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:** Temple University of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine Calicat, MLA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** 2 - Identification of the Role of TULA-2 a Novel Phosphatase in Osteoclast Differentiation and Function

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

8. **Name of Principal Investigator for the Research Project:** Archana Sanjay, 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:

   $ 25,000

   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
Back	MD. Ph.D. Student	100	$25,000

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
Sanjay	Principal Investigator	50
Adapala	Post-Doctoral Fellow	20

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	None	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:
Regulation of bone remodeling by Tula-2 a novel phosphatase	XNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	October 2010	$ 1,912,500 (For 5 years)	$ Not Funded

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?

The Principal Investigator has moved to University of Connecticut Health Center in 2011. She is continuing to work on the project to generate data elucidating the role of the TULA-2 in bone remodeling. She will be submitting grant to NIH in the near future.

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

Yes____X_____ No_____

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

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

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

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

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

Yes_____ No_____X__

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

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

Yes_____X____ No_____

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

The project initiated a collaboration with Dr. Alexander Tsygankov, Depart of Immunology at Temple University. The purpose of the project was to understand the role of of novel protein in bone remodeling.

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_____ No_____X____

If yes, please describe the collaborations:

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.

Purpose of the study:

Osteoporosis and other bone-related maladies afflict 150 million Americans and constitute a significant burden to ever-rising health care cost. The proposed study was designed to evaluate the role of TULA-2, a novel phosphatase in bone remodeling in mouse skeleton. In addition, ex vivo experiments were performed to investigate the role of TULA-2 in differentiation and function of osteoclasts. The research proposed was to understand the basic biology behind bone resorption and help identify targets for therapeutic interventions to control bone loss.

Specific Aims:

1. Characterize the skeletal phenotype of the mice lacking of TULA-2 (DKO mice)

2. Examine the effect of absence of TULA 2 on osteoclast differentiation, survival and function in the control, and DKO osteoclasts generated in ex vivo cultures

Background:

The balance between bone formation and resorption is required to maintain homeostasis of the skeleton. Osteoblasts are responsible for bone formation whereas osteoclasts are the principal cells that resorb bone under normal and pathological conditions. Impaired functionality of either of these cell types, results in bone disorders such as osteopetrosis, osteoporosis, and Paget's disease. The balance of osteoblasts and osteoclasts in bone remodeling is important in skeletal development and maintenance for the following three reasons: First, bone remodeling is necessary to repair fatigue damage since bone is constantly under repetitive cyclic loading, which eventually causes fatigue. Loading cycles produce tiny cracks within the bone, and if these tiny cracks are not resolved, they can lead to structural fractures. Second, the adaptation of bone material to any mechanical stresses is an important reason for bone remodeling. Bone remodeling offers the skeleton the capability to handle the stresses and strain placed on it. Finally, bone remodeling is important in the regulation of calcium. Bone is a major reservoir for calcium, and when serum calcium levels become too low, bone resorption by osteoclasts releases bone calcium to balance the demand.

Osteoclast differentiation and function

Osteoclasts are the only cell type in the body that can resorb bone (1). These cells are formed by the fusion of precursor cells of the hematopoietic monocyte-macrophage lineage. Two essential factors, macrophage colony-stimulating factor (M-CSF) and Receptor Activator of Nuclear factor kB (RANKL) are needed for the hematopoietic precursor cells to differentiate into osteoclasts. M-CSF and RANKL are produced by osteoblasts and stromal cells. In order to resorb bone, osteoclasts attach firmly to the bone matrix, thereby isolating the environment between the cell and the underlying surface. The cell then secretes onto this isolated bone surface a mixture of acid and proteinases that collectively degrade the inorganic and organic components of bone (1).

Protein Tyrosine Phosphatases in Osteoclast Signaling

Molecular studies indicate that phosphorylation of proteins on tyrosine residues is critical for the formation and function of osteoclasts. Tyrosine phosphorylation of protein is a reversible

process that is regulated by the intricate relationship between protein tyrosine kinases (PTK) and protein tyrosine phosphatases (PTP). PTKs and PTPs act like molecular on and off switches and help modulate differentiation and function of bone cells.

The well-established roles of tyrosine phosphorylation in regulating bone structure and of PTPs in antagonizing tyrosine kinase activity suggest that PTPs participate in regulating bone structure (2). The importance of PTPs as a class of molecules on osteoclast formation and function is demonstrated by the ability of the broad-spectrum inhibitors of PTP activity orthovanadate, alendronate and phenylserine oxide to inhibit in vitro formation of osteoclast and bone resorption by osteoclasts (2). This project focused on determining the biological role and molecular basis of action of the TULA, a novel phosphatase protein family, which has never been studied in osteoclasts.

TULA Family, a Novel Family of Regulatory Proteins Possessing a Phosphatase Domain
The TULA family members, TULA and TULA-2 are encoded on different chromosomes, were discovered a few years ago by several groups(3-5). Notably, TULA-family proteins belong to the superfamily of histidine phosphatases, sharing a conserved catalytic core centered on a reactive histidine residue. TULA proteins clearly differ from classical cysteine PTPs, such as SHP-1, which is involved in the regulation of osteoclasts. TULA-family proteins exhibit a unique architecture, featuring the ubiquitin-associated (UBA), Src-homology 3 (SH3), and phosphatase domains. In spite of a substantial homology (~60% of identity+similarity), TULA-1 and TULA-2 are quite different. First, TULA-2 is ubiquitously expressed in mammalian cells, whereas TULA-1 is expressed mostly in lymphocytes and possibly, in mast cells. Second, TULA-2 is an active PTP, while the phosphatase activity of TULA-1 is drastically lower (3-5).

Experimental Plan:

Specific Aim 1:
Characterizing the skeletal phenotype of the mice lacking of TULA 2

Mice:
All the mice used in this aim were established on the B6X129 background. Mice deficient in TULA and TULA-2 genes henceforth called double knock out (DKO) mice were obtained from Dr. Alexander Tsygankov's group at Temple University. We employed the DKO and the counterpart control mice to determine the *in vivo* effect of the absence of TULA-2 on osteoclast function during basal conditions of bone modeling. For each end point per genotype a minimum of six independent samples were assessed.

Methods for characterizing the skeletal phenotype:
Skeletal Analysis: High-resolution micro computated tomography (microCT) was performed as described previously (6). Briefly, images of long bones from 8-week old male mice were acquired using a Skyscan 1172, 12 MPix model (Microphotonics, Allentown, PA). Proximal tibiae and distal femora were scanned with the source voltage 59 kV, a source current of 167, an image pixel size of approximately 5.75um and a 0.5mm aluminum filter. Imaging started

at the distal end of the femur or the proximal end of the tibiae and included approximately 7 mm (1,335 slices) of the total bone length. Using the CTAn software, trabecular bone was separated from cortical bone with a region of interest tool. Trabecular morphometric traits were computed from binarized images using direct 3D techniques that do not rely on prior assumptions from the underlying structures. The volume of interest for trabecular microarchitectural variables was bounded to the endocortical margin, starting 1.5 mm from the proximal tibial condyles in the direction of the metaphysis, and then extending from this position for 250 slices (1.5 mm). Upper and lower thresholds of 255 and 80 were used to delineate each pixel as ‘‘bone’’ or ‘‘nonbone,’’ and trabecular bone volume per total volume (BV/TV), mean trabecular thickness (Tb.Th.), mean trabecular number (Tb.N.), and mean trabecular separation (Tb.Sp.) indices were computed.

For histological and histomorphometric analysis, 12-week old mice were used. To measure dynamic bone formation parameters, mice were injected with calcein (30 mg/kg body weight) 10 and 3 days before sacrifice. Tibiae and femora were dissected and fixed in 3.7% formaldehyde in phosphate-buffered saline, preserved in 70% ethanol, and embedded in methlymethacrylate resin. Sections (5 μm) were deplasticized and stained with von Kossa or were left unstained for the measurement of calcein labeling. Some sections were processed for TRAP staining as per manufacturer's instructions (Sigma). For histomorphophometric analysis, to assess changes in bone structure and remodeling, tibial sections were measured in the proximal metaphysis beginning 340 μm below the chondro-osseous junction of the secondary spongiosa using image analysis software (BIOQUANT Osteo II, Bioquant Image Analysis Corp., Nashville, TN) as described by Parfitt et al. (7). Osteoblast numbers per bone surface (Ob.N./B.S.) on trabecular surfaces was determined in toluidine blue stained sections. Osteoclast numbers per bone surface (Oc.N./B.S.;$TRAP^+$ cells) and osteoclast surfaces on bone surfaces (Oc.S./B.S.) were determined on trabecular surfaces in the secondary spongiosa of TRAP stained sections. Bone formation rate was calculated from calcein labeled sections.

Results:

Gross radiological analysis of long bones from 12 week-old DKO mice showed decreased bone density in the femur and tibia as compared to the age-matched control WT mice (Figure.1A).

MicroCT analysis of hind limb long bones indicate that bone volume was significantly decreased in the DKO mice (Figure 1B). In the DKO long bones, although trabecular separation was not affected, a significant decrease in trabecular thickness and number was observed, compared to WT samples (Figure 1 C). Histological examination of von Kossa-stained sagittal sections of the proximal tibia from age-matched WT and DKO mice indicated a significant decrease in mineralized trabecular bone in DKO mice as compared to WT (Figure 2A). This result was confirmed with histomorphometry of von Kossa-stained bone sections (data not shown). Also, no differences were observed in cortical thickness (data not shown). A decrease in bone volume can result from either a decrease in bone formation by osteoblasts or an increase in bone resorption by osteoclasts. Therefore, in order to determine which cell type contributed to the decreased bone volume in DKO mice, we compared the

number and activity of both osteoblasts and osteoclasts in DKO and WT mice. Toulidene blue staining of the long bones indicated that the numbers of osteoblasts were comparable in WT and DKO samples (Figure 2 C). Similarly, bone formation rate as measured by calcein double labeling was also comparable between the WT and DKO mice (Figure 2 D). On the other hand, quantification of TRAP stained cells in the cancellous bone indicated that there was a two-fold increase in osteoclast numbers and osteoclast surface/bone surface, in DKO mice as compared to WT (Figure 2 B, E, and F).

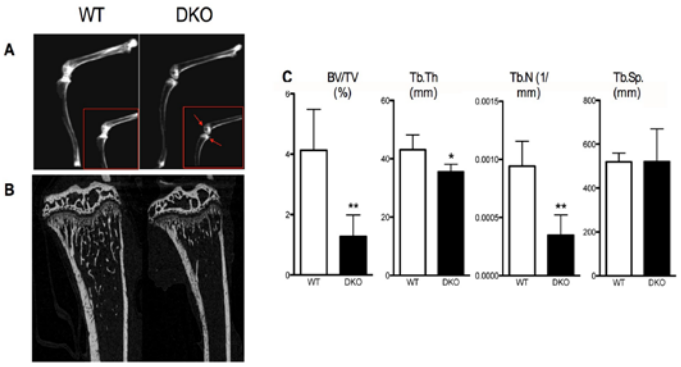

Figure 1. DKO mice have decreased bone volume. **A**. Long bones from 12 wk old WT and DKO mice were X-rayed to qualitatively assess bone density. Insets in lower right of each panel shows the femur-tibia junction; arrows indicate sites with a qualitative decrease in bone density in the DKO samples. **B.** Micro-CT analysis of tibia from WT and DKO mice shows representative sagittal sections of tibia. **C.** Histograms show the trabecular parameters of tibia. N= 4 per genotype, 8 week old male mice, Mean ± SD shown; *$p<0.05$, **$p<0.01$ compared to WT

Specific Aim 2 .
Examine the effect of absence of TULA 2 on osteoclast differentiation, survival and function in the control, and DKO osteoclasts generated in ex vivo cultures

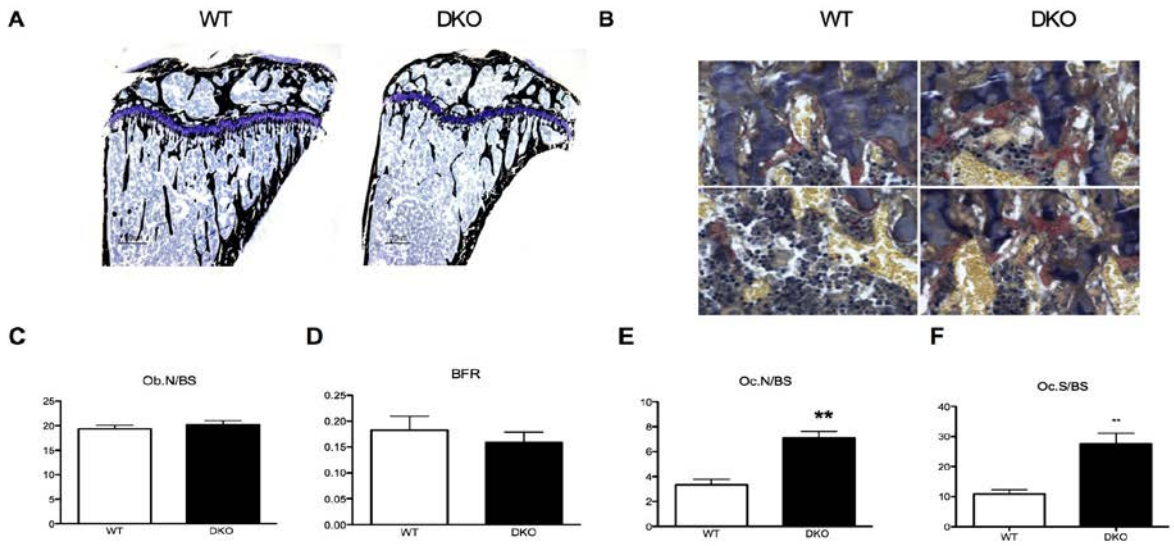

Figure2. Absence of TULA proteins results in decreased bone volume due to increased osteoclast numbers. A. Representative histological sections of proximal tibiae of 12-week old WT and DKO mice stained with Von Kossa and counterstained with Toluidine Blue shows decreased mineralized bone in the metaphyseal trabecular region of DKO samples. Images were taken using a 10x objective. **B.** Tibial sections from 12-week old WT and YF were stained for TRAP (400x mag). Two different sections for each genotype are shown. **C.** Quantification of number of osteoblasts/bone surface (Ob.N./BS), and **D.** dynamic parameters of bone formation were assessed using tibias harvested from 8-week old WT and YF mice that were injected with calcein as described in the methods. Bone formation rate/bone surface (BFR/BS, $\mu m^2/\mu m/day$) is shown. **E**. Osteoclast numbers/bone surface (Oc.N./BS %), and **F.** osteoclast surface/bone surface (Oc.S./BS). N= 4 mice per genotype; Mean ± SD shown; **$p<0.01$, compared to WT.

<u>Methods for characterizing the cellular phenotype:</u>

Cell culture: For generation of osteoclast-like cells (OCLs), bone marrow was isolated from tibia and femur of 4-6week old mice. Following overnight incubation, the non-adherent cells were plated at 2.5 x $10^5/cm^2$ in α-MEM medium containing 10% FBS, and 20 ng/mL M-CSF. Subsequently cells were treated with M-CSF (20 ng/ml) and RANKL (50 ng/ml) for additional 5-6 days. For some experiments, OCLs were also generated by the co-culture method as previously described (8,9). Briefly, mouse primary osteoblastic cells were obtained from 1-day-old mouse calvaria by enzymatic digestion. Bone marrow cells (10^5 cells/cm^2) were co-cultured with calvarial cells (2 x 10^4 cells/cm^2) on tissue culture plates or

collagen gel-coated plates in the presence of 10 nM 1, 25-dihydroxy vitamin D3 and 1 μM prostaglandin E2 (Sigma).

RT-PCR analysis: The expression levels of osteoclast differentiation and fusion markers were analyzed by quantitative real-time PCR. Sequences for the primers and details of the procedure are described previously (8,9).

Determination of Serum Collagen Telopeptide: Serum was prepared from blood collected by cardiac puncture. Concentrations of C-telopeptide (CTX), a degradation product of type-I collagen, in serum of 12-week-old mice were determined using the Rat Laps ELISA (Osteometer BioTech A/S, Herlev, Denmark).

Pit Formation Assay: Functionally active OCLs were formed in co-cultures, as described above. An aliquot of the crude OCL preparation was transferred onto hydroxyapatite coated dishes and cultured for an additional 48 h. The resorbed area was measured using an image analysis system linked to a light microscope (Bioquant Osteo II). A similar aliquot was cultured onto a 24 well dish and cells were TRAP stained after 6 h.

Survival Assay: Following differentiation with MCSF and RANKL on day 5, one set of 96-well plate was fixed with 10% formaldehyde in PBS. Other sets were either kept i□α-MEM or were treated with RANKL (50ng/mL) for 24 hrs. Cells were fixed and TRAP stained using a commercial kit (Sigma, St. Louis). Total number of TRAP+ multinucleated cells were counted and expressed as percentage of the number of cells at the start of the experiment on day 5.

Results:

Next, in order to determine the intrinsic effects of the loss of TULA-2 during osteoclast differentiation, bone marrow cells from mice deficient in TULA and TULA-2 (DKO) were Used. Bone marrow precursors were differentiated into mature osteoclasts in the presence of

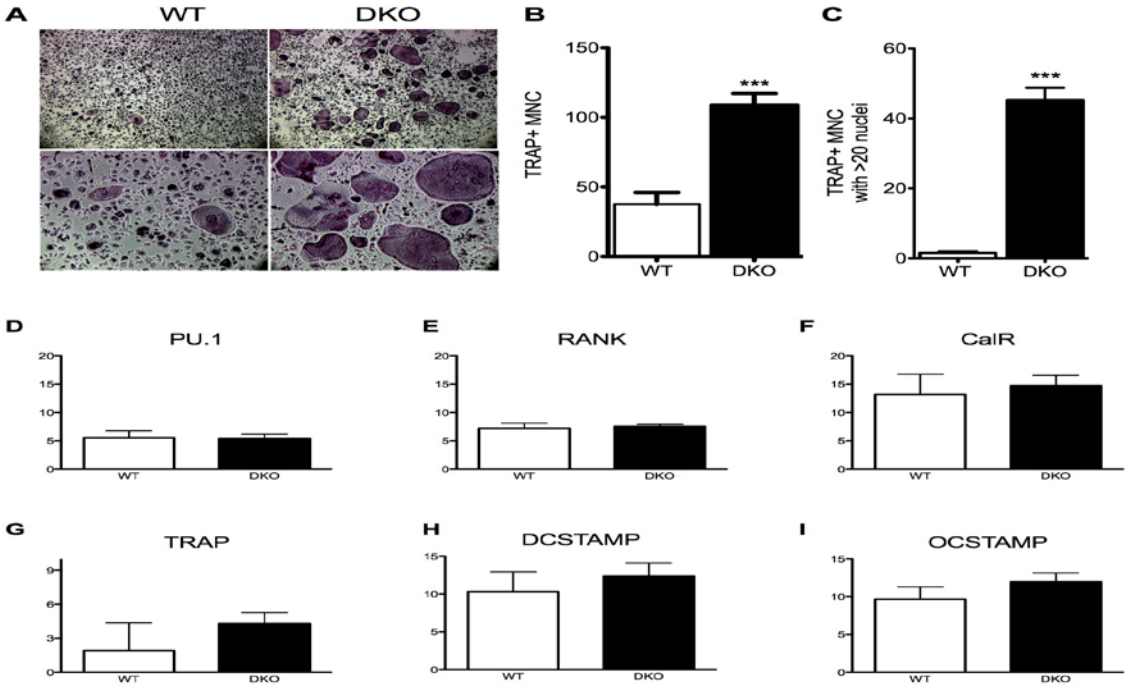

Figure 3. Absence of TULA proteins results in increased osteoclast numbers in ex vivo cultures. A. Non-adherent bone marrow precursors were cultured in the presence of M-CSF for 2 days and for additional 3 days in the presence of M-CSF and RANKL. TRAP staining showed increased in numbers of TRAP + cells in DKO cultures on day 5. Photomicrographs show TRAP stained OCLs at 4x (upper panels) and 20x (lower panels) magnification. **B.** The numbers of TRAP+ multinucleated osteoclasts (MNCs) in the DKO cultures (black bars) were significantly greater than in WT cultures (white bars) at day 5**. C.** Cells with more than 20-50 nuclei were increased in numbers DKO cultures. **D-I.** Bone marrow cells were cultured in the presence of MCSF (20 μg/ml) and RANKL (50 μg/ml) for 5 days and expression of the following was examined by real-time PCR analysis: osteoclast differentiation markers **D**. PU.1; **E**. RANK; **F**. CalcR; and **G.** TRAP; and fusion markers **H.** DC-STAMP and **I.** OC-STAMP was determined by real-time PCRs**.**

M-CSF and RANKL. Following TRAP staining, the total number of TRAP positive cells and the number of osteoclasts with more than 20 nuclei were counted. As indicated in Figure 3 A and B, both total numbers of cells and cells with 20+ nuclei were significantly increased in DKO cultures. To test if the observed increase in osteoclasts in the DKO cultures is due to

enhanced differentiation or fusion of osteoclast precursor cells, expression of osteoclast differentiation markers was examined by real-time PCR analysis. No significant differences were found between the expression levels of PU.1, RANK, Calcitonin Receptor and TRAP, which are used as differentiation markers (Figure 3 D-G). Similarly, expression of DC-STAMP and OC-STAMP, fusion markers, was comparable between the WT and DKO cells (Figure 3 H and I).

An increase in osteoclast numbers could be due to enhanced ability of cells to survive. Therefore, we next examined the ability of mature osteoclasts to survive in cultures in response to MCSF or RANKL. Two sets of osteoclast cultures were derived from bone marrow, as described above. Upon the appearance of large multinucleated cells, one set was fixed and the second set was incubated in serum-free media supplemented MCSF or RANKL for an additional 24 hours. Cells were then TRAP-stained and the percent survival was calculated by normalizing the number of TRAP-stained osteoclasts counted after the additional 24 hour incubation period to the number of osteoclasts fixed immediately after the appearance of osteoclasts. Comparable survival was observed between WT and DKO cultures under all conditions (Figure 4).

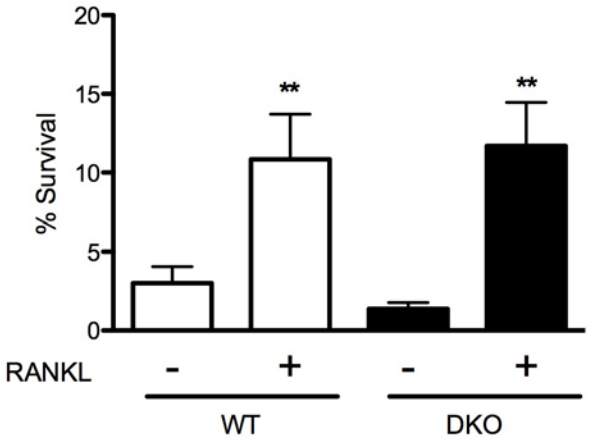

Figure 4. Osteoclast survival in in vitro assay is unaffected in the absence of TULA-2. Osteoclasts were derived from the bone marrow as described in the methods. Osteoclasts were either untreated or treated with RANKL (50 ng/ml) for 24 h. After treatment, cells were fixed and TRAP stained**.** Histogram represents the data expressed as percentage of initial number of TRAP + MNCs surviving after 24 hours. (WT white bars; DKO black bars). * $p < 0.05$ as compared to untreated cells.

We next examined the effect of absence of TULA-2 on osteoclast function. Serum levels of the C-terminal collagen telopeptide (CTX), a serum biomarker of osteoclast activity, were increased in DKO mice as compared to WT mice (Figure 5A). To confirm this defect of DKO osteoclast function, we then examined bone resorption using *in vitro* pit formation assay. DKO and WT OCLs were generated by co-culture with osteoblasts on collagen gel, as described previously (8,9). After 5 days in culture, a portion of the crude OCL preparation was placed on hydroxyapatite-coated tissue culture plates for an additional 48 h. The resorbed area was quantified using image analysis and normalized to the number of OCLs. DKO OCLs resorbed more surface area/cell than the WT OCLs (Figure 5B), confirming the cell autonomous nature of the observed defect in osteoclast function. Thus, the decreased bone volume in the adult DKO mice and increased bone resorption *in vivo* and *in vitro* is, at least in part, due to impaired osteoclast function. Taken together, these results suggest that bone resorption under basal conditions is affected in DKO mice.

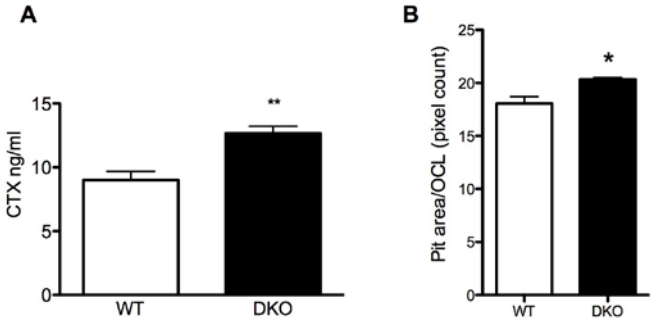

Figure 5. Absence of TULA-2 in osteoclasts results in increased activity both *in vivo* and *in vitro* A. Measurement of serum collagen telopeptide (CTX) demonstrated increased osteoclast activity in the DKO mice (N=4 male mice per genotype; Mean ± SD shown; **p<0.01, compared to WT). **B.** Photomicrographs of the resorbed area. C. Bar graph demonstrating pit formation activity of DKO osteoclasts. Osteoclasts were generated by co-culture method, as described in the methods. After removing adherent cells, resorbed area was quantified and normalized for the number of osteoclasts. Data are presented as mean SE (n=9). **p<0.001 compared to the WT samples.

Summary of the Project :

Precise regulation of osteoclast signaling is critical for maintaining bone homeostasis. Therefore, proteins playing important roles in osteoclast signaling represent potential therapeutic targets for treating various diseases that disrupt proper hemostasis. Signaling mechanisms in osteoclasts are complex and very far from being understood. It is clear, however, that protein tyrosine phosphorylation plays a central role in the activation of osteoclasts. Protein tyrosine phosphorylation is regulated by both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). The role of PTPs in osteoclast differentiation and function has received less attention and is less understood than the role of PTKs. TULA-2 is a recently discovered phosphatase of the TULA family in osteoclasts and provides evidence that it exerts a positive effect on skeletal remodeling. Therefore, TULA-2 represents a novel and potentially important target for therapeutic intervention. Our results

show that the absence of TULA proteins in mice results decreased bone volume largely due to increased osteoclast numbers and function. These results suggest that TULA-2 negatively regulates osteoclast formation and function.

Understanding of the biological functions of TULA-family proteins and their molecular mechanisms should greatly advance both basic knowledge of osteoclast function and therapeutic modalities in bone-related disorders.

References;

1. Teitelbaum SL 2000 Bone resorption by osteoclasts. Science **289**(5484)**:**1504-1508.
2. Granot-Attas S, Elson A 2008 Protein tyrosine phosphatases in osteoclast differentiation, adhesion, and bone resorption. Eur J Cell Biol **87**(8-9)**:**479-490.
3. Tsygankov AY 2008 Multidomain STS/TULA proteins are novel cellular regulators. IUBMB Life **60**(4)**:**224-231.
4. Tsygankov AY 2009 TULA-family proteins: an odd couple. Cell Mol Life Sci **66**(17)**:**2949-2952.
5. Tsygankov AY 2012 TULA-family proteins: A new class of cellular regulators. J Cell Physiol.
6. Brennan T, Adapala NS, Barbe MF, Yingling V, Sanjay A 2011 Abrogation of Cbl-PI3K interaction increases bone formation and osteoblast proliferation. Calcified tissue international **89**(5)**:**396-410.
7. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR 1987 Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res **2**(6)**:**595-610.
8. Adapala NS, Barbe MF, Langdon WY, Tsygankov AY, Sanjay A 2010 Cbl-phosphatidylinositol 3 kinase interaction differentially regulates macrophage colony-stimulating factor-mediated osteoclast survival and cytoskeletal reorganization. Annals of the New York Academy of Sciences **1192:**376-384.
9. Adapala NS, Barbe MF, Langdon WY, Nakamura MC, Tsygankov AY, Sanjay A 2010 The loss of Cbl-phosphatidylinositol 3-kinase interaction perturbs RANKL-mediated signaling, inhibiting bone resorption and promoting osteoclast survival. The Journal of biological chemistry **285**(47)**:**36745-36758.

Abstracts presented at Local and National Meetings

1.Back, S, Adapala, S, Holland, D, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Temple University Research Symposium, Philadelphia, PA 2009.

2.Back, S, Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. American Physician Scientists Association and American Association of Physicians Joint Meeting, Chicago, IL, 2010.

3.Back, S, Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Dawn Marks

Research Day, Temple University, Philadelphia, PA 2010.

4.Back, S, Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay TULA-2: A Novel Tyrosine Phosphatase, Regulates Syk Phosphorylation in Osteoclast Signaling Pathways. American Physician Scientists Association and American Association of Physicians Joint Meeting, Chicago, IL, 2011.

5.Back, S, Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay TULA-2: A Novel Tyrosine Phosphatase, Regulates Syk Phosphorylation in Osteoclast Signaling Pathways. Central Society for Clinical and Translational Research Poster Session, Chicago, IL, 2011.

6.Back, S, Adapala, S, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Orthopedic Research and Education Foundation/Orthopedic Research Society Philadelphia Region Resident Research Symposium, University of Pennsylvania, Philadelphia, PA, 2012.

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. TULA-2, a novel histidine phosphatase, regulates bone remodeling by modulating osteoclast function.	Back SH, Adapala NS, Barbe MF, Carpino NC, Tsygankov AY, Sanjay A.	Cell Molecular Life Sciences	September 2012	☐Submitted ☐Accepted ✓Published

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

Yes_____X____ No_____

If yes, please describe your plans:

We plan to submit a review article detailing the role of phosphatase in bone remodeling.

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 Archana Sanjay, Ph.D.	POSITION TITLE Assistant Professor, Department of Othopaedic Surgery, New England Musculoskeletal Institute University of Connecticut Health Center, Farmington CT, 06030
eRA COMMONS USER NAME asanjay	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Jiwaji University, Gwalior, India	B.Sc.	1985	Biology
Devi Ahilya University, Indore, India	M.Sc.	1987	Biochemistry
Bombay University, India	Ph.D.	1994	Biochemistry

A. Positions and Honors

Employment

1994-1996 Post Doctoral Fellow, NYU Medical Center, New York, NY.
1997-1998 Postdoctoral Associate, Yale University School of Medicine, New Haven CT.
1998-2001 Postdoctoral Fellow, Yale University School of Medicine, New Haven CT.
2001-2004 Associate Research Scientist, Yale University School of Medicine, New Haven CT.
2005- May 2011 Assistant Professor (Approved for Tenure), Temple University School of Medicine, Philadelphia, PA.
2005- May 2011 Assistant Professor (Approved for Tenure), Thrombosis Research Center, TUSM, Philadelphia, PA.
2011 June-Present Assistant Professor (Tenure track), University of Connecticut Health Center, Farmington CT

Honors and Awards

1984 Merit List, Jiwaji University, Gwalior, India
1986 Merit List, Devi Ahilya University, Indore, India
1988-1993 Research Fellowship Council of Scientific and Industrial Research (CSIR), Gov. of India
1997-2000 Postdoctoral Fellowship, Arthritis Foundation, Atlanta, Georgia, USA
1999 American Society of Bone and Mineral Research (ASBMR), Travel Award
2001 International Bone Mineral Society (IBMS), Travel Award
2003 International Bone Mineral Society (IBMS), Travel Award
2007 European Calcified Tissue Society (ECTS), Travel Award
2009 Young Investigator of the Year Award from American Society for Bone and Mineral Research to Dr. Naga Suresh Adapala (Post-doctoral fellow in my lab)
2009 Travel Award from American Society for Bone and Mineral Research to Ms. Tracy Brenan (Graduate Student in my lab)
2010 Travel Award from American Society for Hematology to Ms. Claudia Lorena Buitrago (Graduate Student in my lab)
2010 Travel Award from American Society for Bone and Mineral Research to Dr. Naga Suresh Adapala (Post-doctoral fellow in my lab)

2011 Travel Award from American Society Physician Scientist to Steven H. Back (MD. Ph.D. student in my lab)

C. Selected Peer-reviewed Publications (9 Selected from 40 peer-reviewed publications)

1. **Sanjay A,** Miyazaki T, Itzstein C, Purev E, Horne WC and Baron R. Identification and Functional Characterization of a SrcSH3 domain-binding Site on Cbl. FEBS J. 2006 273:5442-5456. PMID: 17094785
2. Destaing O, **Sanjay A,** Itzstein C., Horne W, De Camille P. and Baron R. The kinase activity of c-Src is essential in the regulation of podosome dynamics and their organization in osteoclasts. Mol. Biol. Cell, 9, 394-404, 2008. PMID: 17978100
3. **Sanjay, A.,** Nakajima, A., Chiusaroli, R., Adapala, N S, Itzstein, C., Horne WC and Baron R. The loss of Cbl-b increases osteoclast bone-resorbing activity and induces osteopenia. J Bone Miner Res. 24:1162-72, 2009. PMCID: 26976221
4. Daniel JL, Dangelmaier CA, Mada S, Buitrago L, Jin J, Langdon WY, Tsygankov AY, Kunapuli SP, **Sanjay A.** Cbl-b is a novel physiologic regulator of glycoprotein VI-dependent platelet activation. J Biol Chem. 285: 17282-9, 2010. PMCID: 2878491
5. Adapala NS, Barbe MF, Langdon WY, Tsygankov AY, **Sanjay A.** Cbl-PI3K Interaction Differentially Regulates MCSF-mediated Osteoclast Survival and Cytoskeletal Reorganization. Ann N Y Acad Sci. 1192: 376-384, 2010. PMCID: 2978603
6. Adapala N, Barbe MF, Langdon WL, Nakamura M, Tsygankov AY, **Sanjay A.** The loss of Cbl-PI3Kinase interaction promotes survival but decreases osteoclast bone-resorbing activity. J. Biol. Chem. 285:36745-36758. 2010 PMID: 20851882
7. Brennan T, Adapala NS, Barbe MF, Yingling V, **Sanjay A.** Abrogation of Cbl-PI3K interaction increases bone formation and osteoblast proliferation. Calcif Tissue Int. 89:396-410, 2011.PMCID: 3191294
8. Buitrago L, Langdon WY, Kunapuli SP* and **Sanjay A*** Tyrosine phosphorylated c-Cbl regulates platelet functional responses mediated by outside-in signaling. Blood. 17:5631-40, 2011. PMCID: 3217362. ***Either author could be cited in as corresponding author***
9. Back SH, Adapala NS, Barbe MF, Carpino NC, Tsygankov AY, **Sanjay A**. TULA-2, a novel histidine phosphatase, regulates bone remodeling by modulating osteoclast function. Cell Mol Life Sci. 2012 Nov 13. [Epub ahead of print] PMID: 23149425

D. Research Support

1. Ongoing

NIH NAIMS-1RO1AR055601 (PI Sanjay) 2008-2013
Title: Regulation of Osteoclast function: Role of Cbl-PI3K complex
(40% Effort)

2. Completed Research Support (in last 5 years)

NIH NHLB- 2R01 HL076799-05 (PI Sabri) 2008-2010
Title: Inflammatory proteases, ubiquitin proteasome system, and cardiac myocyte death
Role: Co-Investigator (10% Effort)

NIH NAIMS-2RO1AR047432 (PI Popoff) 2008-2011
Title: The Role of CTGF in Bone Cell Development and Function
Role: Co-Investigator (5% Effort)

NIH NAIMS-2RO1AR048892 (PI Safadi) 2009-2011
Title: The Role of Osteoactivin in Osteoblast Development and Function
Role: Co-Investigator (5% Effort)

Pennsylvania Department of Health (PI Sanjay) 2009-2010
Title: Role of TULA protein in bone remodeling
The goal of this project is to identify the role of TULA-2, a novel phosphatase in skeletal biology especially its role in osteoclast function and differentiation.
Role: Principal Investigator

Pennsylvania Department of Health (PI Sanjay) 2006-2007
Title: Role of tyrosine kinase Src in bone remodeling
The goal of this project was to analyze the role of tyrosine kinase Src in osteoblast differentiation.

BIOGRAPHICAL SKETCH

NAME Steven H. Back	POSITION TITLE Graduate Student
eRA COMMONS USER NAME sback	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR (s)	FIELD OF STUDY
Carnegie Mellon University, Pittsburgh P.A.	B.S.	2005	Chemical Engineering
Temple University, Philadelphia, P.A.	M.D in training .	2013	Medicine
Temple University, Philadelphia, P.A.	Ph.D.in training	2013	Anatomy and Cell Biology

A. Positions and Honors

Employment/Experience:

2007-Present Graduate Student, Temple University, Philadelphia, P.A.

Professional Memberships

2001- 2005 American Institute of Chemists (AIC)
2001-2005 American Institute of Chemical Engineers (AICHE)
2009-Present Member: American Physician Scientists Association (APSA)
2010-Present Member: American Society of Bone and Mineral Research (ASBMR)

Awards and Honors:

2002-2005 Carnegie Mellon University (CMU) Dean's List
2002-2005 CMU Department of Chemical Engineering Scholarship
2003 AICHE Donald F. Othmer Sophomore Academic Excellence Award
2004 AICHE Pittsburgh Section Scholarship
2005 Valedictorian Chemical Engineering Carnegie Mellon University
2005 American Institute of Chemists Foundation Award
2010 American Physician Scientists Association (APSA) Travel Award
2011 Central Society for Clinical and Translational Research Travel Award
2011 Temple University SOM Department of Anatomy &Cell Biology Research Day Winner
2012 Orthopedic Research and Education Foundation Philadelphia Region Resident Research Symposium at the University of Pennsylvania Poster Session Competition Winner

Abstracts presented at Local and National Meetings

1.**Back, S,** Adapala, S, Holland, D, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Temple University Research Symposium, Philadelphia, PA 2009.

2.**Back, S,** Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. American Physician Scientists Association and American Association of Physicians Joint Meeting, Chicago, IL, 2010.

3.**Back, S,** Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Dawn Marks Research Day, Temple University, Philadelphia, PA 2010.

4.**Back, S,** Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay TULA-2: A Novel Tyrosine Phosphatase, Regulates Syk Phosphorylation in Osteoclast Signaling Pathways. American Physician Scientists Association and American Association of Physicians Joint Meeting, Chicago, IL, 2011.

5.**Back, S,** Adapala, S, T.N. Newman, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay TULA-2: A Novel Tyrosine Phosphatase, Regulates Syk Phosphorylation in Osteoclast Signaling Pathways. Central Society for Clinical and Translational Research Poster Session, Chicago, IL, 2011.

6.**Back, S,** Adapala, S, M.F. Barbe, N. Carpino, A.Y. Tsygankov and A. Sanjay Identifying the Role of TULA-2, a Novel Phosphatase in Bone Remodeling. Orthopedic Research and Education Foundation/Orthopedic Research Society Philadelphia Region Resident Research Symposium, University of Pennsylvania, Philadelphia, PA, 2012.

C. Publications

1.**Back SH**, et al. TULA-2, a Novel Histidine Phosphatase, Regulates Bone Remodeling by Modulating Osteoclast Function. Cell Mol Life Sci. 2013 Apr;70(7):1269-84.

D. Research Support

Formula Fund for Tobacco Grant
Role of TULA-2, a Novel Phosphatase in Bone Remodelling
Role Researcher
$ 25,000 for 2009-2010