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:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

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:** 4100047645

6. **Project Number and Title of Research Project:** 8 - Murine Induced Pluripotent Stem Cells: Differentiation and Bone Formation

7. **Start and End Date of Research Project:** 1/1/2009-6/30/2010

8. **Name of Principal Investigator for the Research Project:** Christopher Niyibizi, 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:

   $ 438,262 includes equipment

   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
Feng Li	Postdoctoral fellow	19% -Fy 08/09	$6,783
Feng Li	Instructor	28%-FY09/10	$9,096
Katherine Ludlow	Work study student	08/09	$382
Katherine Ludlow	Work study student	09/10	$402
Jacqueline Maddox	Wage Payroll lab Tech	09/10	$2,701

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
Niyibizi	PI	2%

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
LSRII Flow Cytometer analysis equipment	Numerous research projects at the Penn State College of Medicine have made extensive use of the LSRII since its purchase – almost 20,000 samples from 22 investigators in 8 departments.	$391,882

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:
Transplantability of iPSC derived cells	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	06/2010	$ 426,525	$383,873

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:

The application to continue the studies will be submitted to the National Institute of Health in July 2013.

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

We plan to submit an R01 grant based on preliminary data generated from the Cure Funding.

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			1
Female				
Unknown				
Total	**1**			**1**

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

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

    The grant funding helped in acquisition of a new multicolor LSRII FACS analysis instrument used for analyses in this work. The Becton Dickinson LSR II is a high-end research grade flow cytometry analyzer with the unique capability of detecting many cellular biomarkers simultaneously, and it was purchased by an equal contribution of TSF/CURE funds and other institutional funds. This instrument allows for the detection of up to 15 different markers in each sample in addition to providing information on size and granularity of all cell populations present. This format provides great flexibility in choosing and or utilizing markers and fluorochromes that are available to the researcher. The instrument provides both qualitative and quantitative analysis of complex populations of cells derived from tissue

culture, experimental animals and human specimens. The end result is the ability to detect minor and major changes in cell populations that are associated with disease progression and prevention.
The BD LSR II has greatly increased the capabilities of investigators at Penn State Hershey to define the role of specific, and often very rare cell populations in a variety of diseases including cancer, malaria, virus infections, diabetes and other autoimmune diseases. No major research center could be competitive in today's environment without a similar instrument. Some specific examples of the type of studies performed include the identification of rare innate immune cells that are associated with recovery from virus infections or that promote kidney disease in diabetic patients. These cells can only be reproducibly identified using 8 or more markers. Additional markers are then used to evaluate the function (protective or disease promoting) of these very rare cells. Other studies are identifying the types of immune cells that are associated with elimination of cancer in both experimental animals and humans following administration of new types of therapies so that these cells may be better harnessed to combat cancer. Alternatively, some studies use this approach to identify the characteristics of tumor cells important for sensitivity to therapy or for causing metastasis. These studies have resulted in new publications and are facilitating the submission of new grant applications to achieve continued progress in the treatment, diagnosis and prevention of important human diseases. Over 20 different basic and clinical research laboratories from across Penn State Hershey have been regular users of this machine, analyzing a total of close to 20,000 samples last year alone, demonstrating its broad applicability to multiple disciplines and diseases.

In addition, the funding generated preliminary data that was used to apply for an NIH R21 grant.

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:

The health research funds led to a collaborative effort with researchers at Penn State University Park. This involved collaboration with an engineer at the University Park campus generating scaffolds that can be used to culture cells and eventually grow tissues.

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.

The goals of the project were to assess the potential of stem like cells called induced pluripotent stem cells (iPS) to differentiate toward mesenchymal stem cells (MSCs) and to make bone and cartilage in vivo, thus to understand the future application of the cells for the repair and regeneration of musculoskeletal tissues.

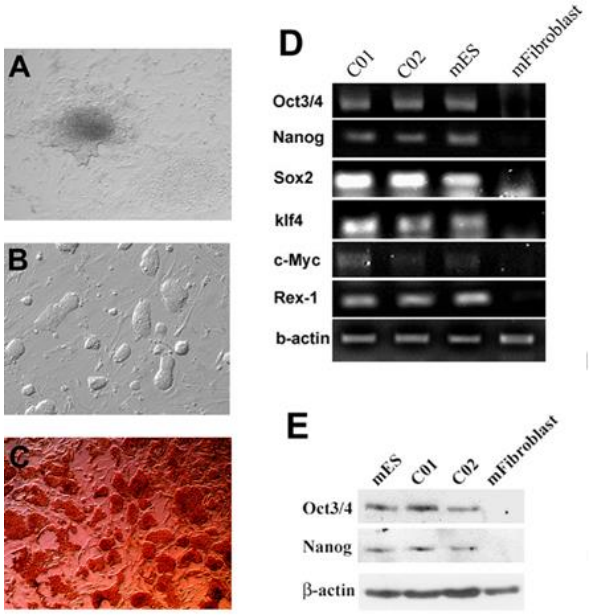
With aging, the yields of adult derived stem cells decrease and some studies have shown that the cells may exhibit reduced proliferation and differentiation potential. Embryonic stem cells (ESC) derived from the inner cell mass of the blastocyst can give rise to any cell type of the body and can be expanded indefinitely without loosing their pluripotency, thus these cells are better suited for regenerative medicine. Because of ethical concerns however, little progress has been made in harnessing the power of these cells. Recently, it has been demonstrated that mouse and human fibroblasts can be reprogrammed into an ESC–like state by introducing combinations of four transcription factors Oct-3/4, Sox2, c-Myc and Klf4. The therapeutic potential of such induced pluripotent stem (iPS) cells remains undefined. The project proposed to generate mouse specific iPS and to assess them for mesenchymal stem cell differentiation (MSCs) and for bone and cartilage formation in vivo. The central hypothesis of the project was that iPS cells can be directed to MSC differentiation and will give rise to osteoblasts, chondrocytes and adipocytes and will make bone in vivo.

To test the above hypothesis, the following aims were proposed:
Aim 1, generate and characterize mouse iPS from tail tip fibroblasts.
To accomplish this aim, we would construct retroviruses containing the four defined factors (Oct-3/4, Sox2, c-Myc and Klf4); these factors would be transfected into tail tip fibroblasts harvested from the 4-day old mice. The transduced cells would be cultured following ESC protocols and then assessed for ESC like state

Aim 2, test the hypothesis that iPS cells can be differentiated into MSCs and the cells will form bone and cartilage in vivo.
In this aim, the iPS cells would be assessed for MSC differentiation and then be evaluated for osteoblast and chondrocyte differentiation in vitro and for bone and cartilage formation in vivo when the cells are incorporated in scaffolds and implanted into the mice from which the reprogrammed fibroblasts were harvested.
The results from the project would provide important information regarding the future application of ESC like stem cells generated from reprogrammed fibroblasts for musculoskeletal tissue repair and regeneration.

Results:
Studies proposed in aim 1 were accomplished as stated. iPSC were generated as proposed. The cells were confirmed to exhibit ES like charcateristics using several criteria.
Generation of the murine iPSC from the tail tip fibroblasts
To assess the feasibility of generating iPSC cells, we harvested tail tip fibroblasts from 4 day old mice. The cells were transduced with 4 factors (Oct-4, Sox2, c-Myc and Klf4) carried in pMX based retrovirus in presence of polybrene. After 6 days, the transduced cells were trypsinized and plated on murine embryonic fibroblasts (MEF). Fourteen days after transduction, clones resulting from reprogrammed fibroblasts were observed (Fig. 1A). The clones were picked and

expanded on murine embryonic stem cells fibroblasts (MEF) and are shown in figure 1B. The morphological appearance and growth on MEF is typical for ESC.



Next we examined the reprogrammed cells for characteristics of ESC state. One of the characteristics of undifferentiated ESC is expression of alkaline phosphatase activity. The reprogrammed fibroblasts we generated here expressed ALP activity indicating that the cells exhibited one of the characteristics of ESC like state (Fig. 1C), The reprogrammed cells also expressed genes associated with ESC shown by gene expression analysis and protein production by western blotting (Fig 1D, E). The primers used were designed to detect endogenous genes not the exogenous factor genes delivered by the retrovirus. These data clearly demonstrate that the reprogrammed fibroblasts exhibit an ESC like state.

Figure 1. iPSC clones generated from reprogramming tail tip fibroblasts exhibit ESC characteristics.
*Fibroblasts generated from the 4 day old murine tail tip fibroblasts were transduced with a retrovirus containing 4 factors and at 6 days after transduction clones were observed (**A**). Picked clones at 14 days cultured on MEF (**B**). The iPSC express ALP activity characteristic of ESC(**C**). iPSC express ESC genes important for pluripotency (**D**). Western blot shows synthesis of proteins associated with ESC (Oct3/4 and Nanog (**E**). The data clearly demonstrate that the cells we have generated exhibit ESC like state. mES=murine ESC, C01 and C02=clones 1 and 2 respectively.*

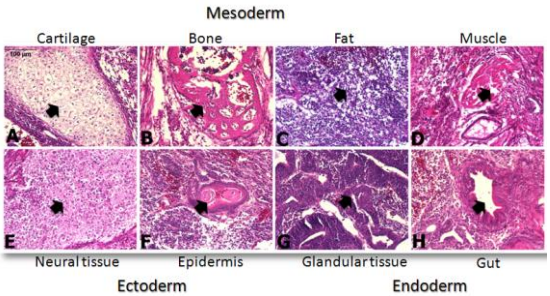


Figure 2. Teratoma formation by iPSC. *Immunodeficient mouse recipients were injected with mouse iPSC intramuscularly. Resulting teratomas demonstrate the following features in ectoderm, mesoderm and endoderm: mesoderm: cartilage (A), bone (B), fat (C), muscle (D); Ectoderm: neural tissue (E), Epidermis (F); Endoderm: glandular tissue (G), gut (H). All images were obtained from the same tumor. Tissue sections were stained with haematoxylin.*

Differentiation of iPSC into 3 germ layers

One of the important characteristics of ESC is the ability to give rise to 3 germ layers; mesoderm, ectoderm and endoderm. iPSC were assessed for the ability to give rise to the 3 germ layers as a further confirmation of iPSC we have generated here for ESC like state. iPSC injected into the thigh muscles of SCID mice generated well-differentiated teratoma-like masses containing all three embryonic germ layers (Fig. 2).

iPSC give rise to cells that exhibit MSCs characteristics and deposit calcium in vitro
In aim 2 of the project, we assessed the cells for differentiation toward MSCs and bone formation as proposed. To assess this feasibility, iPSC derived EBs were differentiated by treatment with retinoic acid (RA) and treatment with TGF-beta1 for 2 days. The resulting cells (Fig. 3A) were assessed by flow activated cell sorting (FACS) for the expression of the putative MSCs surface antigens and calcium deposition in vitro. The results showed that iPSC directed EBS upon differentiation gave rise to cells expressing putative MSCs surface antigens (Fig. 3A, B). The cells expressed CD73 and CD105 in addition to other surface antigens, the putative markers of MSCs.

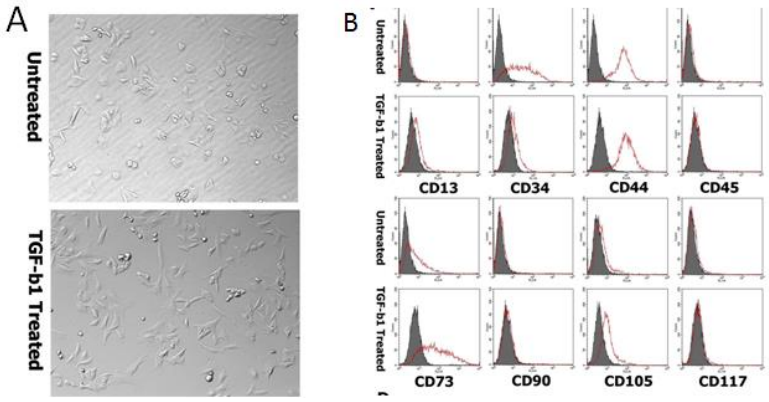


Fig. 3. Morphological appearance and expression of surface antigens associated with MSCs by Differentiated iPSC in presence or absence of TGF-beta1**.** *A) iPSC differentiated into small and spindle shaped cells upon differentiation. TGF-beta1 treated and untreated iPSC appear to contain cells that differ morphologically. B) The cells express surface antigens attributed to MSCs upon differentiation in vitro. These data support suggest iPSC have potential to give rise to MSCs.*

Osteogenic differentiation

iPSC differentiated by retinoic acid treatment and 2-day treatment with TGF-beta1 and maintained in osteogenic medium, deposited calcium in vitro as indicated by Alizarin red and Von Kossa staining (Fig. 4A, B). These data imply that the cells differentiated into osteoblasts at least in vitro. Interestingly, iPSC briefly treated with TGF-beta1 deposited more calcium when cultured in osteogenic medium than untreated cells (Fig 4A, B, C). Osteoblast differentiation was further confirmed by analysis of expression of osteoblasts associated genes (Fig. 4D). Taken together, the data suggest that brief treatment of iPSC with TGF-beta1 enhances differentiation of iPSC toward MSCs thus generating cells with high potential for differentiation toward osteoblasts lineage. Our present approach will be to enrich iPSC into MSCs by sorting cells based on CD73 after brief TGF-beta1 treatment. This surface antigen is highly expressed by differentiating iPSC and has been shown previously to enrich for cells with MSCs characteristics from differentiating human ESC (38). The CD73 cells sorted from differentiated hESC were shown to give rise to osteoblasts, chondrocytes, adipocytes and engraftable myoblasts.

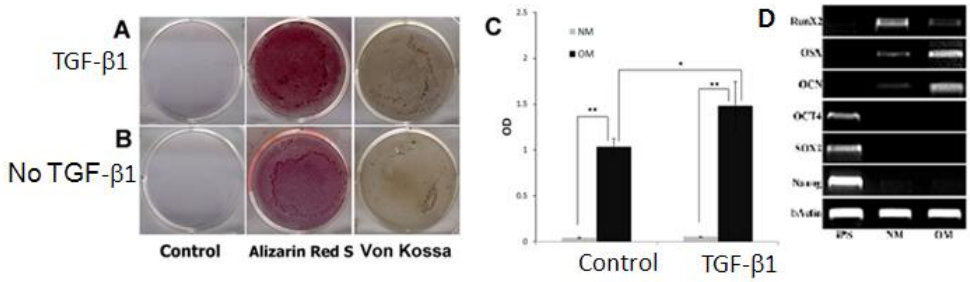


Figure 4. Mineralization and osteoblast gene expression by differentiating iPSC ***A****) iPSC directed EBS treated with RA and TGF- β1 cultured in osteogenic medium and stained with Alizarin Red S;* ***B****) iPSC cultured in osteogenic medium without TGF-β1 supplementation and stained with Alizarin Red S.* ***C****) Relative calcium deposition in vitro by iPSC cultured in presence or absence of TGF-β1. iPSC cultured in presence of TGF- β1 for 2 days, deposited more calcium than non TGF-β1 treated.* ***D****) Expression of osteoblasts genes by differentiating iPSC cultured in osteogenic medium. NM=Normal medium no osteogenic supplements, OM=osteogenic medium, iPSC undifferentiated iPSC.*

We did not have sufficient funds ($30,000 direct costs) to complete all the studies proposed in aim 2 to test the potential of cells to make bone and cartilage in vivo. However, we did complete related studies in which cells were implanted in mice and shown to make bone in vivo. The PA Cure funds were also used to support studies with adult derived stem cells demonstrating that MSCs harvested from bone marrow generate factors that play a role in inducing other cells to migrate and differentiate. These data were published acknowledging partial support by the PA CURE grant.

Summary of Research Completed during the funding period

For the funding period, we focused on generating bone cells from the induced pluripotent stem cells (iPSC) as we proposed. We discovered that when the cells are treated with a growth factor called Transforming growth factor beta 1 (TGF-beta1) the cells give rise to cells found in bone marrow and other musculoskeletal tissues that we call Mesenchymal stem cells (MSCs). These mesenchymal stem cells can be coaxed to become bone or cartilage cells. So far we showed that the cells can become bone cells and deposit the mineral calcium in vitro. We also demonstrated that adult derived cells produce factors that contribute to cell differentiation and recruitment. The results generated from Tobacco Cure laid an important platform for funding from outside sources. The findings from the studies were presented at the following Orthopaedic Research Society meetings:

Abstract Presentations:

Li F, **Niyibizi C** TGF-beta1 Treatment Enhances Induction of Osteogenic Lineage in Murine Induced Pluripotent Stem Cells (iPS). 56th Annual Orthopeadic Research Society, New Orleans March 5-9, 2010.

Li, Feng, Whyte, Noelle, **Niyibizi, Christopher**. Factors Produced by Differentiating Multipotent Mesenchymal Stromal Cells Support Migration, Differentiation of Exogenous

MSCs: Implications for Paracrine actions by MSCs . 57th Annual Orthopeadic Research Society, Long Beac1.

F. Li, N. Song, **C. Niyibizi**. Paracrine Effects by Differentiating Human Bone Marrow Mesenchymal Stem Cells. Trans Orth Res Soc. Jan 25, 2013, San Antonio TX.

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, 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. Derivation of Murine Induced Pluripotent Stem Cells and Assessment of Their Differentiation Toward Osteogenic Lineage	Li, Bronson, Niyibizi	J Cellular Biochem	Aug 2009	☐Submitted ☐Accepted X Published
2. Differentiating multipotent mensenchymal stromal cells generate factors that exert paracrine activities on exogenous MSCs: Implications for paracrine activities in bone regeneration	Li, Whyte, Niyibizi	Biochemical & Biophysical Research Communications	Aug 2012	☐Submitted ☐Accepted X Published

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

Yes_____ No___X_____

If yes, please describe your plans:

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Niyibizi, Christopher	Associate Professor
eRA COMMONS USER NAME niyibizi	

Univeristy	*Degree*		
University of Massachusetts, MA	BA	1976	Biology
Rutgers University, New Brunswick, NJ	MSc.	1980	Biochemistry
McGill University, Montreal, Canada	Ph.D.	1985	Biochemistry

B. Positions and Honors.

Positions and Employment

1984-1985 Research Fellow, Harvard Medical School, Biological Chemistry, Boston, MA.
1985-1989 Postdoctoral Research Associate, University of Washington, Dept. Of Orthopaedics Seattle, WA.1989-1992.

1990-1992 Research Assistant Professor, Orthopaedics, University of Washington Seattle, WA.

1992-2000-Assistant Professor, Orthopaedic Surgery University of Pittsburgh, Pittsburgh, PA.

2001-2003 Associate Professor, University of Pittsburgh, Orthopaedic Surgery, Pittsburgh, PA.
2003-2004 Associate Professor Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey PA.
2005-Present Associate professor (tenure) Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey PA.
2005-present Associate Professor (secondary), Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey PA.

Other Experience and Professional Memberships

Member American Association of Bone and Mineral Society Research 2006-present
Member American Society for Matrix Biology 2004-present
Member Orthopaedic Research Society 1993-Present

Awards and Honors

Regular Member NIH Pathobiochemistry Study Section 2003-2004
Regular member NIH Skeletal Biology Structure and Regeneration Study Section 2004-2007
Regular member NIH Physiology and Pathobiology of Musculoskeletal, Oral and skin Systems (F10B) Study Section, 2007-2010
Adhoc Reviewer**:** NIH Musculoskeletal Tissue Engineering study section**:** 2007
Adhoc Reviewer: American Association of Bone and Mineral Research Bridge grants Sept. 2007
Mentor; Young Investigator award; Association of Bone and Mineral Research Society, 2006
Young Investigator Award, Chem. and Biol. of Mineralized Tissues (1988)
University of Massachusetts Scholarship (1975-1976)
Windowshop Scholarship Fund (1975)

First Award NIH, 1994-2000
Certificate for Outstanding Mentor of Minority summer Research Program: American Chemical Society, summer 1998.
Elected Team member of Consultants, Tissue Informatics Inc. 1999-2003
Member Orthopaedic Research Society Program Committee 2004-2005
Topic Chair of Progenitors/Stem cells: Orthopaedic Research Society 2005-2007

C. Selected peer-reviewed publications from a total of 58 from (2000) (in chronological order).

1. Niyibizi C, Wallach C, Mi Z, Robbins P. Approaches to Skeletal Gene Therapy. Crit. Rev Eukary Gene Expression 2002; 12:163-73.
2. Adachi N, Sato K, Usas A, Cummins J, Niyibizi C, Fu FH, Ochi M, Huard J. Muscle derived cell based ex -vivo gene therapy for the repair of full - thickness articular cartilage defects. J Rheum 2002; 29:1920-30-63.
3. Niyibizi C, Wang S, Mi Z, Robbins PD. The fate of mesenchymal stem cells transplanted into immunocompetent neonatal mice: Implication for skeletal gene therapy via stem cells. Mol Ther 2004; 9: 955.
4. Niyibizi C**,** Wang S, Mi Z, Robbins P. Gene therapy approaches for Osteogenesis Imperfecta. Gene Therapy 11:408-416, 2004.
5. Kawamura K, Chu CR, Sobajima S, Robbins PD, Fu FH, Izzo NJ, Niyibizi C. Adenoviral-mediated transfer of TGF-beta1 but not IGF-1 induces chondrogenic differentiation of human mesenchymal stem cells in pellet cultures.Exp Hematol. 2005; 33:865-72.
6. Wang X, Li F and Niyibizi C. Progenitors Systemically Transplanted into Neonatal Mice Localize to Areas of active bone formation, Stem Cells, 2006; 24: 1869-1878.Li F, Wang X, Niyibizi C. Bone marrow stromal cells contribute to bone formation following infusion into femoral cavities of a mouse model of osteogenesis imperfecta. Bone. 2010; 47:546-55.
7. Li F, Wang X, Niyibizi C. Distribution of single-cell expanded marrow derived progenitors in a developing mouse model of osteogenesis imperfecta following systemic transplantation. Stem Cells. 2008; 25:3183-93.
8. Liao X, Li F, Wang X, Yanoso J, Niyibizi C. Distribution of murine adipose-derived mesenchymal stem cells in vivo following transplantation in developing mice**.** Stem Cells Dev. 2008; 17:303-14.
9. Maddox J (Yanoso), Li F, Liao X**,** Niyibizi C. Effects of culturing on the stability of putative adipose derived stem cell markers. Open Stem Cell J. 2009; 1:54-61.
10. Niyibizi C, Li F. Potential implications of cell therapy for osteogenesis imperfecta. International Journal of Clinical Rheumatology, 2009, 4: 57-66.
11. Feng Li, Sarah Bronson, Christopher Niyibizi. Derivation of murine induced pluripotent stem cells (iPS) and assessment of their differentiation toward osteogenic lineage. J Cell Biochem. 2010, 109:643-652.
12. Li F, Wang X, Niyibizi C. Bone marrow stromal cells contribute to bone formation following infusion into femoral cavities of a mouse model of osteogenesis imperfecta. Bone. 2010; 47:546-55.
13. Maddox JR, Ludlow KD, Li F, Niyibizi C. Breast and Abdominal Adipose Multipotent Mesenchymal Stromal Cells and Stage-Specific Embryonic Antigen 4 Expression. Cells Tissues Organs. 2012 Jan 10. [Epub ahead of print].
14. Feng Li, Noelle Whyte, Christopher Niyibizi. Differentiating multipotent mesenchymal stromal cells generate factors that exert paracrine activities on exogenous MSCs: Implications for paracrine activities in bone regeneration. Biochem Biophys. Res Commun.http://dx.doi.org/10.1016/j.bbrc.2012.08.095.
15. Li F, Niyibizi C. Cells derived from murine induced pluripotent stem cells (iPSC) by treatment with members of TGF-beta family give rise to osteoblasts differentiation and form bone in vivo. BMC Cell Biol. 15, 13:35, 2012.