

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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Allegheny-Singer Research Institute
2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 06/30/2010
3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Rebecca E. Pfeifer
4. **Grant Contact Person’s Telephone Number:** 412-359-3137
5. **Grant SAP Number:** 4100047623
6. **Project Number and Title of Research Project:** 03 – A High Fidelity Rat Model of Pulmonary Arterial Hypertension
7. **Start and End Date of Research Project:** 01/01/2009 – 06/30/2010
8. **Name of Principal Investigator for the Research Project:** Michael J. Passineau, PhD
9. **Research Project Expenses.**

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

\$ 52,569.44

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	Position Title	% of Effort on Project	Cost
Passineau	Principal Investigator	2.64%	\$ 4,181.48
Machen	Research Assistant	21.48%	\$11,871.36
Zorelias	Research Assisant	21.95%	\$ 9,406.56

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	Position Title	% of Effort on Project
None		

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 _____ No X _____

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

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:
A System for Long-Term Gene Expression in the Pulmonary Vascular Endothelium	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input checked="" type="checkbox"/> Nonfederal source (specify: Gilend Sciences Research Scholars Program)	8/2009	\$100,000	\$100,000
Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin	<input type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input checked="" type="checkbox"/> Nonfederal source (specify: Entelligence Young Investigators Award Program_)	12/2009	\$100,000	\$100,000

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

Yes No _____

If yes, please describe your plans:

We plan to submit an R01 application to NIH in 10/2010 using this model.

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

We will continue to use this model in the grants listed above and in future projects.

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 _____ No

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

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

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

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

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.

Having this animal model up-and-running in our institution has improved our competitiveness for NIH funding and made our institution more attractive to new investigators we are seeking to recruit.

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 are now working with the developer of this model, Dr. R. James White, to scan some of his samples using our Micro-CT techniques.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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 (β) should not print as boxes (\square) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Specific Aim 1: To train CVI staff in the production of this rat model of PAH through an externship with the originator of this model at the University of Rochester.

This aim has been completed in its entirety. In order to perform this aim, two members of the lab staff travelled to Rochester and learned the surgery first-hand in Dr. White's lab. These staff members performed the surgery hands-on over a two day visit, at which point they felt sufficiently confident in their abilities to return to our facility.

Specific Aim 2: To mimic this model of PAH in a cohort of rats within the CVI and confirm congruency of experimental endpoints with those previously published by White et al.

The proposal predicted performing this technique in a cohort of 20 rats, followed by echocardiographic, hemodynamic and histological evaluation.

This aim has been completed in its entirety but has been slightly modified from the original research plan. Specifically, we have postponed the hemodynamic and histological evaluation of the pulmonary vasculature in favor of Micro-CT 3D angiography. This new method of assessing pulmonary vascular changes in animal models is exquisitely sensitive, and directly explores the second most important consideration in clinical PAH (cardiac function being the first, which we assess with echocardiography). Thus, we have set aside older, indirect techniques in favor of direct analysis of pulmonary vascular architecture.

The first cohort of 13 animals was performed between January 1, 2009 and December 31, 2009 with results as follows: 9 pre/intraoperative deaths due to difficulties with intubation; 3 successful surgeries with post-operative deaths and 1 successful survival surgery. The one rat that survived was administered monocrotaline and was monitored for two months, but did not develop signs of pulmonary hypertension.

The final work product of this aim was a cohort of 10 animals in which the model was replicated with a 90% success rate between February 2010 and June 2010. 100% of these animals developed pulmonary hypertension. Validation of the fidelity of replication of the published model was based upon echocardiography and pulmonary vascular angiography and secondarily by mean survival. Figures 1-3 below present these analyses. By all methods of analysis, we have confirmed that our rats developed severe PAH indistinguishable from that reported in White *et al.*

It should be noted that while we have completed both of the specific aims proposed, the histological and hemodynamic analyses outlined in the original proposal have not been performed. This is because such analyses are incompatible with the more sophisticated Micro-CT angiography, which requires opening the heart to access the pulmonary artery and

renders tissue unusable for histological analysis. Having replicated the model as proposed, we have extracted more value from expanding Dr. White's findings to 3-D structural than we would have by simply replicating the histological analyses in his original report.

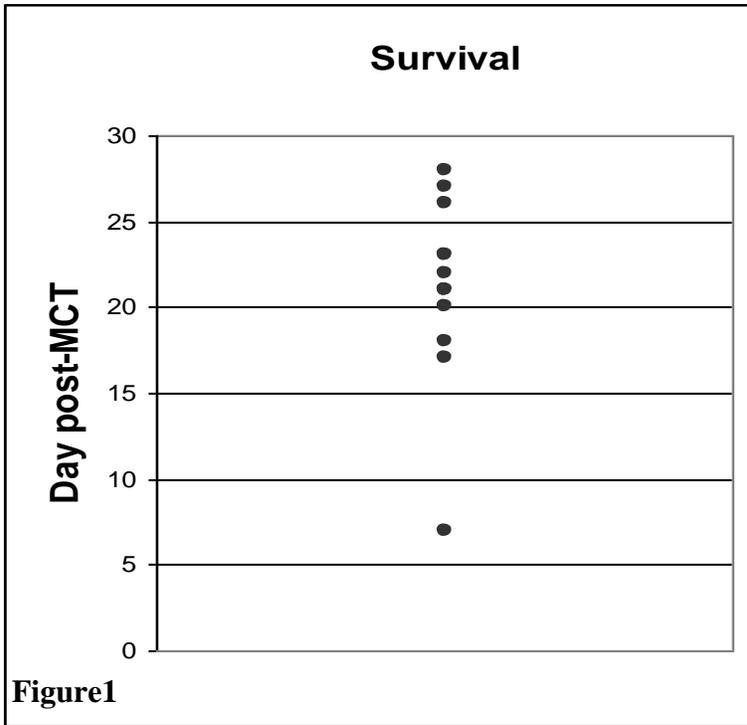


Figure 1: Survival post-MCT in PAH animals. Survival post-MCT for each individual animal in our final cohort of PAH animals. All animals showed post-mortem evidence of PAH.

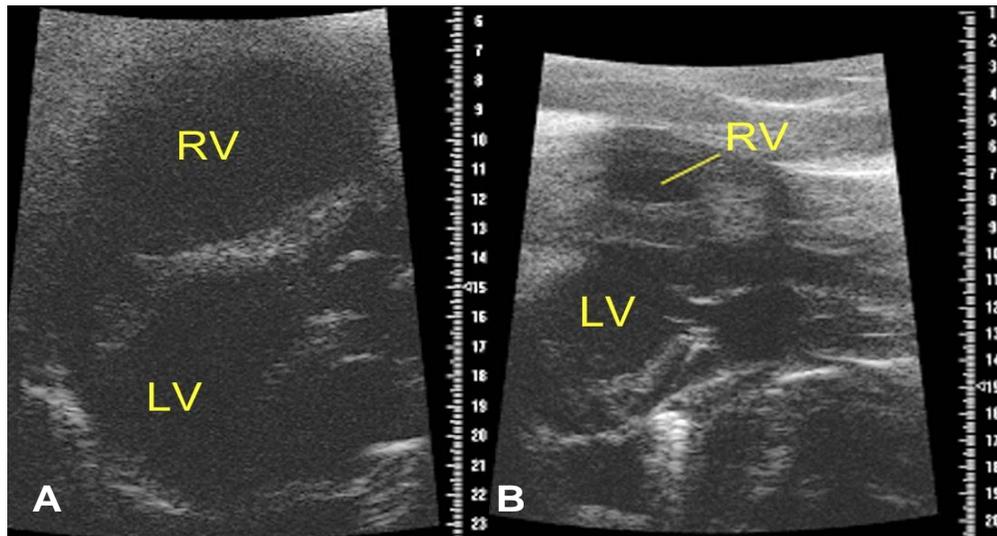


Figure 2: Echocardiography of rats hearts showing right ventricular (RV) enlargement consistent with pulmonary hypertension. (A) is a PAH rat 28 days following MCT, and (B) is a naïve rat serving as a normal control.

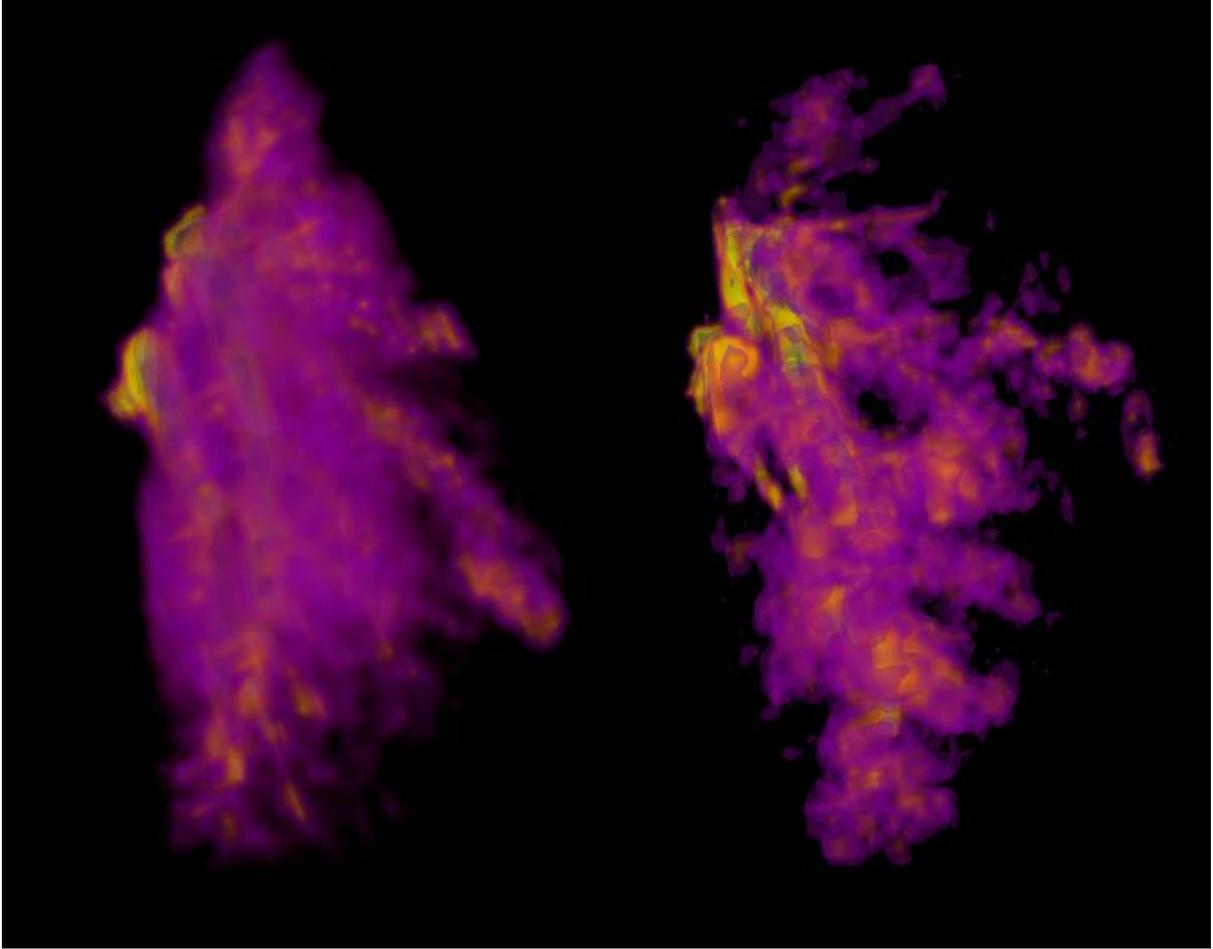


Figure 3: Micro-CT angiography of the right lung of a normal rat (left) and a PAH rat 20 days post-MCT. Note the “cluster of grapes” appearance of the PAH lung, which is a common finding.

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
 No

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

Yes
 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

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, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the “Cognition and MRI in Older Adults” research project (Project 1), and two publications for PI Zhang for the “Lung Cancer” research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI

Project 1 – Smith – Publication 2 – Cognition and MRI

Project 3 – Zhang – Publication 1 – Lung Cancer

Project 3 – Zhang – Publication 2 – Lung Cancer

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				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> 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 will publish the results of the two funded studies listed in 11(A)

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

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 _____ No _____ X _____

If yes, please describe your plans:

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

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Passineau, Michael Joseph	POSITION TITLE Assistant Professor		
eRA COMMONS USER NAME (credential, e.g., agency login) mjpassineau			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
Cedarville College	B.A.	06/95	Bible/Chemistry
University of Miami	Ph.D.	12/01	Neuroscience
University of Alabama at Birmingham	Postdoctoral	12/05	Gene Therapy
University of Alabama at Birmingham	Postdoctoral	2/07	Gene Therapy

A. Personal Statement

B. Positions and Honors

Positions and Employment

2003-2005	Postdoctoral Fellow, Gene Therapy Center, Division of Human Gene Therapy, UAB
1/2006-3/2007	Postdoctoral Scholar, UAB School of Dentistry
3/2007-6/1/2008	Instructor, Department of Oral and Maxillofacial Surgery, UAB School of Dentistry
4/2007-6/1/2008	Associate Scientist, UAB Gene Therapy Center
8/2007-6/1/2008	Associate Scientist, UAB Center for Metabolic Bone Disease
6/1/2008-	Research Scientist, Allegheny-Singer Research Institute
6/1/2008-	Assistant Professor, Drexel University School of Medicine

Other Experience and Professional Memberships

2007-	Member, International Association for Dental Research
2008-	Member, American Association for the Advancement of Science
2008-	Member, American Society for Cell and Gene Therapy
2009-	Member, American Thoracic Society
2010-	Member, American Heart Association

Honors

2006	Colgate Research in Prevention Award (International Association for Dental Research)
2006	Bloc Travel Award (American Association for Dental Research)

2003-2005 Ruth L. Kirchstein National Service Award
1999 Finalist, Student Research Competition, National Neurotrauma Society
1994 American Heart Association Summer Fellow

C. Selected Peer-reviewed Publications (Selected from 42 peer-reviewed publications)

Most relevant to the current application

- Raymond L. Benza, Dawn M. Pekarek, Joseph P. Barchue, Jose A. Tallaj, Michael J. Passineau, Christopher S. Coffey, Hernan E. Grenett (2009). TGF- β Polymorphisms, gender, and age and their effect on acute cardiac rejection. *Journal of Heart and Lung Transplantation* 2009 Oct;28(10):1057-62.
- Passineau M and Curiel DT (2005). Gene transfer and expression in the vascular endothelium. In Aird W (ed.) *Endothelial Biomedicine*, Cambridge University Press, Cambridge UK.
- Everts M, Kim-Park SA, Preuss MA, Passineau MJ, Glasgow JN, Pereboev AV, Mahasreshti PJ, Grizzle WE, Reynolds PN, Curiel DT. Selective induction of tumor-associated antigens in murine pulmonary vasculature using double-targeted adenoviral vectors. *Gene Ther.* 2005 Jul;12(13):1042-8.

Additional recent publications of importance to the field (in chronological order)

- Passineau MJ, Zourelis L, Machen L, Edwards PC, Benza RL. Ultrasound-assisted non-viral gene transfer to the salivary glands. *Gene Ther.* 2010 May 27.
- Passineau M, Siegal GP, Everts M, Pereboev A, Jhala D, Wang M, Zhu ZB, Kim-Park SA, Curiel DT, Nelson G. The Natural History of a Novel, Systemic, Disseminated Model of Syngeneic Mouse B Cell Lymphoma. *Leuk Lymphoma.* 2005 Nov;46(11):1627-1638.
- Breidenbach M, Rein DT, Everts M, Glasgow JN, Wang M, Passineau MJ, Alvarez RD, Korokhov N, Curiel DT. Mesothelin-mediated targeting of adenoviral vectors for ovarian cancer gene therapy. *Gene Ther.* 2005 Jan;12(2):187-93.

D. Research Support

Ongoing Research Support

5R00DE018188-03
05/31/2011
NIH

06/01/2008 –

Salivary Gland-Based Gene Therapy for Lysosomal Storage Diseases

The major goals of this project are to address the biochemical defect underlying Fabry disease by developing and evaluating salivary gland based gene therapy approach using human α -Gal A (Galactosidase) and Adeno-Associated virus (AAV) vector in mouse model.

Role: PI

3R00DE018188-03S110
05/31/2011
NIH

09/25/2009-

This grant is a competitive revision supplement to 5R00DE018188-03 (above), funded through the American Reinvestment and Recovery Act to expand the original aims of the project by applying a newly-developed gene transfer technique. This technique will be tested in parallel with viral vectors per the existing project, in the Fabry mouse model.

Role: PI

Gilead Sciences Research Scholars Program 1/1/2010-12/31/2011

Gilead Sciences

A System for Long-Term Gene Expression in the Pulmonary Vascular Endothelium

This proposal seeks funding to develop, validate, and utilize an AAV gene therapy vector with the potential to achieve long-term, selective gene expression in the pulmonary vasculature.

Role: PI

Entelligence Young Investigators Award Program 8/1/2010-7/31/2011

Actelion Pharmaceuticals

Gene Therapy to Drive Endogenous Biosynthesis of Prostacyclin

The overall goal of this proposal is to design and test a gene therapy strategy for pulmonary arterial hypertension (PAH). PAH is a rare, deadly, and incurable disease with a mean survival of 2.8 years from onset of symptoms, if left untreated. The challenge to be addressed by this proposal is the need for a therapeutic regimen that allows for endogenous production of prostacyclin therapy within the patient's own body, throughout the entire lifetime of the patient.

PROCHYMAL 403 for Acute MI

07/27/2009-Open

Ended

Osiris Therapeutics

A Phase II, multi-center, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of PROCHYMAL* (ex vivo cultured adult human mesenchymal stem cells) intravenous infusion following acute myocardial infarction. Our lab serves as the unblinded drug preparation arm for this site.

Role: Co-Investigator

Completed Research Support

1K99DE018188-01

06/01/2007-

5/31/2008

Salivary Gland-Based Gene Therapy for Lysosomal Storage Diseases

The major goals of this project are to address the biochemical defect underlying Fabry disease by developing and evaluating salivary gland based gene therapy approach using human α -Gal A (Galactosidase) and Adeno-Associated virus (AAV) vector in mouse model.

Role: PI

4100047623

01/01/2009 – 6/30/2010

Pennsylvania Department of Health

A High-fidelity Rat Model of Pulmonary Arterial Hypertension

The project's aim is to deploy and validate a rat model of pulmonary arterial hypertension in our laboratories as a substrate for testing gene therapy interventions in this disease.

Role: PI