Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 41. Development of a Nanotechnology Platform for Delivery of siRNA to cancer cells

7. **Start and End Date of Research Project:** 7/20/2012 – 6/30/2013

8. **Name of Principal Investigator for the Research Project:** James R. Connor, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 107,100

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Connor, James	PI	2%	$9,870
Achuthamangalam B. Madhankumar, PhD	Co-PI	45%	$63,962

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Pang, Min	Graduate Student	100%
(department match)		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes___X_____ No_____ _____

If yes, please indicate the source and amount of other funds:

Departmental match of $30,000

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
National Institutes of Health (Rich/Connor – Multi-PI) 1R01CA169117091A1 *Glioblastoma Therapy through Ferritin Targeting*	XNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	12/2012	$1,539,448.	$1,231,606.

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans: We expect to apply for a renewal of the current NIH grant.

12. Future of Research Project. What are the future plans for this research project?

We anticipate publishing 3-4 papers over the next 3 years as the data coalesce into a strong story. The longer term goal is to generate interest in this nanotechnology as a therapeutic option for brain tumors.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes____X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian			1	
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project is additional research that will support the development of a nanomedicine program through our Penn State Cancer Institute.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes____X_____ No_____

If yes, please describe the collaborations:

We have developed a collaboration with Dr. Jeremy Rich, a neurooncologist at Cleveland Clinic.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No__X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic

plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goal: We will formulate liposomes and conjugate them with interleukin (IL-13) to take advantage of the selective expression of IL-13 receptors on glioma cells, including glioma stem cells. The liposomes will encapsulate small interference RNA (siRNA) for ferritin. We have previously demonstrated that ferritin is expressed in cancer cells and that when ferritin expression is decreased through delivery of siRNA the cancer cells become more vulnerable to chemotherapeutic agents and radiation. We have demonstrated we can deliver the siRNA by direct intratumoral injection. Although this is a clinically viable option, we could improve the utility and applicability of this approach if our liposomes can be injected intravenously. Thus, our goal for this project is to develop stable liposomes that can be injected intravenously and be selectively taken up by cancer cells in an intracranial model. We have already shown this approach will work with liposomes carrying a chemotherapeutic agent and will modify this liposomal design to carry siRNA.

Status: The data generated with these funds resulted in an improvement of our NIH grant from a score in the 17th percentile to the 1st percentile. The grant was funded.

Hypothesis: Conjugating human interleukin (IL)-13 cytokine to liposomes will selectively target the high grade glioma tumor cells expressing IL-13Rα2 receptor and improve transfection efficiency. In addition, we hypothesize that siRNA delivery through liposomes will protect the therapeutic siRNA from degradation by RNase. The data generated have supported the hypothesis. The data will be discussed in the context of each aim *infra vide*.

Aim 1: Formulation and characterization of tumor targeted liposomes for effective transfection of glioma cells with siRNA for heavy chain ferritin. The objectives of this aim were successfully met.

DC-Cholesterol/DOPE lipids (30:70 w/w), and rhodamine labeled phospholipids [L-α-phosphatidylethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt)] were purchased from Avanti Polar Lipids (Alabaster, AL, USA), BCNU (Carmustine) was purchased from Sigma (USA). The non-specific (NS) siRNA was from Qiagen, Inc. The H-ferritin antibody was a gift from Dr. Paolo Arosio (Milan, Italy). Female adult athymic nude mice were purchased from Charles Rivers Laboratories, Inc (USA). Chamber slides were obtained from Nalgene Nunc International, Inc (USA). MTS/PMS reagent (3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium/phenazine methosulfate assay) was

purchased from Promega Inc.

To formulate the liposomes, lipids were dissolved in methanol/chloroform (1:1 v/v) and subsequently rotary evaporated to obtain a lipid film, which was dried over N_2 and further dried at 4° C in a desiccator overnight. The lipid film was hydrated in PBS (2.8 mg/mL w/v) and sonicated. A polycarbonate membrane of gradually decreasing pore size was used to produce small unilamellar vesicles by extrusion through a two-stacked, 0.1µm polycarbonate membrane and subsequently with a 0.05 µm membrane using a nitrogen pressure–operated extruder (Lipex extruder, Northern Lipids, Inc., Vancouver, British Columbia, Canada). All the extrusions were performed 10 times at an operating pressure of 800 p.s.i. (5,440 kPa). To study cellular uptake, the C-liposomes were labeled with rhodamine by adding a 1% (molar ratio) of rhodamine-labeled phospholipids [L-a-phosphatidylethanolamine-N-(lissamine Rhodamine B sulfonyl) (ammonium salt) to the liposome formulation.

The size distribution of liposomes was determined by light scattering analysis through an ALV/DLS/SLS-5022F compact gonimeter system (ALV, Germany). The zeta potential and poly dispersity index (PDI) of liposomes were measured by PALS Zeta Potential Analyzer (Ver. 3.16, Brookhaven Instruments Corp). The PDI represents the distribution of particle size of the liposomes in a given medium or buffer in which they are suspended.

To evaluate the potential cytotoxicity of the liposomes, U251 cells, 4,000/well were seeded in 96-well plates and allowed to attach overnight. The cells were transfected with 0, 2, 4 or 6 µL of the C-liposome stock in DMEM serum free media. After 3 hours, the medium was replaced with complete medium. An MTS assay was performed a 24, 48 and 72 hours post transfection. The cytotoxicity of C-liposomes was also evaluated in mice. Athymic nude mice were divided into three groups, with three mice in each group. Control mice were injected with 50 µL of PBS. For the treatment groups, 6 µL of C-liposomes and 6 µL/1.5 µg of liposomes:siRNA complex was injected intraperitoneally (IP) once per week for a total of six weeks. After 6 weeks, 300 µL of the blood was collected through tail vein. The liver and kidney toxicity, total bilirubin, blood urea nitrogen (BUN), creatine, aspartate amino transferase (AST, also called SGOT), alanine aminotransferase (ALT, also called SGPT) and alkaline phosphatase were determined using an automated chemistry analyzer and kits from Thermo Electron Inc, according to the manufacturer's instruction.

To determine the best ratio of liposomes and siRNA, liposomes and siRNA-FITC were mixed on ice under the following six conditions: (ratio of C-liposomes:siRNA v/w µL/µg) 0:1, 2:1, 3:1, 4:1, 5:1, 6:1. The mixtures were equilibrated at room temperature for 5 minutes, and then DNA loading buffer was added. The samples were then loaded onto a 1% agarose gel and electrophoresed at 100 volts for 18 minutes in TAE buffer. The image was taken by an Image Reader (Fuji Film Las-3000).

Aim 2: Establish efficacy of H-ferritin siRNA delivery through tumor targeted liposomes. The objectives of this aim were met successfully.

First, we demonstrated the *in vitro* sensitization to temozolomide by H-ferritin down-regulation

in human glioma cell line U251 as shown in our preliminary data (Fig. 1 and 2).

Although the cell culture data and the subcutaneous models established proof of concept, clinical application of our nanotechnology platform requries and the goal of this proposal was to demonstrate that we can successfully deliver siRNA liposomes to the intracranial tumor in a therapeutically relevant model. To accomplish this, we transduced patient-derived glioma cancer stem cells (GSCs) with retroviruses encoding firefly luciferase. These cells were intracranially implanted and monitored with bioluminescent imaging. At tumor onset, H-ferritin siRNA liposomes or non-specific siRNA liposomes were intravenously injected into the mouse via the tail vein. Our data shows that H-ferritin siRNA increases survival and tumor volume either as a monotherapy or in combination with TMZ (Fig 3 and 4).

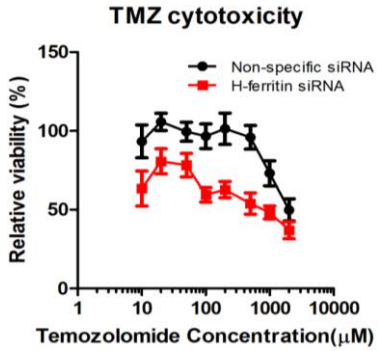

Fig. 1. H-ferritin down-regulation sensitizes U251 human glioma cells to the anti-cancer drug temozolomide (TMZ). Cells were treated with different concentrations of TMZ at 24 hours after transfection with H-ferritin siRNA. An MTT cell proliferation assay was performed 24 hours after temozolomide treatment to evaluate cell viability which was decreased by H-ferritin knockdown.

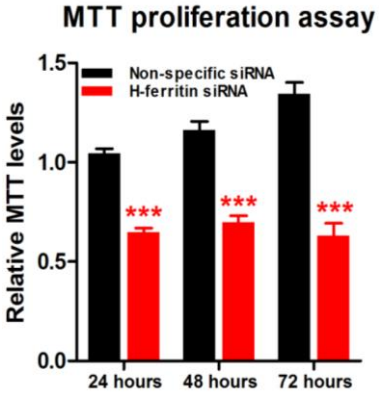

Fig. 2. H-ferritin siRNA enhanced radiation efficacy in glioma cells *in vitro*. U251 human glioma cell viability was monitored by MTT cell proliferation assay at different time points post radiation. Cells were subjected to 20 Gy of radiation at 24 hours post transfection with either non-specific siRNA or H-ferritin siRNA. Cells with radiation exhibited enhanced cytotoxicity when combined with H-ferritin knockdown. ***P<0.0001.

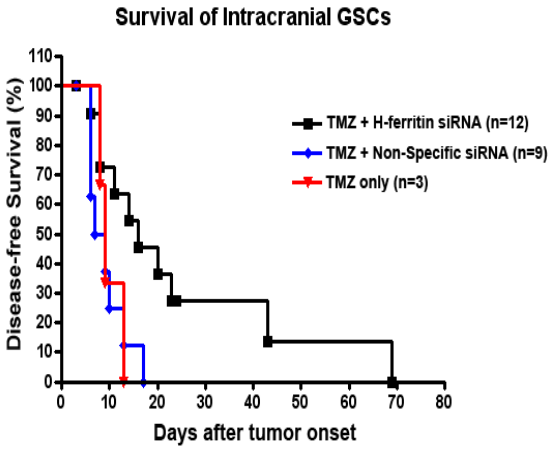

Fig. 3. H-ferritin siRNA + TMZ treatment in vivo. 1000 GSCs were intracranially implanted into athymic nu/nu mice. At tumor onset (determined by MRI) all mice were IP injected with TMZ (25mg/kg) twice a week. Two groups received a single IV liposomal treatment (either H-ferritin siRNA or non-specific siRNA; 80ug/kg). Kaplan-Meier curve demonstrates the increase in survival with H-ferritin siRNA (p= 0.0132).

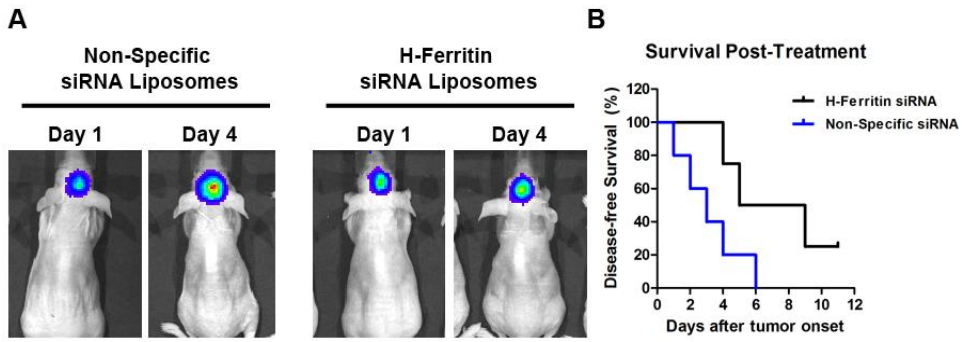

Fig. 4. H-ferritin siRNA liposomes in vivo. GSCs expressing luciferase were intracranially implanted into athymic nu/nu mice and monitored daily using the Xenogen IVIS imaging system for tumor development. At tumor onset, non-specific or H-ferritin siRNA liposomes were injected via tail vein. (A) Images of tumor size at Day 1 and Day 4 of treatment. (B) Kaplan-Meier curve of survival post-treatment after tumor onset ($p < 0.06$)

To begin to understand the mechanism by which the siRNA for ferritin was increasing sensitivity to the therapeutic options we began a series of cell culture studies. As shown in the next sequence of studies. We predicted an increase in DNA lesions induced by TMZ and radiation in the presence of siRNA for H-ferritin and expect to see these lesions appear at lower concentrations for both of the conventional therapeutic agents. In glioma stem cells (GSCs) radioresistance results from activated DNA damage response, we interrogated DNA damage and repair mechanisms following ferritin siRNA exposure. In figure 5, we use plasmid DNA to demonstrate that radiation exposure induces the supercoiled formation in the absence of H-ferritin and the protection is specific to H-ferritin, which may imply an unabated transcription activity.

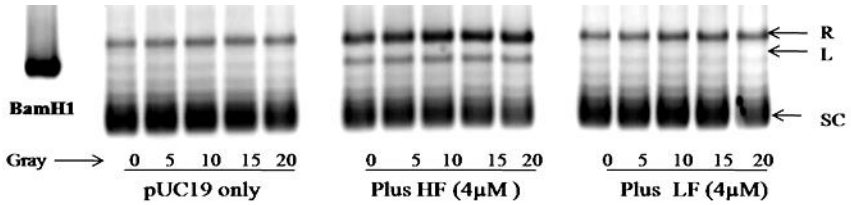

Fig. 5. H-ferritin, but not L-ferritin, inhibits the supercoiled formation of DNA plasmid under radiation. DNA plasmid pUC19 was treated with 4 μM of recombinant H-ferritin (HF) or L-ferritin (LF) and then exposed to incremental doses of radiation. The conversion to relaxed (R) and linear (L) forms was observed in the presence of H-ferritin (middle panel) but not L-ferritin (right panel). Higher H-ferritin concentration was associated with increased DNA in relaxed and linear forms (middle panel). (SC: DNA in supercoiled form).

Since DNA double strand breaks (DSBs) induced by ionizing radiation is the major cause of radiotherapy-induced cytotoxicity we interrogated the activity of DNA DSBs response proteins. Our data in Figure 6 show that H-ferritin down-regulation repressed the activity of DNA DSBs

repair protein ATM induced by radiation in glioma cells, further suggesting a significant role for H-ferritin in DNA protection.

Fig. 6. H-ferritin down-regulation impairs DNA repair mechanism induced by radiation in human glioma cells. U251 cells were exposed to 20 Gy of radiation at 48 hours post transfection of H-ferritin siRNA. The activity of DNA repair protein ATM was evaluated by the phosphorylation at S1981 position.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible

subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We anticipate that at least 3 papers will be generated in the next 3 years.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its

impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response. None

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:

Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes____X_____ No_____

If yes, please describe your plans:

We hope to find a commercial partner who would be interested in the development of our nanoliposome/siRNA delivery system for clinical application. We are in discussion with our Office of Technology Transfer.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
James R. Connor, Ph.D.	Distinguished Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
James_R_Connor	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Thomas More College, Ft. Mitchell, KY	B.A.	06/75	Psychology
Wright State University, Dayton, OH	M.S.	06/78	Physiology
University of California, Berkeley, CA	Ph.D.	06/81	Anatomy
Boston University Medical Center, Boston, MA	Postdoc	06/81-06/83	Neuroscience

A. Personal Statement

My research program on iron metabolism and mechanisms of iron homeostasis in the brain has been funded from the NIH for over 25 years and my interest in this area has been ongoing for over 30 years. I have an internationally recognized research program as evidenced by an H-index of 64. Over the past 10 years, our projects have developed a relevance to cancer. We have developed a targeted nanotechnology program to deliver chemotherapeutic agents, siRNA and contrast enhancement agents to brain tumors. We have the skill set and interest to significantly contribute to a positive outcome of the proposed research project.

B. Positions and Honors

Professional Positions:

1983-1987 Research Biologist, Veteran's Administration Medical Center, Washington, DC
1983-1987 Assistant Research Professor, Department of Physiology, The George Washington University School of Medicine
1987-1990 Assistant Professor, Department of Anatomy Pennsylvania State University College of Medicine (PSUCOM) Neuroscience & Anatomy, (PSUCOM)
1990-1996 Associate Professor, Department of Neuroscience & Anatomy, (PSUCOM)
1996- Professor, Department of Neuroscience & Anatomy, (PSUCOM)
1999-2002 Vice Chair Department of Neuroscience & Anatomy, (PSUCOM)
2002-2003 Interim Chair Department of Neuroscience & Anatomy, (PSUCOM)
2004- Professor Neurosurgery, (PSUCOM)
2006 Co-Director, Neuro-oncology Experimental Therapeutic Group (PSU Cancer Institute)
2007 University Distinguished Professor (PSUCOM)

Grant Review Experience:

NIH, NIEHS, Veterans Administration, Department of Defense USDA, NSF, MRC, Wellcome Trust, Israel Academy of Science, Alzheimer's Association, Hong Kong Research Council, American Federation for Aging Research, American Heart Association

Honors and Awards:

Samuel Hinkle Society Outstanding Young Investigator Award, Pennsylvania State University College of Medicine (1990)

Mentored two graduate students awarded Marion Kies Award by the American Society for Neurochemistry for outstanding doctoral thesis (1999, 2010)
Elected Chair of the East Coast Iron Club (2000-2002)
Dean's Lecturer Pennsylvania State University College of Medicine/M.S. Hershey Medical Center (2003)
Distinguished Alumni Award, Thomas More College (2007)
Named University Distinguished Professor Penn State University (2007)
Honorary Professor, Department of Neurology, Tianjin First Center Hospital, Tianjin, China (2008-)

Editorial Advisory Boards:
Journal of Neuroscience Research (1994-); GLIA (2001-); *Fluids and Barriers of the CNS* (2004-); *Current Neurovascular Research* (2004-); *CNS Drug Discovery* (2005-); *Recent Patents on Biotechnology* (2008-); *Journal of Neurochemistry* (Handling Editor, 2011-), *PlosOne (2012-).*

C. Selected Peer-reviewed Publications:

1. Lee S, **Connor JR**. Regulation of Hfe by stress factors in BV-2 cells. *Neurobiology of Aging* 26(6):803-812, 2004.
2. Wang X, Lee S, Simmons Z, Boyer P, Scott K, Liu W, **Connor J**. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related cellular consequences. *Journal of Neurological Sciences* 227(1) 27-33, 2004.
3. Surguladze N, Thompson K, Beard J, **Connor JR**, and Fried M. Interactions and reactions of ferritin with DNA. *Journal of Biological Chemistry* 279(15):14694-14702, 2004.
4. Surguladze N, Patton SM, Cozzi A, Fried MA, **Connor JR.** Characterization of nuclear ferritin and mechanism of translocation. *Biochemical Journal* 388 (Pt 3):731-740, 2005.
5. Zhang X, Surguladze N, Slagle-Webb B, Cozzi A, **Connor JR.** Cellular iron status influences the functional relationship between microglia and oligodendrocytes. *GLIA* 54(8):795-804, 2006.
6. Madhankumar AB, Slagle-Webb B, Mintz A, Sheehan JM, **Connor JR.** Interleukin-13 receptor-targeted nanovesicles are a potential therapy for glioblastoma multiforme. *Molecular Cancer Therapeutics* 5(12):3162-3169, 2006.
7. Lee SY, Patton SM, Henderson RJ, **Connor JR.** Consequences of expressing mutants of the hemochromatosis gene (JFE) into a human neuronal cell line lacking endogenous HFE. *The FASEB Journal* 21(2):564-576, 2007.
8. Fisher J, Devraj K, Ingram J, Slagle-Webb B, Madhankumar AB, Klinger M, Simpson IA, **Connor JR.** Ferritin: A novel mechanism for delivery of iron to the brain and other organs. *American Journal of Physiology – Cell Physiology*, 293(2):C641-C649, 2007.
9. Madhankumar AB, Slagle-Webb B, Wang X, Yang Q, Antonetti D, Miller P, Sheehan J, **Connor JR**: Efficacy of IL-13 receptor targeted liposomal doxorubicin in the intracranial brain tumor model. *Molecular Cancer Therapeutics* 8(3):648-654, 2009.
10. Mitchell RM, Lee SY, Randazoo WT, Simmons Z, **Connor JR**: Influence of HFE variants and cellular iron regulation on monocyte chemoattractant protein-1. *J Neuroinflamm* 6:6, 1-12 Feb, 2009. PMCID: 2656486.
11. Hall EC, Lee SY, Simmons Z, Neely EB, Nandar W, **Connor JR**: Prolyl-peptidyl isomerase, Pin1, phosphorylation is compromised in association with the expression of the HFE polymorphic allele, H63D. *Biochimica et Biophysica Acta* 1802:389-395, 2010.

12. Alkhateeb A, **Connor JR**. Nuclear Ferritin: A new role for ferritin in cell biology. Biochimica et Biophysica Acta 1800 (2010) 793–797.
13. Liu X, Madhankumar AB, Sheehan J, Surguladze N, Slagle-Webb B, **Connor JR**. "H-ferritin siRNA Delivered by Cationic Liposomes Increases Chemotherapeutic Sensitivity for Treating Glioma." Cancer Res; 71(6) March 15, 2011.
14. Lee SY, Liu S, Mitchell RM, Slagle-Webb B, Hong YS, Sheehan JM, **Connor JR**. 2010. HFE polymorphisms influence the response to chemotherapeutic agents via induction of p16INK4A. *Int J Cancer*. 129, 2104-2114, 2011.
15. Alkhateeb A, Leitzel K, Ali S, Campbell-Baird C, Evans M, Fuchs E, Kostler W, Lipton A, **Connor JR**. Elevation in serum Inflammatory biomarkers predicts response to trastuzumab-containing therapy. *PloS One*, 7(12):e51379, 2012.