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):** 1/1/2010 - 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:** 17. Parathyroid Hormone and Prostate Cancer Colonization of Bone

7. **Start and End Date of Research Project:** 4/21/2010 - 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Ronald Gomes, 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:

   $ 46,041

   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
Sheehan	Grad Student	30% -January thru June 2011	$4,318
Muthusamy	Post-doctoral Fellow	30% - May thru December 2010	12,059

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
Muthusamy	Postdoctoral Fellow	10%
Gomes	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
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_____ No____X_____

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:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

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

Yes_____ No____X_____

If yes, please describe your plans:

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

None. The PI has left the institution.

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			1	
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				
Other				1
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_____ No____X_____

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

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:

This project helped support an on going collaboration with Robert A. Sikes at the University of Delaware and his prostate cancer research group.

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 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 broad, long term objective of this project is to identify the requirement(s) for PTH-enhanced bone formation in osteosclerotic PCa colonization and establishment in murine bone. There are

two specific aims of this project: 1) Identify the effect of intermittent parathyroid hormone (PTH)-zolendric acid administration on SCID/Beige bone; 2) Isolate and characterize bone marrow endothelial cells from SCID/Beige bone.

Under aim I, our working hypothesis is that PTH, zolendric acid co-administration is anabolic to bone. To test this hypothesis we will employ the following approach. Twenty-four, 8-week old male SCID/Beige immune-compromised mice will be divided into four groups of six. Each group will be treated as follows: *Group one*, vehicle (control); *group two*, human PTH 1-34 (40 μg/kg per day); *group three*, zolendric acid (ZA) (3 μg/day); *group four*, human PTH 1-34 combined with zolendric acid (ZA). Mice in each group will be treated intermittently with 3 rounds. An intermittent treatment round is defined as: one subcutaneous injection each day for 5 days followed by two days of no injections. Thus, 3 treatment rounds span a total of 21 days.

Under aim II our working hypothesis is that human prostate cancer cell adhesion to murine bone marrow endothelial cells is enhanced following intermittent exposure to PTH, *in vitro*. To test this hypothesis we will employ the following approach. Six, 8-week old, male SCID/Beige immune-compromised mice will be used for the isolation and characterization of bone marrow vascular endothelial cells from the femur and lumbar spine. For endothelial cell isolation we propose to employ immunomagnetic selection using Dynabeads (Invitrogen) conjugated with mouse CD31, according to Fei et al., 1990, and Dong et al., 1997. Murine bone marrow endothelial cells (mBMECs) will be characterized: 1) morphologically; 2) by their ability to form tube-like structures when seeded on Matrigel; 3) by FACS analysis for their expression of von Willebrand factor, CD31, CD34, VCAM-1, and VE-cadherin, and PTH 1R. Cultures will be expanded for use in cell-cell adhesion assays.

The successful completion of these aims will establish the feasibility to conduct future experiments that seek to identify: 1) the requirement for enhanced bone formation in the colonization and establishment of human osteosclerotic PCa cells in murine bone; 2) the requirement for enhanced bone resorption in the colonization and establishment of human osteosclerotic PCa cells in murine bone; 3) mechanisms of osteosclerotic PCa cell colonization and establishment in murine bone.

The broad, long term objective of this project was to identify the requirement(s) for PTH-enhanced bone formation in osteosclerotic PCa colonization and establishment in murine bone. There are two specific aims of this project: 1) Identify the effect of intermittent parathyroid hormone (PTH)-zolendric acid administration on SCID/Beige bone; 2) Isolate and characterize bone marrow endothelial cells from SCID/Beige bone.

Although objective 2 was completed, objective 1 was not undertaken as the result of Dr. Muthusamy's departure from the laboratory. Accordingly, we initiated research to identify a role for TRPV6 ion channels and prostate cancer cell early colonization to murine bone that supported the completion of Ph.D. candidate Sheehan's dissertation. In this study, we tested the hypothesis that TRPV6 ion channels were required for the colonization of PCa cells to murine bone. To test this hypothesis, SCID/Beige mice were pre-administered PTH 1-34 to enhance bone turnover and osteoblastic PCa cell uptake in bone and animals were subsequently inoculated with C4-2 cells engineered to express reduced levels of TRPV6. Skeletal tissue

sections including long bones and lumbar vertebrae were analyzed 8 weeks post-inoculation. Our hypothesis predicts that PCa cell uptake and colonization would be enhanced following PTH pre-administration, and that PCa cells expressing reduced levels of TRPV6 would poorly colonize murine bone under similar conditions.

Summary of Research Completed

Our central hypothesis is that TRPV6 ion channels regulate PCa uptake and colonization in murine bone. We propose to test this hypothesis by addressing the following specific aim: Identify a role for TRPV6 ion channels in the establishment of PCa bone metastases. Under this aim SCID/Beige mice were administered intermittent PTH 1-34 and injected with PCa cells with reduced levels of TRPV6 expression. Eight weeks post injection, serum was collected and long bones and lumbar vertebrae were harvested for analysis. Bone tissue sections were stained with antibodies specific to prostate specific antigen (PSA) and pan-cytokeratin to detect and localize PCa cells within the bone marrow.

Methods:

Materials

Human PTH 1-34 was purchased from Bachem Inc. (Torrance, CA). Polyclonal antibodies for wide spectrum screening of pan-cytokeratin and PSA were purchased from Dako North America, Inc. (Carpinteria, CA), and BioGenex (San Ramon, CA), respectively. Enzyme-Linked Immunosorbent Assays (ELISA) for PSA were purchased from Anogen Inc. (Mississauga, Ontario, Canada). Unless otherwise stated, all other reagents were purchased from Sigma Chemicals, Co (St. Louis, MO).

Preparation of PTH

PTH 1-34 was reconstituted in 10 mM acetic acid/sterile PBS solution and stored at -80°C at a concentration of 400 μg/ml in siliconized tubes. For injections, the stock solution was diluted to 40 μg/ml in sterile PBS. Control animals were injected with equivalent volumes of acetic acid diluted in PBS.

Animals

Forty eight, six week old, male, C.B-17 SCID/Beige mice were purchased from Taconic Laboratories (Rockville, MD), and stabilized in the animal research facility at the Penn State College of Medicine. Mice were housed 5 per cage, maintained under specific pathogen-free conditions, in a continuously HEPA filtered room, maintained between 21-22°C, with 40-60% humidity on 12 hr light and dark cycles and given free access to food and water. Animal body weights were recorded before the start and then every other day during the experiment.

Experimental Design

At 8 weeks of age, mice were divided into 8 groups of equal average body weight. Groups 1-4 (n=6/group) served as treatment groups and were administered intermittent doses of PTH 1-34 (40 μg/kg). Groups 5-8 (n=6/group) served as vehicle control animals and were administered equivalent amounts of acetic acid diluted in PBS. All injections were administered subcutaneously, once per day for five consecutive days, followed by two days without injections. This treatment cycle was repeated 3 times, lasting 21 days. On day 23, animals were anesthetized

with a ketamine (20 mg/ml)/xylazine (2mg/ml) cocktail (5µl/g) and received intracardiac injections of established C4-2 PCa cell lines (250,000 in 20 µl). Groups 1 and 5 received WT cells, Groups 2 and 6 received control shRNA cells, encoding a non-specific scrambled shRNA sequence, Groups 3 and 7 received TRP-11 cell line, which had a decrease in TRPV6 expression of ~60%, and Groups 4 and 8 received TRP-12 cell line, which had a decrease in TRPV6 expression of ~63%. After PCa cell inoculation, animals received an additional 5 days of intermittent PTH 1-34 or vehicle injections. Eight weeks post-PCa cell injection mice were administered ketamine/xylazine cocktail and blood was collected via cardiac puncture before euthanasia via cervical dislocation. Gross necropsies were performed, followed by the extraction of the heart, lungs, spleen, liver, hind limbs and lumbar vertebrae. Soft tissues were weighed, fixed overnight in 4% formaldehyde and stored at 4°C in 70% ethanol. Hindlimbs and lumbar vertebrae were cleaned of all soft tissue, and fixed for 24-48 hrs. in 4% formaldehyde, then subsequently decalcified for 14-days in 10% EDTA, pH 8 (4°C) and stored at 4°C in 70% ethanol until paraffin embedding. This experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Pennsylvania State University College of Medicine.

Prostate Specific Antigen ELISA

Blood samples were kept at 4°C overnight to clot then centrifuged at 10,000 G for 10 min to separate out serum. Serum was analyzed for the presence of PSA using an ELISA according to the manufacturer's protocol (Anogen, Mississauga, Ontario, Canada). PSA serum amounts were calculated from a standard curve created from known concentrations of PSA. Animals were considered PSA positive if serum levels exceeded 4.5 ng/ml.

Immunohistochemistry

Localization of pan-cytokeratin and PSA positive cells within harvested tissues were performed by indirect immunohistochemistry employing polyclonal antibodies. Sections were deparaffinized and rehydrated by passage through xylene and graded alcohol series to water, then blocked using 3% (v/v) normal goat serum and incubated overnight (4°C) with primary antibodies specific to PSA and pan-cytokeratin. Bound antibodies were visualized using ImmPACT DAB Peroxidase substrate (Vector Labs, Burlingame, CA) as a chromagen. Sections were counter-stained with either Hematoxylin or Methyl Green. Antibody specificity was assessed by labeling tissue sections with secondary antibodies only.

To determine if there was a statistically significant difference in the number of PSA positive cells in animals from different treatment groups, PSA positive cells were counted in the marrow spaces of the proximal femurs, distal femurs, and proximal tibiae. Three 20X images were captured per animal using a Nikon EFD3 inverted light microscope and PSA positive cells per µm2 were quantified using NIS Elements-D 3.2 software (Melville, NY).

Statistical Analysis

All experiments were conducted in at least triplicate and repeated at least three times. Graphpad PRISM version 5.0 (GraphPad Software, San Diego CA) was used for statistical analysis. One-way ANOVA with a Bonferroni post hoc test was performed with experiments of multiple groups to compare all columns of data. Fisher's exact test was used to determine statistical

significance of PSA and pan-cytokeratin positive animals. All data are expressed as mean ± SD and were considered significant if $p \leq 0.05$.

Results:

End Stage Body and Tissue Weights

Throughout the experimental time course animals gained an average of 3.05 g (26.4 ± 0.85 g mean initial and 29.4 ± 1.145 g mean final body weight). At no time during the study were differences in mean body weight observed between treatment groups (Figure 1). Five mice did not survive the duration of the study. Two mice died the day of PCa cell inoculations, and three more died one week later. All animal deaths occurred, unexpectedly within PTH treatment groups. Two animals were injected with WT cells while the other was injected with control shRNA cells.

Wet weights of lung, liver, heart, and spleen were measured and there were no differences in mean weight of any of the organs between the different treatment groups (Figure 2). Two mice developed lung tumors which increased their lung weight and were considered outliers (Figure 2A). One animal with a lung tumor (WT/PTH treatment group) also had increased spleen (Figure 2C) and liver wet weight (Figure 2D).

Prostate Specific Antigen Serum Analysis

Serum PSA analysis identified two mice expressing PSA above the 4.5 ng/ml threshold (Figure 3). One mouse also had a prominent lung tumor. All other animals had PSA levels that ranged from 2.1-2.8 ng/ml and were considered PSA negative.

Immunohistochemistry

Lung tissue sections from the animals that developed lung tumors were probed with antibodies specific to PSA and while the majority of the tumor in each case was not PSA positive, areas surrounding vascular elements were positive for PSA. Lung tumor tissue sections exhibited a dense cellular morphology and very few alveoli and bronchi were present. In contrast, tumor negative lung tissues were also negative for PSA positive cells (Figure 4).

Serial sections of femurs, proximal tibiae, and lumbar vertebrae were probed with antibodies specific to PSA and pan-cytokeratin, markers for PCa epithelial cells. PCa cells were identified in small clusters (3-5 cells) within the marrow spaces of bone tissue sections in close proximity to growth plates and vascular elements. In regard to long bones, 100% of animals administered PTH and injected with WT C4-2 cells had PSA and pan-cytokeratin positive cells visualized in the marrow spaces of the distal femur and proximal tibia. In contrast, 20% of animals administered vehicle and injected with WT C4-2 cells had PSA and pan-cytokeratin positive cells visualized within the marrow spaces of long bones (Table 1 and Figure 5). These results are consistent with our previous report.

PSA and pan-cytokeratin positive cells were visualized in 60% of the animals injected with control shRNA cells and administered PTH while 50% of animals administered vehicle had PSA and pan-cytokeratin positive cells which was not statistically different. In contrast, only 20% of animals injected with TRP-11 cells and administered PTH had PSA and pan-cytokeratin positive cells observed in the long bones; this difference was statistically significant. However, TRP-12 colonization of bone was similar to animals injected with control shRNA cells (60%) (Table 1). PSA

positive cells were quantified by individual region (proximal femur, distal femur, and proximal tibia) to determine if there was a decrease in cell number when cells with reduced TRPV6 expression colonized bone. There was no difference observed (data not shown); there were also no differences observed when all three regions were combined (Figure 8).

In comparison to long bones, there were disparities in our observations of lumbar vertebrae. PSA and pan-cytokeratin positive cells were visualized in the marrow spaces of the lumbar vertebrae in 67% of animals administered PTH and injected with WT C4-2 cells. Conversely, 40% of animals administered vehicle and injected with WT C4-2 cells had PSA and pan-cytokeratin positive cells visualized within the marrow spaces of lumbar vertebrae. PSA and pan-cytokeratin positive cells were visualized in 100% of the animals injected with control shRNA cells and administered PTH while 58% of animals administered vehicle had PSA and pan-cytokeratin positive cells identified in the lumbar vertebrae. In contrast, only 40% of animals injected with TRP-11 and TRP-12 cells and administered PTH had PSA and pan-cytokeratin positive cells observed in the lumbar vertebrae (Table 2). There was no difference in the number of PSA and pan-cytokeratin positive animals between groups.

Group	PSA + Long Bones	Cytokeratin + Long Bones	Average % of PCa Positive Long Bones
WT/Veh	1/5	1/5	20%*
WT/PTH	6/6	6/6	100%
Con/Veh	3/6	3/6	50%
Con/PTH	3/5	3/5	60%
TRP-11/Veh	2/5	2/5	40%
TRP-11/PTH	1/5	1/5	20%*
TRP-12/Veh	5/6	6/6	92%
TRP-12/PTH	3/5	3/5	60%

Table 1. Number of animals per group with PSA and pan-cytokeratin positive cells present in marrow spaces of the femur and proximal tibia (Long bones). The right most column gives the average percentage of animals per group that were both PSA and pan-cytokeratin positive. * indicates significantly different compared to the WT/PTH group, $p \leq 0.05$.

Group	PSA+ Lumbar	Cytokeratin + Lumbar	Average % of PCa Positive Lumbar
WT/Veh	2/5	2/5	40%
WT/PTH	4/6	4/6	67%
Con/Veh	5/6	2/6	58%
Con/PTH	5/5	5/5	100%
TRP-11/Veh	2/5	3/5	50%
TRP-11/PTH	2/5	2/5	40%
TRP-12/Veh	3/6	6/6	75%
TRP-12/PTH	2/5	2/5	40%

Table 2. Number of animals per group with PSA and pan-cytokeratin positive cells present in marrow spaces of the lumbar vertebrae. The right most column gives the average percentage of animals per group that were both PSA and pan-cytokeratin positive.

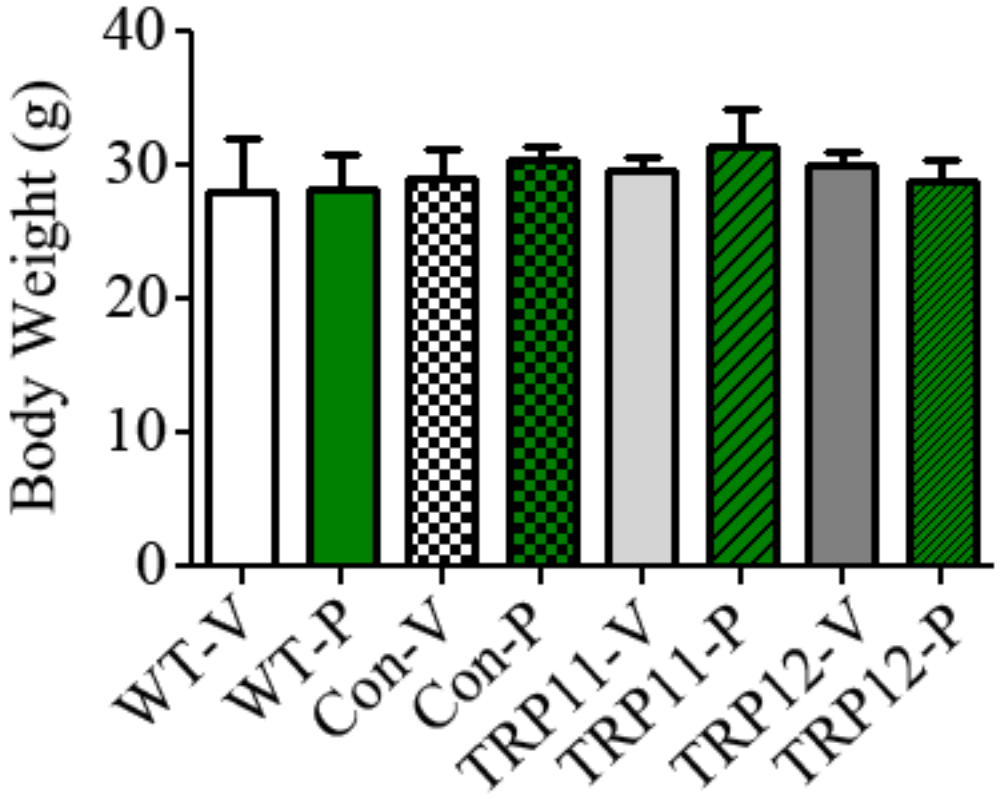

Figure 1. Terminal body weights did not differ among treatment groups. Animal weights were taken on the final day of the study. No differences were observed in animal weights between groups. The bars represent the mean weight (g) +/- SD per group. n=5-6/group, V= vehicle, P= PTH

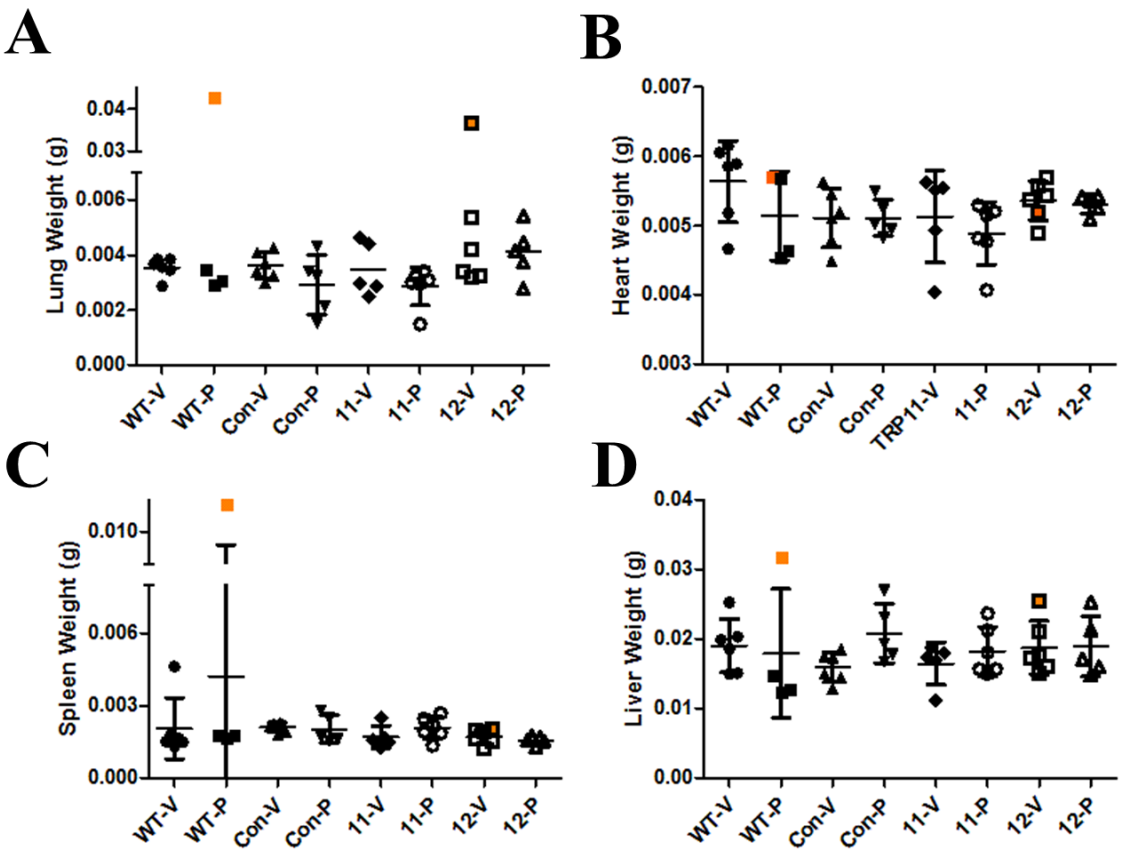

Figure 2. Scatter plots of soft tissue wet weights. A) Lung tumors were visualized in two animals, indicated by orange color, increasing their overall weight. No other differences in lung tissue weight were observed across groups. B) No differences in heart wet weight were observed among groups. C) No differences in spleen wet weight were observed, however, one animal with a prominent lung tumor also had an enlarged spleen (indicated in orange). D) No differences in wet weights of the right lobe of the liver were observed. n=5-6/group, V= vehicle, P= PTH

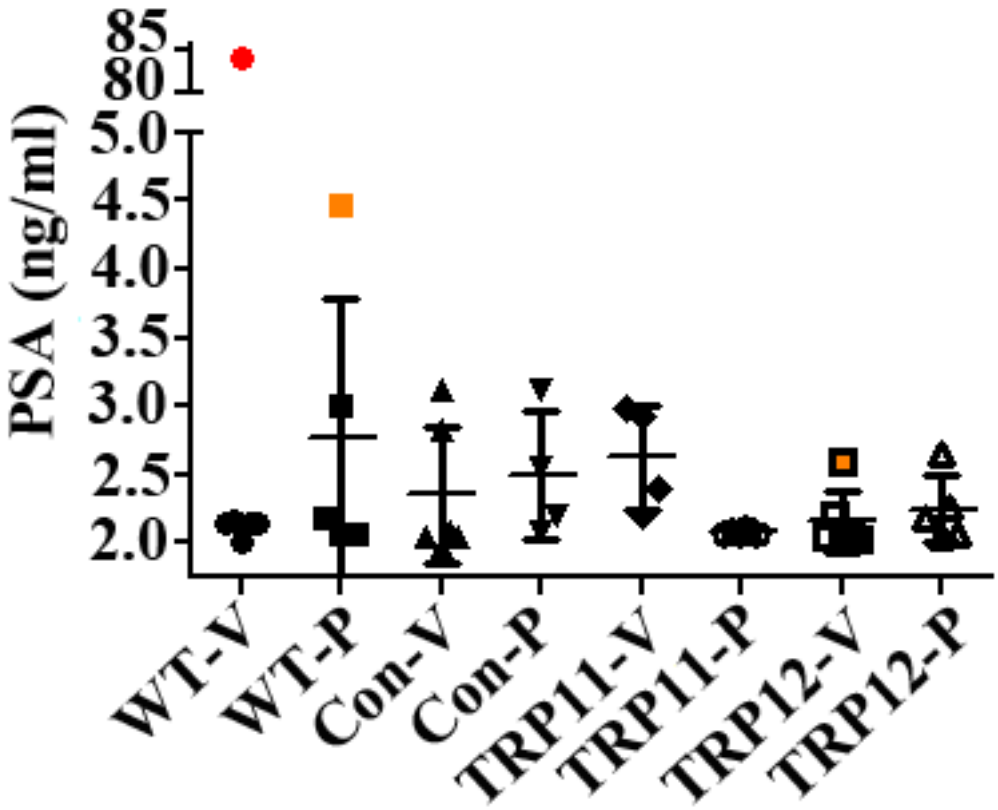

Figure 3. Scatter plot of PSA serum levels across treatment groups. Serum collected 8 weeks after PCa cell inoculation was analyzed by ELISA for PSA. Two animals were 4.5 ng/ml or above, suggesting they were PSA positive (indicated by the red circle and orange square). For the other animals, serum PSA levels ranged from 2.1-2.8 ng/ml and were considered PSA negative. Another animal with an observed lung tumor was not PSA positive (indicated by the black outlined orange square). There were no significant differences in serum PSA among groups. n=5-6/group, V= vehicle, P= PTH

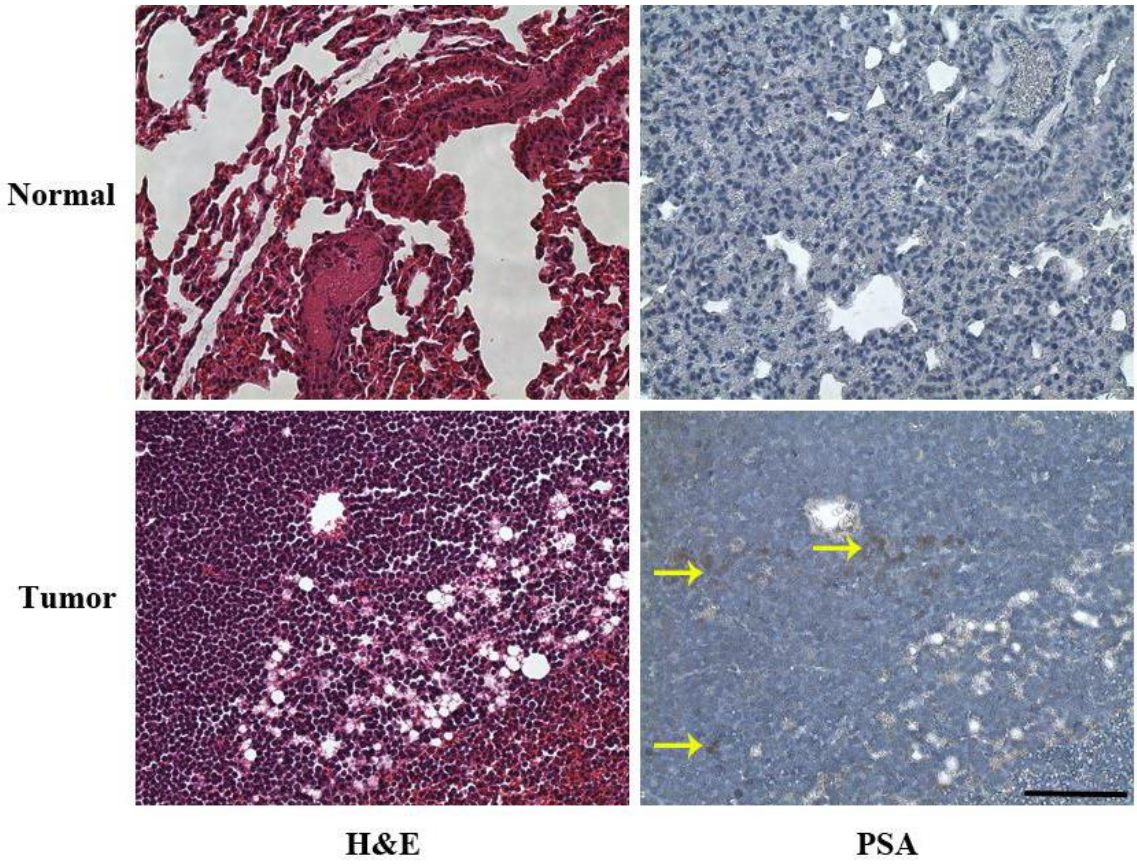

Figure 4. Lung tissue morphology. Paraformaldehyde fixed lung tissue was stained with hematoxylin & eosin (H&E) and probed with antibodies specific for PSA. 20X images representing normal tissue (top row) and tumorous tissue (bottom row). Positive staining is indicated by the yellow arrows. Scale bar = 0.1 mm (black bar bottom right image)

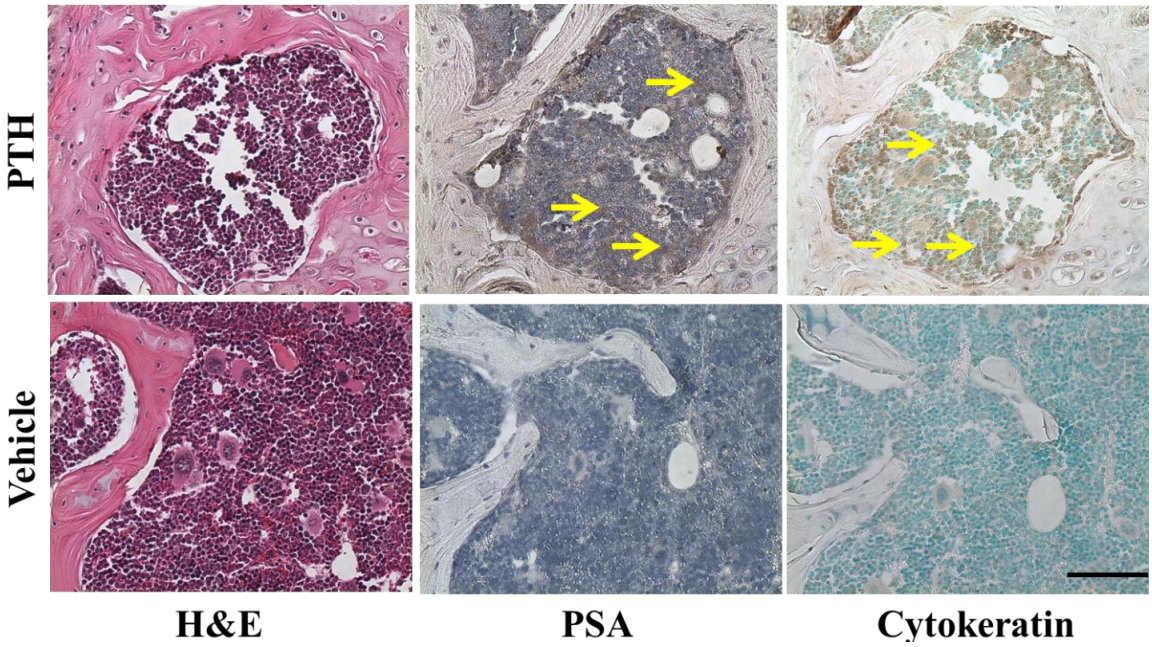

Figure 5. Representative PSA and pan-cytokeratin positive cell localization in long bones of mice inoculated with control shRNA cells. Long bone tissue sections were deparaffinized and rehydrated by passage through xylene and graded alcohol series to water, then blocked using 3% (v/v) normal goat serum and incubated overnight (4°C) with primary antibodies specific to PSA and pan-cytokeratin or stained with H&E. Bound antibodies were visualized using ImmPACT DAB Peroxidase substrate as a chromagen. Sections were counter-stained with either Hematoxylin or Methyl Green. Positive cells are marked by the yellow arrows indicating brown pericellular staining. The labels on the left indicate administration of PTH or vehicle (Veh). Scale bar = 0.1 mm (bottom right image)

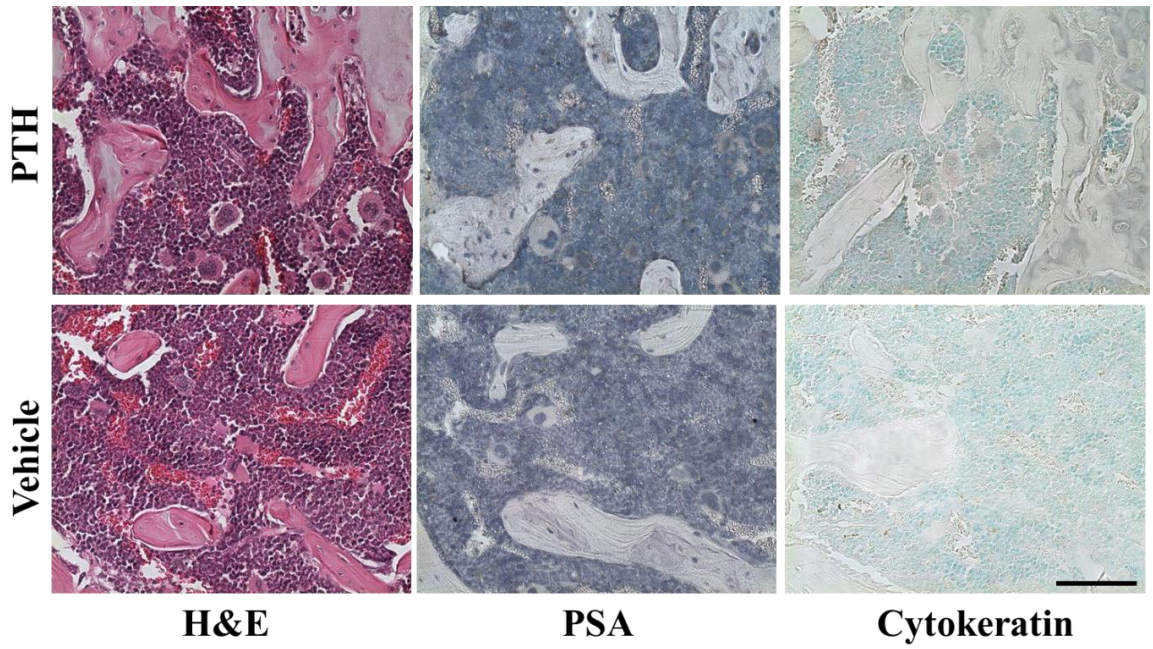

Figure 6. Representative PSA and pan-cytokeratin positive cell localization in long bones of mice inoculated with TRP-11 cells. Long bone tissue sections were deparaffinized and rehydrated by passage through xylene and graded alcohol series to water, then blocked using 3% (v/v) normal goat serum and incubated overnight (4°C) with primary antibodies specific to PSA and pan-cytokeratin or stained with H&E. Bound antibodies were visualized using ImmPACT DAB Peroxidase substrate as a chromagen. Sections were counter-stained with either Hematoxylin or Methyl Green. Positive cells are marked by the yellow arrows indicating brown pericellular staining. The labels on the left indicate administration of PTH or vehicle (Veh). Scale bar = 0.1 mm (bottom right image)

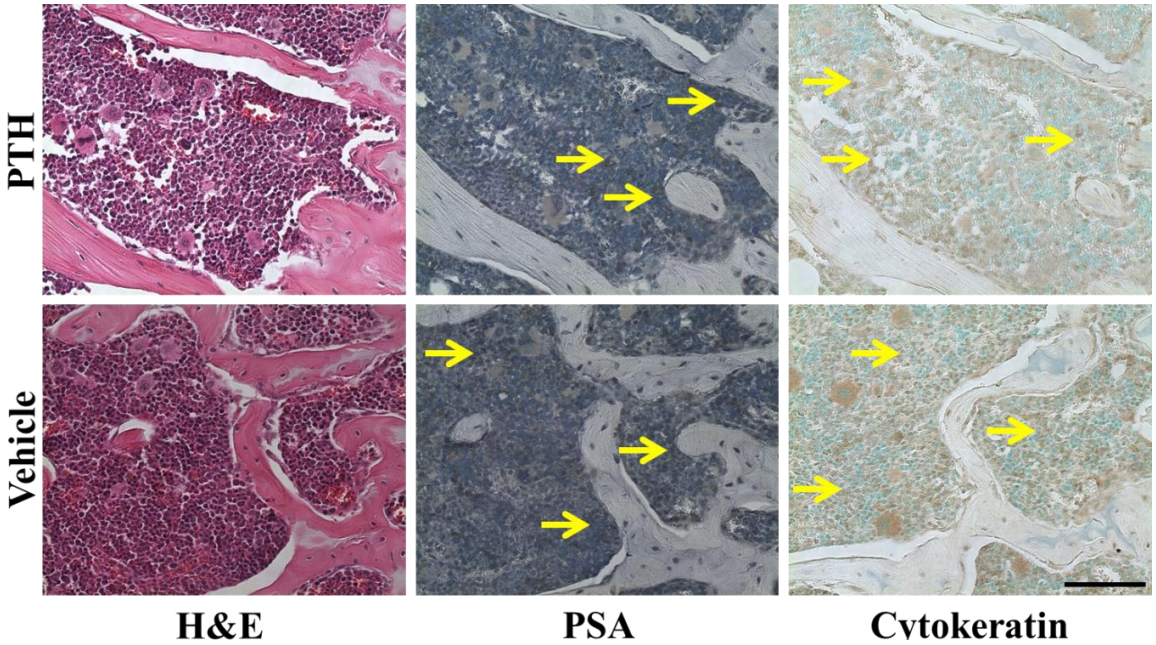

Figure 7. Representative PSA and pan-cytokeratin positive cell localization in long bones of mice inoculated with TRP-12 cells. Long bone tissue sections were deparaffinized and rehydrated by passage through xylene and graded alcohol series to water, then blocked using 3% (v/v) normal goat serum and incubated overnight (4°C) with primary antibodies specific to PSA and pan-cytokeratin or stained with H&E. Bound antibodies were visualized using ImmPACT DAB Peroxidase substrate as a chromagen. Sections were counter-stained with either Hematoxylin or Methyl Green. Positive cells are marked by the yellow arrows indicating brown pericellular staining. The labels on the left indicate administration of PTH or vehicle (Veh). Scale bar = 0.1 mm (bottom right image)

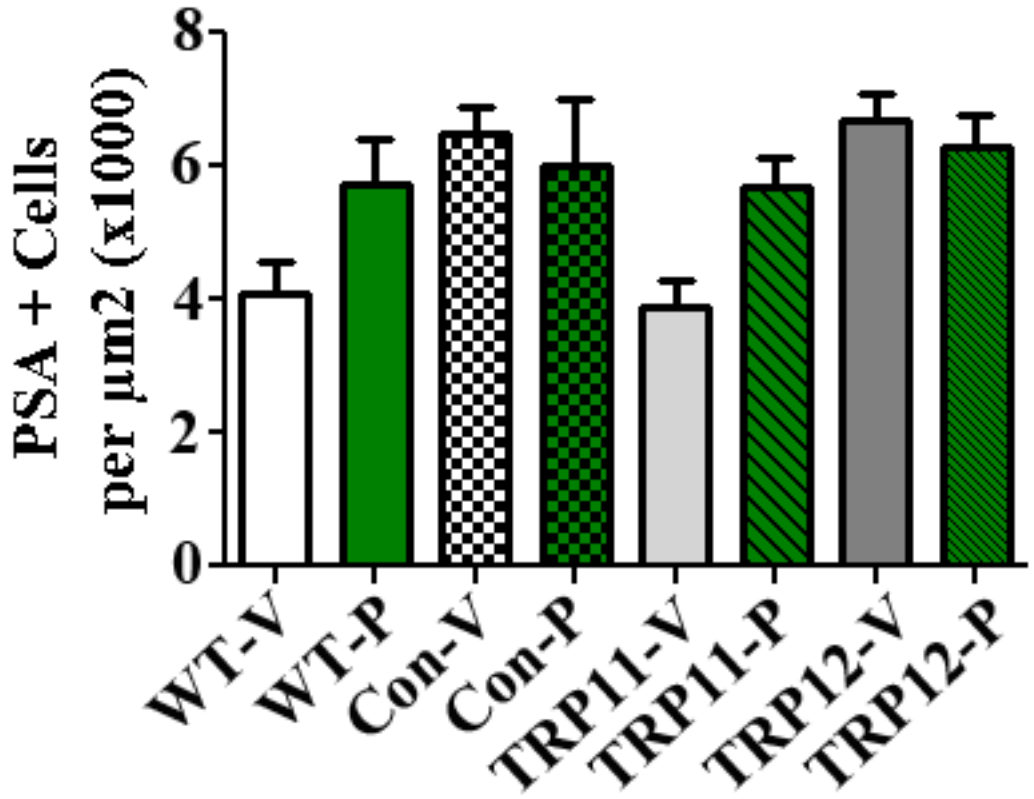

Figure 8. Number of PSA positive cells quantified in long bones did not differ among treatment groups. Images were taken of the proximal femur, distal femur, and proximal tibia of tissue sections probed with antibodies specific to PSA. PSA positive cells were quantified across three images from each region for each animal. Each bar represents the mean number +/- SD of PSA positive cells per μm^2 per treatment group.

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. None				☐Submitted ☐Accepted ☐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.

BIOGRAPHICAL SKETCH

NAME: Ronald R. Gomes, Jr.	POSITION TITLE: Assistant Professor
ERA Commons User Name rgomes	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
University of California San Diego, La Jolla, CA	B.A.	June, 1989	Psychology
University of California Berkeley, Berkeley, CA	M.A.	June, 1995	Human Biodynamics
University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX	Ph.D.	Dec.,1998	Integrative Biology/Physiology
University of Delaware, Newark, DE	Post-doc.	Feb., 1999-2004	Bone & Cartilage Developmental Biology

A. Personal Statement.

The goal of the proposed research is to identify how osteoblastic prostate cancer colonizes bone, from the circulation, and the contribution of parathyroid hormone in this process. To accomplish this task we will employ, over the next five years, an approach established in my laboratory, to identify mechanisms supporting parathyroid hormone-induced colonization and establishment of human osteosclerotic/osteoblastic prostate cancer in murine bone. Ground work for this project was initiated toward the end of my successful, individual NRSA supported postdoctoral fellowship when my present collaborator, Dr. Sikes and I began discussing approaches aimed at addressing his need for novel model systems that would allow him to identify and investigate mechanisms of prostate cancer bone colonization. Our efforts, despite the absence of significant funding, gathered momentum as I moved into a faculty position at Penn State's College of Medicine, and have thus far generated two related manuscripts and a book chapter. My broad background in psychology, physiology, molecular and cellular biology, and postdoctoral training in cartilage and bone biology, will suit me well in accomplishing the specific tasks detailed in the attached application. The present application, builds logically on my background, previous work, and will employ an established, productive collaboration that will be further enhanced by consultation with established investigators (Drs. Cullen, Donahue, Grubbs and Niyibizi), who provide additional expertise in statistics, bone biology, and cell biology, respectively. In summary, this biosketch demonstrates I am a highly motivated young investigator with an established record of project creativity, success, peer support, and that I possess the qualifications to take on and successfully accomplish the proposed project.

B. Positions and Honors.

Research and Professional Experience

1989-1991	Research Assistant I; Dept. of Kinesiology University of California, Los Angeles
1992	Analytical Chemist I, Syntex, Inc.; Palo Alto, California
1992-1995	Graduate Student Instructor, University of California, Berkeley
1999-present	Peer Reviewer; Journal of Applied Physiology
1999-2004	Postdoctoral Fellow, University of Delaware

2001-2004	NIH-Individual National Research Service Award Fellow
2004-present	Peer Reviewer; Journal of Cell Science
2005-	Assistant Professor, Department of Orthopaedics and Rehabilitation, Division of Musculoskeletal Sciences, Penn State College of Medicine
2006-	Director, Harrisburg High School Summer Research Fellowship Program
2006-	Member of the faculty of the Graduate School, Penn State College of Medicine
2006-	Faculty member, graduate program in Molecular and Cellular Biology
2007-	Appointment by the Dean to the Advisory Committee for Non-Matriculated Ed.
2007-	Faculty member, graduate program in Anatomy
2008-	Member, Penn State Cancer Institute
2009-	Appointment by the Dean to Council on Diversity, Penn State College of Medicine
2009-	Peer Reviewer; Acta Biomaterialia
2009-	Peer Reviewer; Clinical and Experimental Medicine
2009-	Peer Reviewer; DoD Orthopaedic Research Program

Awards:

1987-1989	Minority Biomedical Research Support Award, University of California San Diego
1993	Louise Patterson Award for Academic Excellence, University of California, Berkeley
1993	Travel Award, American Physiological Society/NIDDK, University of Texas, Houston
1994	Louise Patterson Award for Academic Excellence, University of California, Berkeley
1994-1998	American College of Sports Medicine, Graduate Scholarship for Minorities & Women
1995	Louise Patterson Award for Academic Excellence, University of California, Berkeley
1995-1997	NIH Grant #AR41995-02S1: IGF system, eccentric exercise, muscle atrophy and age
1996	Graduate Student Poster Award, The Integrative Biology of Exercise
1999	Poster Award, American Society for Bone and Mineral Research
1999-2002	University of Delaware Biotechnology Institute Postdoctoral Fellowship
2001	New Investigator Award: The Chemistry and Biology of Mineralized Tissues
2001-2004	NIH Individual National Research Service Award (F32 AG20078)
2004	NIH-P20-PR16458
2005	Special Capital Research Equipment Award: A MicroCT System for Longitudinal *Research Involving Small Animals*
2006	Junior Faculty Development Program, the Penn State College of Medicine
2006	Special Capital Research Equipment Award: *Room renovation support for MicroCT*