AD_____
(Leave blank)

Award Number:

W81XWH-10-1-1054

TITLE:
Modulating Wnt Signaling Pathway to Enhance Allograft Integration in Orthopaedic Trauma Treatment

PRINCIPAL INVESTIGATOR:
Amarjit S. Virdi, PhD

CONTRACTING ORGANIZATION:

Rush University Medical Center
Chicago, IL 60612-3839

REPORT DATE:
October 2012

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

- ✓ Approved for public release; distribution unlimited
- ☐ Distribution limited to U.S. Government agencies only; report contains proprietary information

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
October 2012	Annual	30September2011-29September2012

4. TITLE AND SUBTITLE
Modulating Wnt Signaling Pathway to Enhance Allograft Integration in Orthopaedic Trauma Treatment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-1054

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Amarjit S. Virdi, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Rush University Medical Center

Chicago, IL 60612-3839

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research Materiel Command
Fort Detrick, Maryland 21702-

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release: distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The research project was designed to test a novel approach of modulating Wnt signaling pathway in the bone tissue repair by using monoclonal antibodies against sclerostin (Sost) and DKK-1 (donated by Amgen Inc., Thousand Oaks, CA under MTA). Since the previous annual report, the project has progressed at a rapid pace. After overcoming the technical issue with allograft placement, we have performed all the surgical procedures, harvested approximately 85% of the test material (remaining will be completed by mid-December 2012), analyzed approx. 10% of the samples by µCT (data presented in this report), banked the remaining samples. The initial assessment of the data is encouraging as it reveals that the use of anti-Sost or anti-Dkk-1 antibodies enhances new bone formation around the allograft. The mechanical testing the regenerated region has not been performed but likely to support our hypothesis of improved integration.

15. SUBJECT TERMS
Logistic and technical difficulties.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

The scope of this project is to evaluate if the use of a novel anabolic treatment that targets the specific signaling pathways during osteogenesis that promotes bone healing will enhance the integration of allografts to the host bone in an animal model that simulates severe bone loss due to local trauma. In general, it is known that several different growth factors aid bone regeneration. In previous studies we have reported enhanced bone regeneration when growth factors, such as bone morphogenetic protein (BMP), are applied directly at the site of injury (1-10). It is also known that mechanical stimuli at the regenerate also accelerate the healing process. We and others have demonstrated that pulses of low intensity ultrasound, delivering mechanical stimulus, accelerates fracture healing (11-14). However, the focus of the proposed application is to employ a novel approach of modulating the LRP5/Wnt cell signaling pathway which is known to be critically involved in osteogenesis in order to repair large bone defects such as those experienced by soldiers in the battlefield due to ballistics related trauma to the extremities. Monoclonal antibodies raised against sclerostin and dickkopf-1 (Dkk-1) were proposed to be the test reagents employed to modulate the Wnt signaling pathway. An agreement with Amgen Inc. (Thousand Oaks, CA) was established for them to donate the reagents.

In order to carry out this research, we had proposed an animal model of segmental bone defect in the rat femur. In the ongoing research projects in our laboratory we have employed this model to study the efficacy of combining BMP-2 and low intensity pulsed ultrasound to improve new bone regeneration in the gap. In the current study we had proposed to place an allograft in the created gap and to then treat the animals with systemic delivery of anti-sclerostin or anti-Dkk-1 antibodies for the prescribed period of time. The endpoints proposed were x-ray and μCT imaging, mechanical testing and histology.

In addition to the text above (same as last annual report), we have now overcome technical issues highlighted in that report. The overall pace in all aspects of the project now meets our expectations of completing the tasks by the proposed deadline of September 29, 2013 and will be reflected in the final report due October 29, 2013.

Body

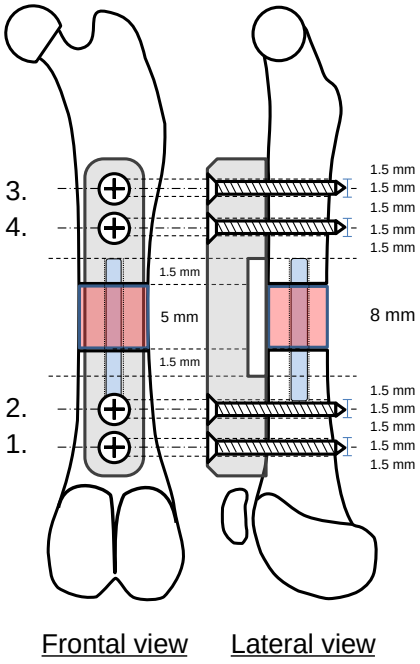
We hypothesized that **neutralizing the LRP5/Wnt pathway inhibitors Sost or DKK1 with monoclonal antibodies will enhance allograft integration to the host bone.** The proposed work in this project was designed to test this hypothesis by addressing two specific aims.

Aim 1: Determine the effect of modulating the LRP-5/Wnt pathway with anti-Sost monoclonal antibody on allograft incorporation in a rat segmental repair model using radiographical, morphological and mechanical endpoints.

Aim 2: Determine the effect of modulating the LRP-5/Wnt pathway with anti-Dkk1 monoclonal antibody on allograft incorporation in a rat segmental repair model using radiographical, morphological and mechanical endpoints.

Within each these aims we proposed to use fresh frozen and freeze-dried allografts to emulate clinical scenarios where banked tissue available for use in patients is processed by these procedures.

As explained in the previous annual report, an accurate and consistent surgical placement of the allograft posed a technical challenge. After a number of attempts we succeeded in achieving a reliable and reproducible protocol that was only minimally different from the proposed model. The modification to the surgical procedure involved placing a polyethylene rod in the intramedullary canal such that it spanned across the allograft and the host bone (**Figure 1**). Steps involved in the surgical procedure are shown in **Figure 2**. The final result was to maintain alignment of allograft with the host bone. Polyethylene is known not to integrate with the bone and would not present any significant variable during mechanical testing.



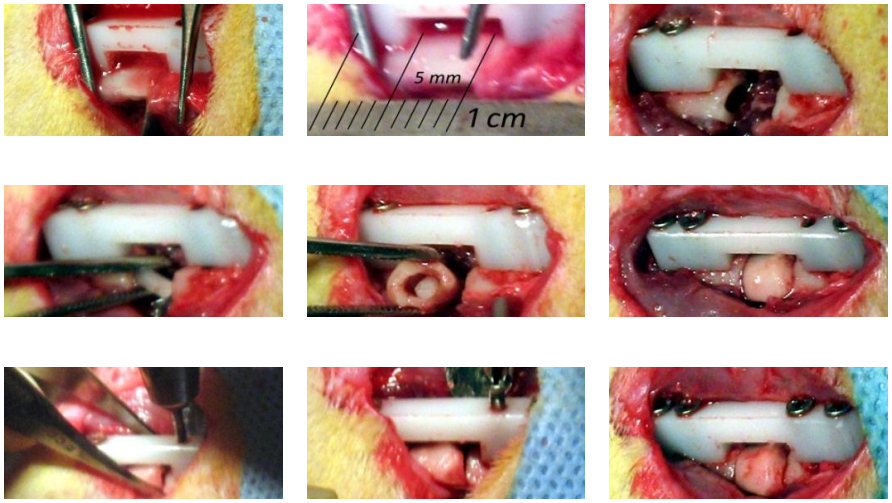


Figure 2: Surgical steps (top left to bottom right) for allograft placement in the segmental defect. Note the internal fixator (white) and relative location of the allograft.

Figure 1: Schematic representation of the allograft (pink) placement in the segmental defect. Note that the allograft is held in place, and thus stays aligned with host bone, using a polyethylene wire (blue). The whole defect is stabilized with an internal fixator (gray) that is secured with four screws.

Using this approach, we have completed all proposed surgical procedures. All but one subgroup (freeze-dried allograft, 4 week time point, saline/anti-Sost/Anti-Dkk1; N=16 each treatment) have been treated, samples harvested and banked for analysis. In vivo radiographs as well ex-vivo radiographs at the time of harvesting have also been completed.

We have analyzed a subset of samples (fresh frozen; N=3) from 4, 8 and 12 week time points for saline, anti-Sost and anti-Dkk1 using µCT scanning. Data is presented below. Quantitative data is presented to show the following outcomes.

Bone volume – this indicates the amount of new bone formed around the allograft and represents the overall quantity and rate of bone regeneration. In general, higher BV correlates with better mechanical competence.
Trabecular Number – this outcome indicates the spread of new bone formation.

Trabecular Thickness – this outcome indicates the physical characteristics of new trabeculae. Thicker trabeculae are mechanically more stronger than thinner ones.
Trabecular Spacing – This is an indicator of the void space between the trabeculae and is inversely related to trabecular thickness.

Pictures below (**Figures 3, 4 and 5**) show radiograph and µCT images for representative samples from each group.

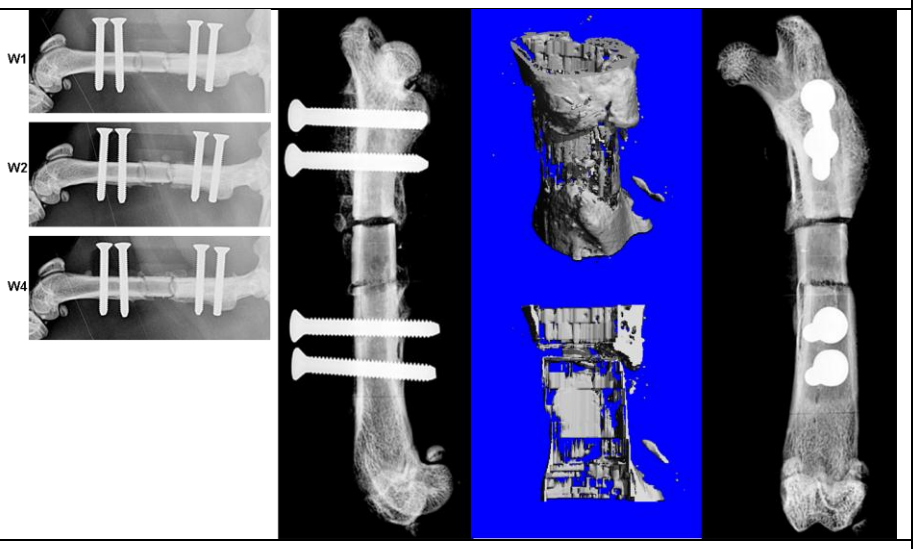
Figure 3: In vivo radiographs, ex vivo radiographs and µCT scans for 4 week time point

Saline treatment

Left to right:
1 – in vivo radiographs
2 – ex vivo radiograph (AP)
3 - µCT showing new bone only
4 – ex vivo radiograph (Lateral)

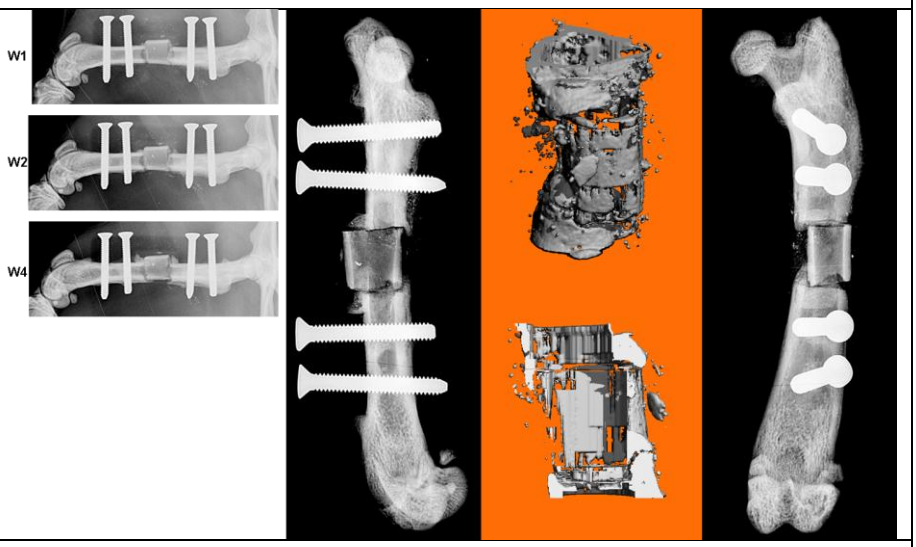
W1, W2 etc. refer to the week at which the in vivo radiograph was taken.

Same layout for all panels in Figures 3, 4 and 5.



Anti-Sost antibody Treatment

Layout as described above.



Anti-Dkk1 antibody Treatment

Layout as described above.

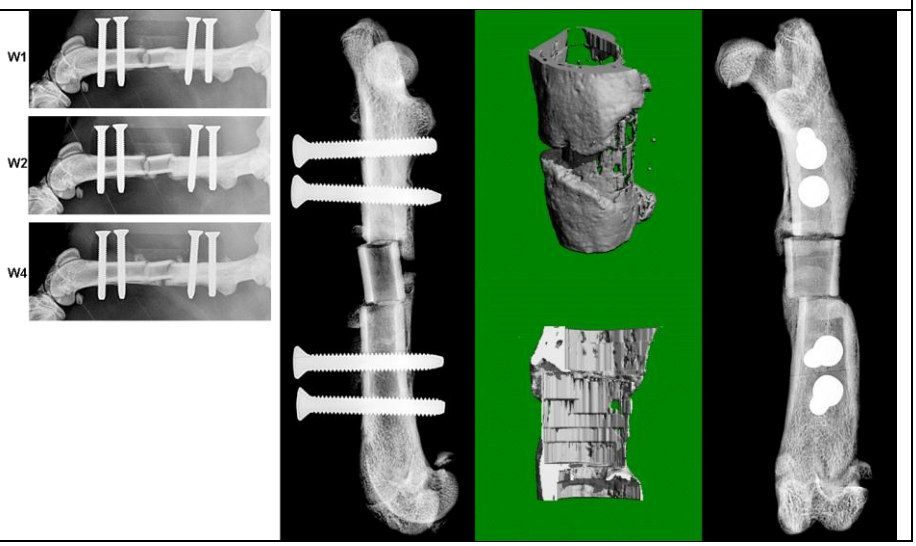
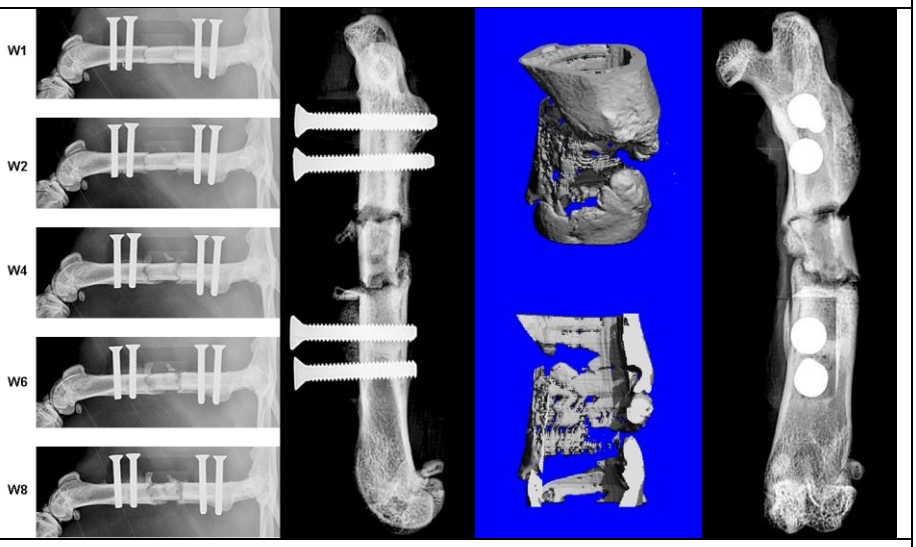


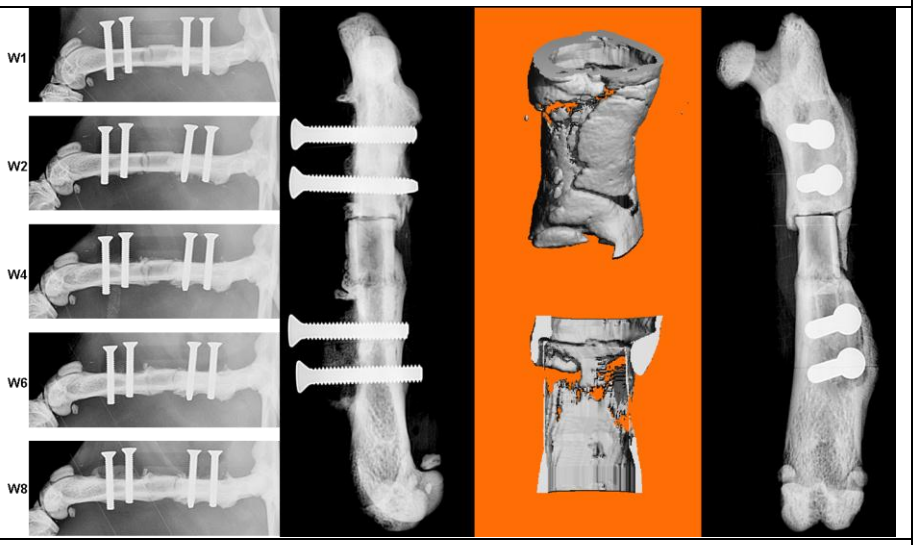
Figure 4: In vivo radiographs, ex vivo radiographs and µCT scans for 8 week time point

Saline treatment

Please see Figure 3 for layout description.



Anti-Sost antibody Treatment



Anti-Dkk1 antibody Treatment

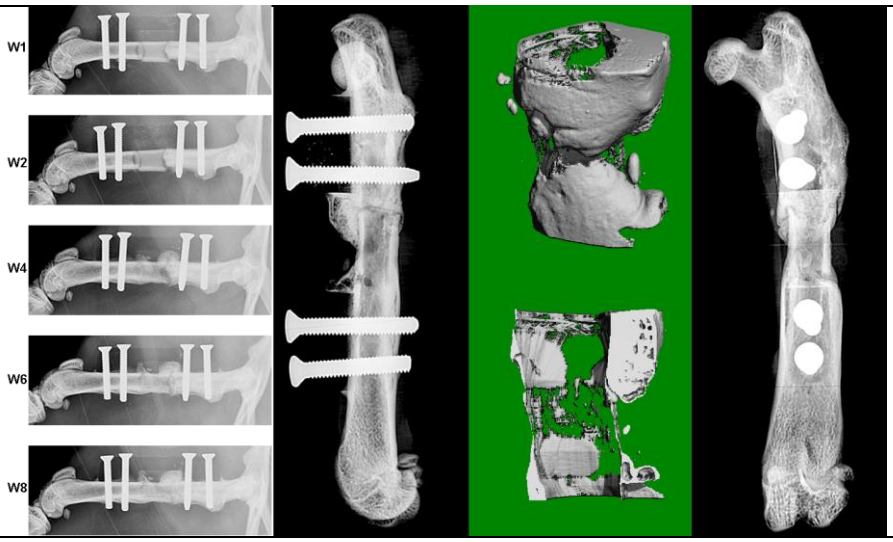
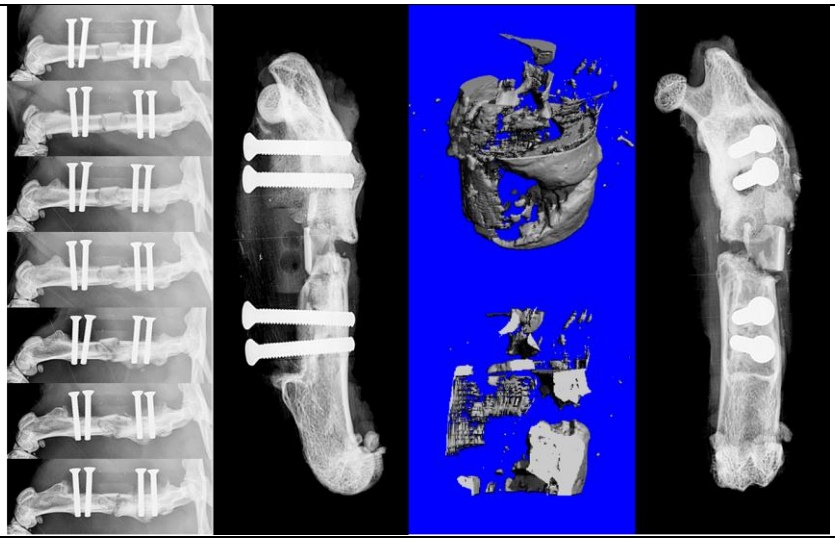


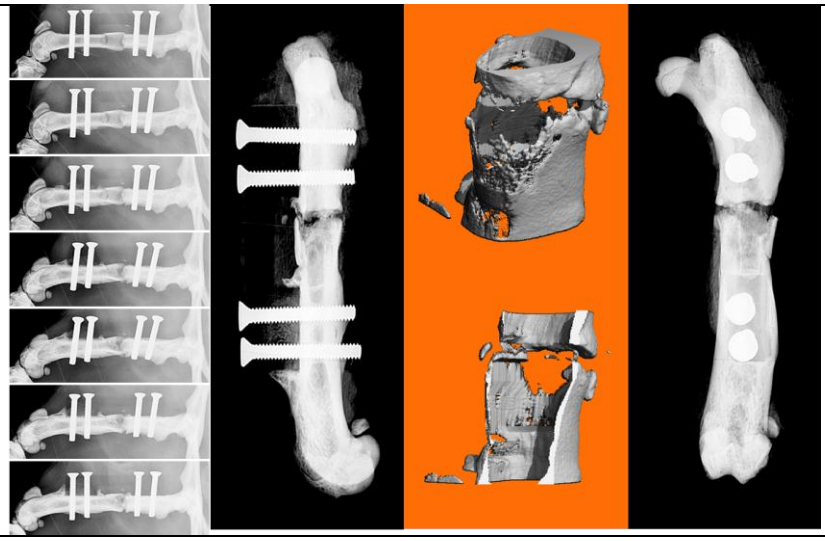
Figure 5: In vivo radiographs, ex vivo radiographs and µCT scans for 12 week time point

Saline treatment

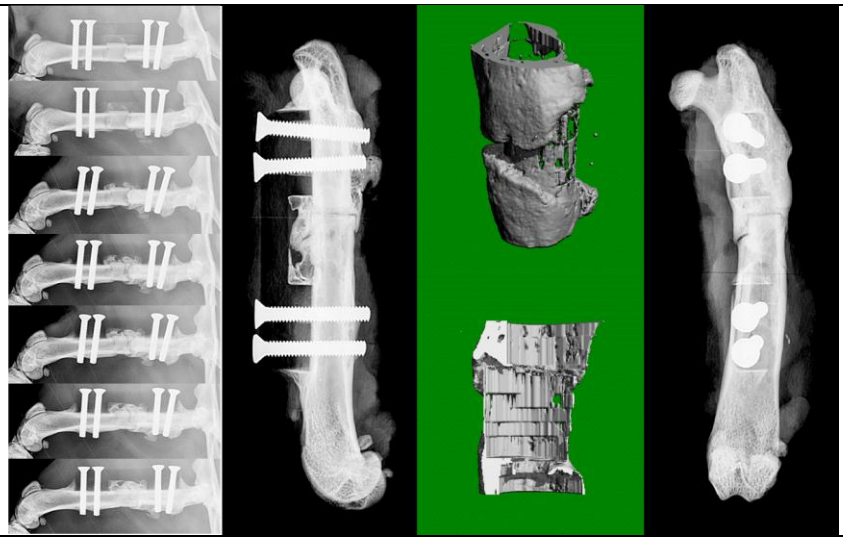
Please see Figure 3 for layout description.



Anti-Sost antibody Treatment



Anti-Dkk1 antibody Treatment



µCT evaluation data was analyzed for four most relevant outcomes. **Figures 6, 7** and **8** depict graphs of this quantitative data for all treatments and time points. No statistical analysis was performed because of small sample sizes. In the final analyses we will have N=15 per treatment, per time point, and we will subject all data to extensive statistical analyses to provide statistical support for our findings.

In general, the data reveals that both anti-Sost and anti-Dkk1 antibody treatments enhanced bone formation when compared with saline treatment. These findings were observed in all outcomes including trabecular thickness where the new bone formation is occurring that may be making the existing trabecular structure mechanically more competent. The planned mechanical testing will provide the necessary support for this observation. If proven true, this would represent a practical means of enhancing repair of large bone defects in orthopedic trauma and can be translated into clinical practice in the near future.

When the data was plotted to study the changes occurring over treatment time (**Figures 9, 10 and 11**), a time effect was observed for bone formation and trabecular thickness. The Y-axis scales for all treatments have been kept constant to allow visual comparison. Again, it is clear that anti-Sost and anti-Dkk1 treatment show higher values than saline treatment. A decline seen for bone formation at 12 week for anti-Sost treatment has been seen before by us (unpublished observation) and may represent bone remodeling that occurs later in the regenerative process.

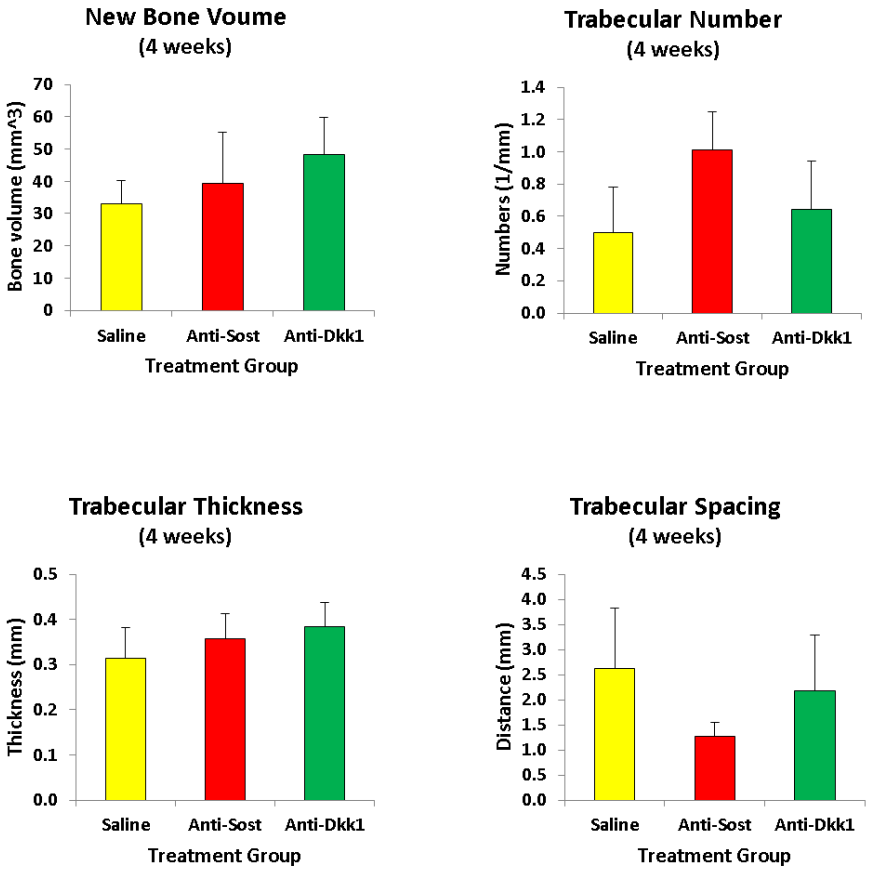


Figure 6: µCT data for 4 week time point.
The bars are color coded for easier interpretation. Yellow = Saline, Red = anti-Sost, Green = anti-Dkk1.

Note – the layout and color coding is the same for Figures 7 – 11.

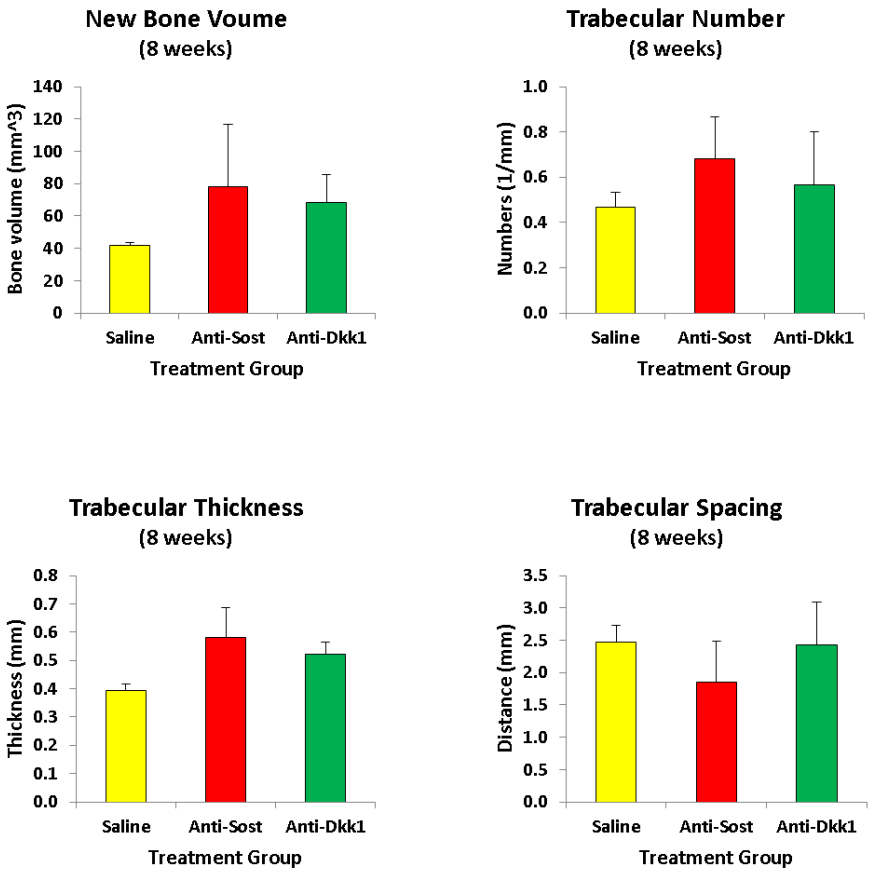


Figure 7: µCT data for 8 week time point. See **Figure 6** legend for color coding.

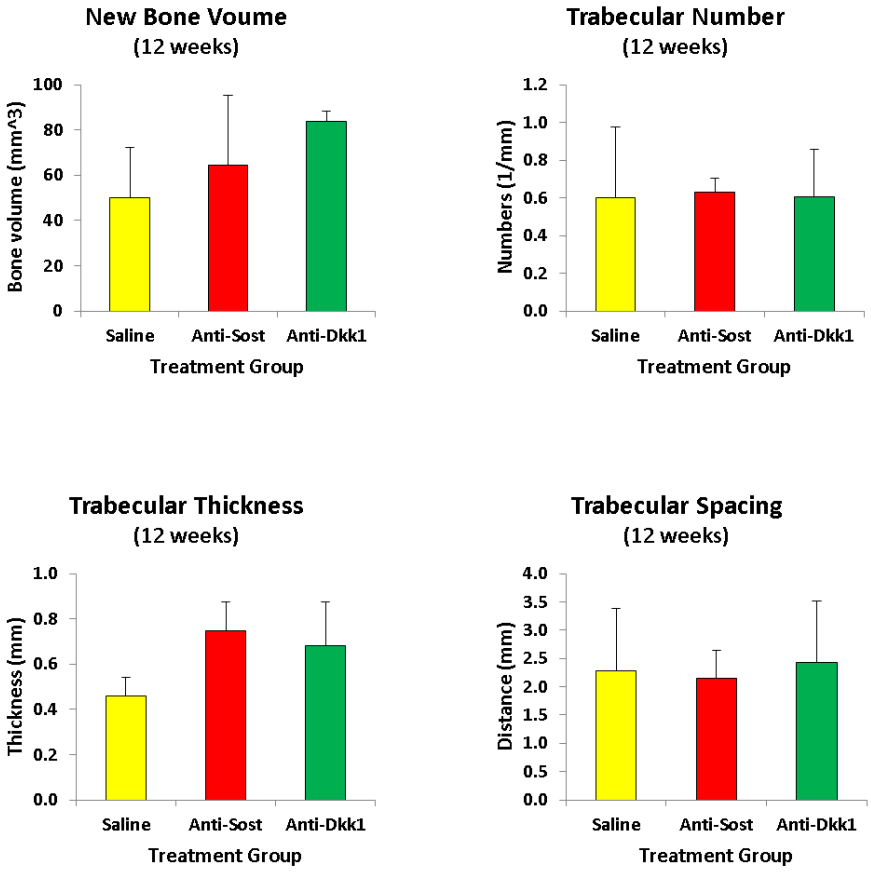


Figure 8: µCT data for 12 week time point. See **Figure 6** legend for color coding.

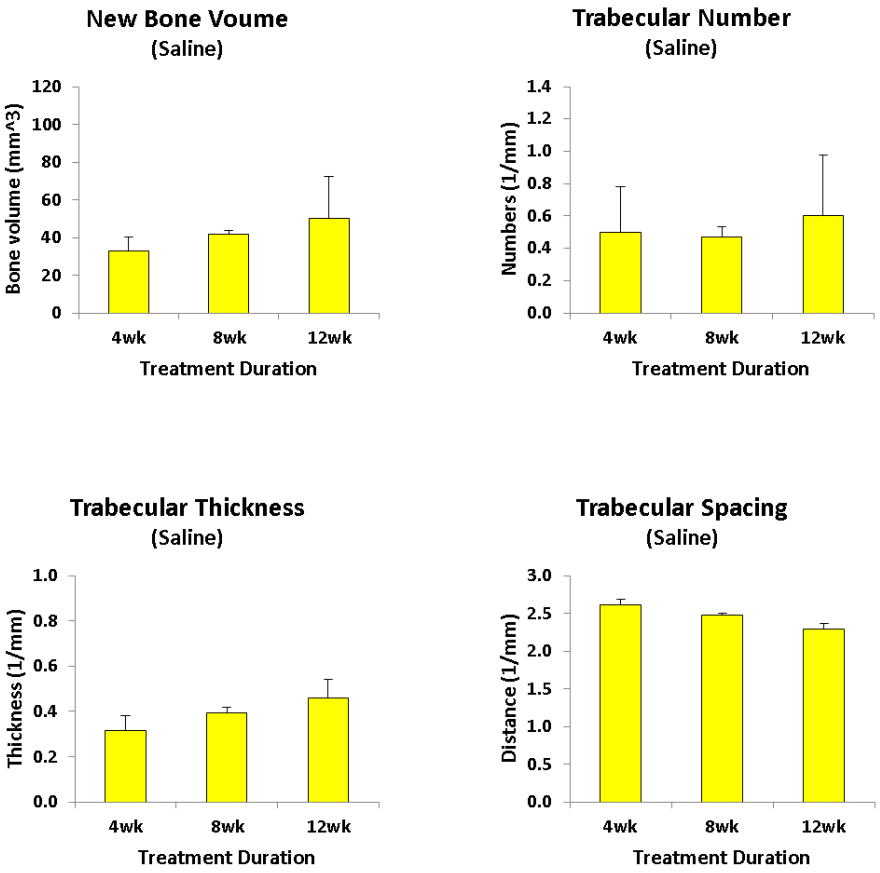


Figure 9: µCT data for saline treatment group at different time points.

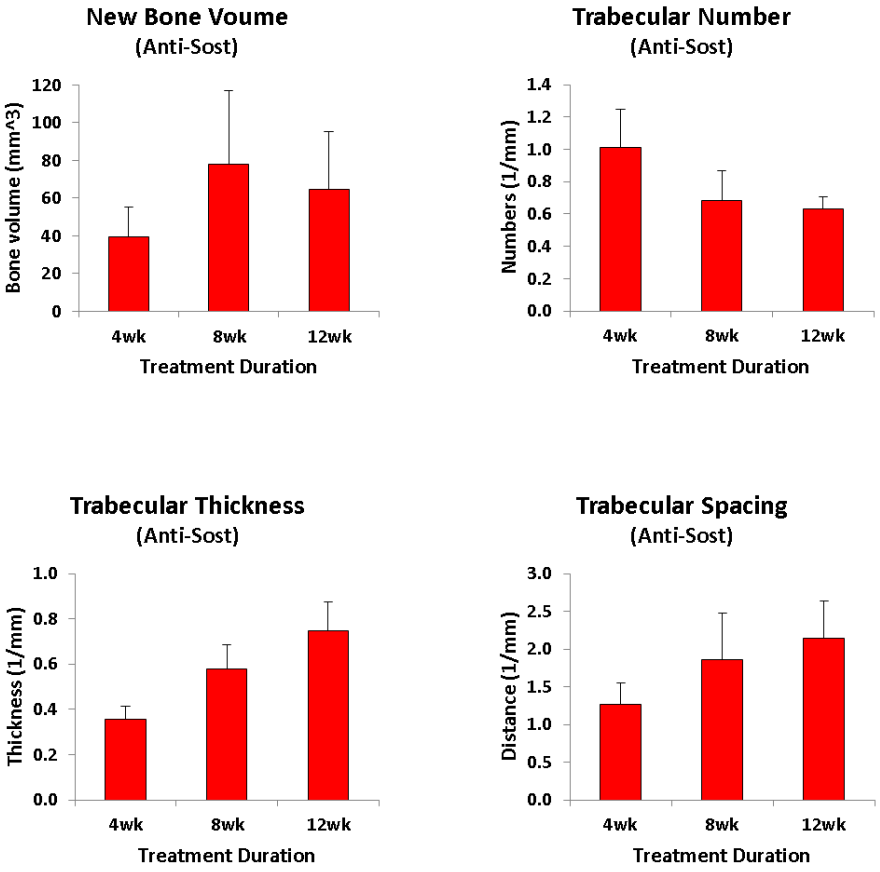


Figure 10: µCT data for anti-Sost antibody treatment group at different time points.

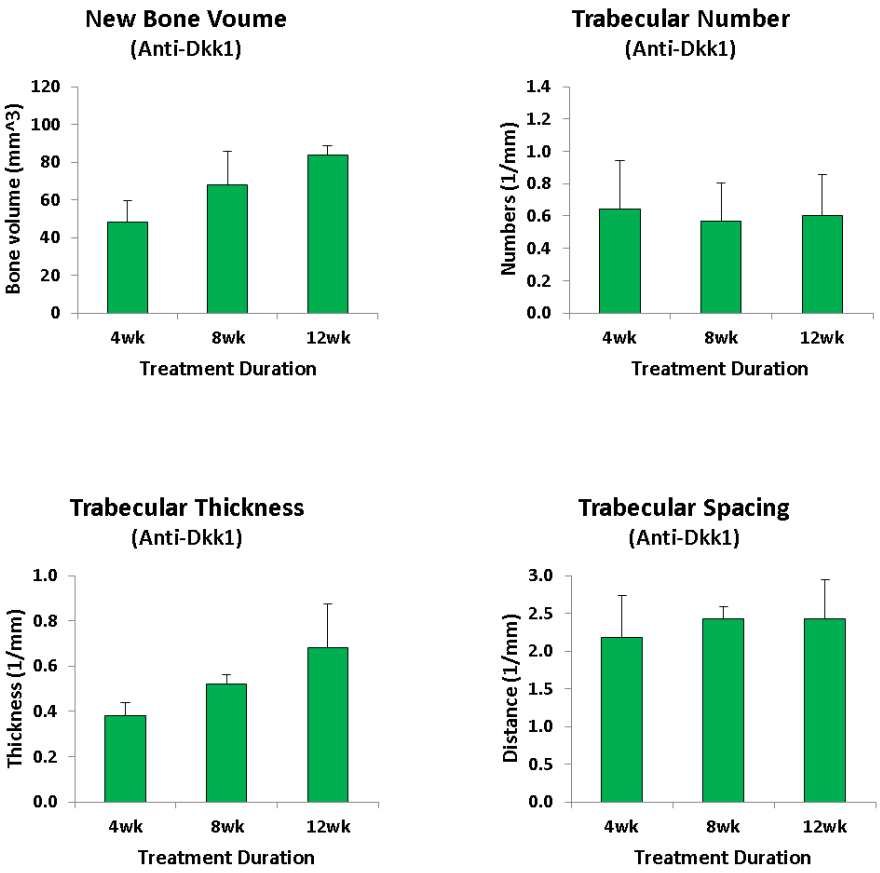


Figure 11: µCT data for anti-Dkk1 antibody treatment group at different time points.

Key Research Accomplishments

- All surgical procedures have been completed.
- Nearly 85% of samples have been harvested and banked.
- Over 90% of the planned radiographs have been obtained.
- 10% of the samples have been analyzed for μCT evaluation.

Reportable Outcomes

- None at this stage.

Conclusion

- The observations made based on a limited data available at this stage indicate that modulating the LRP/Wnt signaling pathway with anti-Sost and anti-Dkk1 monoclonal antibodies enhances new bone formation around allografts in a rat segmental defect model.

References

1. Virdi, A. S., De Ranieri, A., Kuroda, S., Dai, Y., and Sumner, D. R. Anabolic Agents and Gene Expression Around the Bone Implant Interface. J.Musculoskelet.Neuronal.Interact. 2004;4(4):388-9.

2. Kuroda, S., Virdi, A. S., Li, P., Healy, K. E., and Sumner, D. R. A Low-Temperature Biomimetic Calcium Phosphate Surface Enhances Early Implant Fixation in a Rat Model. J.Biomed.Mater.Res.A 7-1-2004;70(1):66-73.

3. De Ranieri, A., Virdi, A. S., Kuroda, S., Healy, K. E., Hallab, N. J., and Sumner, D. R. Saline Irrigation Does Not Affect Bone Formation or Fixation Strength of Hydroxyapatite/Tricalcium Phosphate-Coated Implants in a Rat Model. J.Biomed.Mater.Res.B Appl.Biomater. 2005;74(2):712-7.

4. De Ranieri, A., Virdi, A. S., Kuroda, S., Shott, S., Leven, R. M., Hallab, N. J., and Sumner, D. R. Local Application of RhTGF-Beta2 Enhances Peri-Implant Bone Volume and Bone-Implant Contact in a Rat Model. Bone 2005;37(1):55-62.

5. De Ranieri, A., Virdi, A. S., Kuroda, S., Shott, S., Dai, Y., and Sumner, D. R. Local Application of RhTGF-Beta2 Modulates Dynamic Gene Expression in a Rat Implant Model. Bone 2005;36(5):931-40.

6. Chung, E. H., Gilbert, M., Virdi, A. S., Sena, K., Sumner, D. R., and Healy, K. E. Biomimetic Artificial ECMs Stimulate Bone Regeneration. J.Biomed.Mater.Res.A 12-15-2006;79(4):815-26.

7. Sumner, D. R., Turner, T. M., Urban, R. M., Virdi, A. S., and Inoue, N. Additive Enhancement of Implant Fixation Following Combined Treatment With RhTGF-Beta2 and RhBMP-2 in a Canine Model. J.Bone Joint Surg.Am. 2006;88(4):806-17.

8. Ho, J. E., Barber, T. A., Virdi, A. S., Sumner, D. R., and Healy, K. E. The Effect of Enzymatically Degradable IPN Coatings on Peri-Implant Bone Formation and Implant Fixation. J.Biomed.Mater.Res.A 6-1-2007;81(3):720-7.

9. Barber, T. A., Ho, J. E., De Ranieri, A., Virdi, A. S., Sumner, D. R., and Healy, K. E. Peri-Implant Bone Formation and Implant Integration Strength of Peptide-Modified P(AAM-Co-EG/AAC) Interpenetrating Polymer Network-Coated Titanium Implants. J.Biomed.Mater.Res.A 2007;80(2):306-20.

10. Sena, K., Sumner, D. R., and Virdi, A. S. Effect of Recombinant Human Transforming Growth Factor-Beta2 Dose on Bone Formation in Rat Femur Titanium Implant Model. J.Biomed.Mater.Res.A 3-1-2010;92(3):1210-7.

11. Angle, S. R.; Sena, K.; Sumner, D. R.; Virkus, W. W.; Virdi, A. S. Optimum healing of rat femoral segmental defect with bone morphogenetic protein-2: a dose response study. Transactions of the Orthopaedic Research Society 36, 0287. 2010.

12. Angle, S. R.; Sena, K.; Sumner, D. R.; Virkus, W. W.; Virdi, A. S. Combined use of low intensity pulsed ultrasound and rhBMP-2 to enhance bone formation in a critical sized defect. Transactions of the Orthopaedic Research Society 36, 1506. 2011.

13. Angle, S.; Sena, K.; Sumner, D. R.; Virkus, W. W.; Virdi, A. S. Temporal effects of low intensity pulsed ultrasound (LIPUS) on rhBMP-2 induced bone formation in a critical sized segmental defect in the rat. Transactions of the Orthopaedic Research Society 37, 0414. 2012.

14. Angle, S.; Sena, K.; Sumner, D. R.; Virkus, W. W.; Virdi, A. S. Combined use of low intensity pulsed ultrasound (LIPUS) and rhBMP-2 enhances strength of newly formed bone in a critical sized segmental defect in the rat. Transactions of the Orthopaedic Research Society 37, 0057. 2012.

15. Virdi, A. S.; Liu, M.; Sena, K.; Maletich, M.; McNulty, M.; Ke, H. Z.; Sumner, D. R. Sclerostin antibody increases bone volume and enhances implant fixation in a rat model. J.Bone Joint Surg.Am 24 (Suppl 1). 2012;94(18):1670-80.

16. Virdi, A. S.; Sena, K.; McNulty, M. A.; Ke, H. Z.; Liu, M.; Sumner, D. R. Sclerostin antibody increases peri-implant bone formation in a rat ovariectomy model. Transactions of the Orthopaedic Research Society 36, 190. 2011.

Appendices

- None