals have been submitted.

Facilities, Equipment and Other Resources:

Talamo Inc., is affiliated with the University at Albany-College of Nanoscale Science & Engineering. The company is able to use all of the state of the art facilities at the college. CNSE is located within a 800,000 square foot complex that houses the most advanced 200mm/300mm wafer facilities in the academic world, including over 80,000 square feet of Class 1 capable cleanrooms equipped with 300mm wafer processing tools. Talamo Inc., has priority on all of the instruments in both Dr. Hempel's and Dr. Bergkvist's laboratories. These labs have all of the general laboratory equipment needed for cell growth and maintenance, protein chemistry and biochemical analysis in the proposed research. The company has access to the other laboratories at CNSE that are part of the Nanobioconstellation and can use any instrument depending on availability.

Instruments:

Dynamic Light Scattering
Fourier Transform Infrared Spectrometer

Dynamic Light Scattering
A dynamic light scattering (DLS)/zeta potential instrument owned by Dr. Magnus Bergkvist will be used to determine the size, polydispersity, distribution, and the zeta potential of our synthesized nanoparticles. The DLS instrument records its findings by measuring the intensity of light scattered by the nanoparticles as a function of time.

Fourier Transform Infrared Spectrometer
A Fourier Transform Infrared Spectrometer owned by Dr. Magnus Begkvist will be used to identify the functional groups present in our synthesized nanoparticles. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a

molecular fingerprint of the sample. No two unique molecular structures produce the same infrared spectrum.

The proximity of the CNSE NanoTech complex to the University at Albany main campus (10 min walk) is located give access to a range of other shared facilities and equipment within the SUNY school system. The life-sciences facilities are located on the University at Albany main campus and encompass 194,000 square-feet with 28 laboratories and biological core facilities. The Laboratory Animal Resources Center is a facility at the University at Albany that supports the animal-related needs of University researchers, educators, and students. The locations of the proposed SBIR research will allow for the completion of Phase I testing and in the future will ensure a seamless transition to mass-scale production.

The Phase I research will be split between the labs at CNSE and the Laboratory Animal Resource Center at the University at Albany. The particle synthesis will occur at CNSE. The in-vivo mice testing will occur at the Laboratory Animal Resource Center. Our team has been enrolled in the Occupational Health and Safety Program for animal users and is listed on an active, approved IACUC protocol. The Facilities Manager also led our team through the Facilities Training, thus granting us access to the animal facilities at the University at Albany.

Supplementary Documents:

Talamo Inc., has a letter of support from Francis Cuss, the senior vice president of R&D at Bristol-Myers Squibb.