Comparison of a Novel Oxysterol Molecule and rhBMP2 Fusion Rates in a Rabbit Posterolateral Lumbar Spine Model

Trevor P. Scott M.D.[1]; Kevin H. Phan B.S.[1]; Haijun Tian M.D.[1]; Akinobu Suzuki M.D., Ph.D[1]; Scott R. Montgomery M.D.[1]; Jared S. Johnson M.D.[1]; Elisa Atti Ph.D.[2], Sotirios Tetratis D.D.S., Ph.D.[2]; Renata C. Pereira Ph.D.[3]; Jeffrey C. Wang M.D.[4]; Michael D. Daubs M.D.[5]; Farhad Parhami Ph.D M.B.A.[6*]

1 Department of Orthopedic Surgery, UCLA, Los Angeles, California
2 School of Dentistry, UCLA, Los Angeles, California
3 Department of Pediatric Nephrology, UCLA, Los Angeles, California
4 Department of Orthopedic Surgery and Neurosurgery, USC, Los Angeles, California
5 Department of Orthopedic Surgery and Neurosurgery, UNLV, Las Vegas, Nevada
6 Department of Medicine, David Geffen School of Medicine, UCLA, Los Angeles, California

* Corresponding Author:
Farhad Parhami, Ph.D., M.B.A.
Professor of Medicine
David Geffen School of Medicine at UCLA
Center for the Health Sciences, A2-237
10833 Le Conte Avenue
Los Angeles, CA. 90095
Tel: 310-825-5729
Email: fparhami@mednet.ucla.edu

1

Abstract

The non-union rate following lumbar spinal fusion is potentially as high as 25%. Bone morphogenetic protein-2 (rhBMP2) has been used as a biological adjunct to promote bony fusion. However, recently there have been increasing concerns about BMP. Oxysterol 133 (Oxy133) has been shown to promote excellent fusion rates in rodent lumbar spine models and offers a potential alternative to rhBMP2. The purpose of this study is to compare the fusion rate of rhBMP2 and Oxy133 in a randomized control trial using a posterolateral lumbar rabbit spinal fusion model. 24 male adult white New Zealand rabbits (3-3.5kg) underwent bilateral posterolateral lumbar spinal fusion at L4-L5. Rabbits were divided into 4 groups: control (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). At 4 weeks fusion was evaluated by fluoroscopy, and at 8 weeks the rabbits were sacrificed and fusion was evaluated radiographically, by manual palpation, and with microCT.

Fusion rates at 4 weeks were as follows: group A 25%, group B 67.7%, group C 91.7%, and group D 90.0%. Fusion rates by radiographic analysis at 8 weeks were: group A 40.0%, group B 91.7%, group C 91.7%, and group D 100%. Evaluation of fusion masses by manual palpation of excised spines after sacrifice showed the following fusion rates:, group A were 0% fused, group B were 83.3% fused, group C were 83.3% fused, and group D were 90% fused. These findings in a rabbit model demonstrate that both low and high dose 20 mg Oxy133 appears to promote fusion that is equivalent to than 30 ug rhBMP2 and significantly greater than the control group.

Keywords: Oxysterol; rhBMP2; Spine Fusion; Osteogenesis

Introduction

Non union is a significant post operative problem in both spinal surgery and treatment of long bone fractures; in spinal fusion surgery its incidence can approach 25%[1]. Fracture surgery is performed by orthopedic surgeons for the majority of displaced adult fractures. Spinal fusion surgery is performed by both orthopedic surgeons and neurosurgeons for degenerative disc disease, arthritis or deformity of the lumbar and cervical spine. Painful non-union, or pseudoarthrosis, after spinal fusion surgery can cause significant morbidity and can be challenging to treat surgically. Since there has been a marked increase in the incidence of spinal fusion surgery in the last two decades, pseudoarthrosis has become a significant public health issue[2]. Therefore a large amount of effort has been put into preventing its occurrence after spinal fusion surgery.

Classically the gold standard treatment to promote fusion at the time of spinal surgery has been autogenous bone taken from the anterior iliac crest. However, this surgery has been found to have significant donor site morbidity, and it has been associated with increased blood loss and operative time. Furthermore, multi-level spinal fusions often require greater volume of bone graft than is available from the iliac crest [3-5].

In response to this issue there has been a large amount of research activity developing biologic adjuncts to bone fusion as an alternative to autogenous bone graft. Recombinant bone morphogenetic protein 2 (rhBMP2) was first developed in the 1960's, and in 2002 it was approved by the Food and Drug Administration for use as a bone fusion adjunct in anterior single level lumbar fusions [6]. rhBMP2 proved to be an effective fusion adjunct and was widely adopted over the following decade. However, recently there have been concerns about the quality of the bone in the fusion masses when rhBMP2 is used, and there have been reports of unforeseen complications such as airway edema, seroma formation, soft tissue swelling,

4

retrograde ejaculation, carcinogenicity, bone osteolysis and ectopic bone formation [7-10].

As of yet no effective alternatives to rhBMP2 have been developed. However, certain members of a group of molecules known as oxysterols have been shown to be osteogenic and anti-adipogenic when applied to mesenchymal stem cells in vitro [11]. Our lab has previously developed a novel small molecule osteogenic oxysterol analogue molecule, Oxy133, which induces the expression of osteogenic differentiation markers in vitro when applied to osteoprogenitor cells in vitro, and which we demonstrated as being capable of promoting equivalent bone fusion to rhBMP2 in a rat model. Furthermore, this molecule is relatively simple and inexpensive to produce in large scale. We have proposed that osteogenic oxysterols may be alternatives to rhBMP2 to promote spinal fusion effectively and safely .

The purpose of this study was to examine the effectiveness of Oxy133 to induce spinal fusion in a larger animal model than the previously used rat model, namely a rabbit posterolateral lumbar spinal fusion model. We hypothesized that Oxy133 would have at least equivalent efficacy to rhBMP2 in stimulating spinal fusion.

Materials and Methods

Animals

24 male adult white New Zealand rabbits (3-3.5kg) were purchased from Charles River Laboratories (Wilmington, MA) and were maintained and housed at the UCLA vivarium in accordance with regulations set forth by the UCLA Office of Protection of Research Subjects. The study was performed under a protocol approved by the UCLA Animal Research Committee (ARC). All animals were euthanized using intravenous pentobarbital 100mg/kg 8 weeks after the spinal fusion procedure, and their spines were excised and stored in 70% ethyl alcohol.

Surgical Procedure

Rabbits were premedicated with buprenoprhine 30 minutes prior to surgery and the surgical site was shaved and prepped with betadine and 70% ethanol. The rabbits were anesthetized using 1 ml of xylazine IM and 1.0 - 1.2 ml of ketamine IM before intubation. Anesthesia was maintained with 1-4% isoflurane. Following surgery the animals were extubated. Aseptic technique was utilized for all surgical procedures. Posterolateral intertransverse process spinal fusion at L4–L5 was performed as described in prior studies[12]. The L6 vertebral body was identified using the iliac crest as a landmark. A 6-cm longitudinal midline incision was made through the skin and subcutaneous tissue over L4–L5 down to the lumbodorsal fascia. A 3-cm longitudinal paramedial incision was then made in the paraspinal muscles bilaterally to expose the transverse processes of L4 and L5, which were decorticated with a high-speed burr. The surgical site was then irrigated with sterile saline, and 20 mm x 10 mm x 8 mm piece of mineral collagen matrix (Ossimend, Biomet Spine.) containing normal saline control (NS) rhBMP-2, or Oxy133 were placed bilaterally, with each implant spanning the transverse processes. The implants were then covered with the overlying paraspinal muscles and the lumbodorsal fascia and skin were closed with 4–0 Prolene sutures (Ethicon, Inc., Somerville, NJ). Animals were allowed to ambulate, eat, and drink ad libitum immediately after surgery.

Radiographic Analysis

Radiographs of the lumbar spine were taken of each animal at 4 and 8 weeks after surgery using anterioposterior fluoroscopy at 4 weeks and a Faxitron LX60 cabinet radiography system at 8 weeks for posteranterior images. Radiographs were evaluated blindly by three independent observers employing the following standardized scale: 0, no fusion; 1, bony

bridging; and 2, complete fusion with bridging between transverse processes. The scores from the observers were added together and only a score of 5 or 6 was considered as complete fusion.

Manual Assessment of Fusion

Eight weeks after surgery, animals were euthanized and the spines were surgically removed and evaluated by three blinded independent observers for motion between levels. Nonunion was recorded if motion was observed between the facets or transverse processes on either side. Complete fusion was recorded if no motion was observed bilaterally. Spines were scored as either fused or not fused. Unanimous agreement was required to consider a spine completely fused.

Micro-Computer Tomography

Each removed spine was analyzed by high resolution micro-computed tomography (micro-CT), using a SkyScan 1172 scanner (SkyScan, Belgium) with a voxel isotropic resolution of 20Hm and an X-ray energy of 55kVp and 181mA to further assess the fusion rate and observe the fusion mass as we have previously reported[13]. Three hundred and sixty projections were acquired over an angular range of 180Åã with steps of 0.5 with an exposure time of 220 msec/slice. Five frames were averaged at each rotation step to get better signal to noise ratio. A 0.5 mm aluminum filter was used to narrow down the X-ray beam frequency in order to minimize beam hardening artifact. Virtual image slices were reconstructed using the cone-beam reconstruction software version 2.6 based on the Feldkamp algorithm (SkyScan). These settings produced serial cross-sectional 1024 x 1024 pixel images. Sample re-orientation and 2D visualization were performed using DataViewer (SkyScan). 3D visualization was performed

using Dolphin Imaging version 11 (Dolphin Imaging & Management Solutions, Chatsworth, CA). Fusion was defined as the presence of bridging bone between the L4 and L5 transverse processes. The reconstructed images were judged to be fused or not fused by two experienced independent observers and only a consensus was considered fused. To quantify the density of bone formed within each fusion mass, the tissue volume of the mass (TV), trabecular bone volume within the mass (BV), and BV/TV ratio were calculated. This was performed using CT Analyzer software (SkyScan, Belgium) with measurements across about 1000 axial slices (20um per slice, 20.0 mm length) spanning the entirety of each fusion mass.

Histology

After undergoing micro-CT, two representative specimens from each surgical group were processed. Samples were decalcified, embedded in paraffin and sectioned in the sagittal plane and stained with H&E stain. Photomicrographs of sections were obtained as previously reported using a ScanScope XT System (Aperio Technologies, Inc., Vista, CA). Bony trabeculae bridging adjacent transverse processes were required to confirm fusion.

Statistical Analysis

Statistical analyses were performed using the SPSS program (version 19.0; IBM, New York). All p values were calculated using Fisher's exact test for fusion rates. ANOVA or Kruska Wallis was used for analysis of microCT data. A value of $p < 0.05$ was considered significant for fusion rates, p value < 0.01 was significant for analysis of microCT because of the use of the Bonferroni test.

Results

The 24 rabbits used in this study were divided into 4 groups: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). One of the control rabbits was sacrificed at 4 weeks due to a deep infection and one of the 60 mg Oxy133 treated rabbits expired from complications secondary to anesthesia at the time of surgery. Bone formation and spinal fusion were assessed at various time points post-operatively through radiographic analysis, and after sacrifice using manual assessment, microcomputed tomography, and histology.

Radiographic Analysis

Due to the size of the rabbits radiographic imaging at 4 weeks was performed via fluoroscopy as this was the only imaging modality at our institution which could accommodate the live rabbits. At this time point fusion was seen unilaterally in 3 of 6 group A rabbits (25% fusion). Of the 6 rabbits in group B, 2 had fusion unilaterally and 3 rabbits had fusion bilaterally (67.7% fusion). In group C, 5 of 6 rabbits had bilateral fusion and 1 rabbit had fusion unilaterally (91.7% fusion). In group D, 1 of 5 rabbits had fusion unilaterally and the other 4 rabbits all had bilateral fusion (90% fusion). Groups C and D had significantly greater fusion rates than group A at four weeks ($p=0.004$ and $p= 0.003$, respectively); the fusion rate of group B was not significantly greater than the control group at the 4 week time point (Figure 1, Table 1).

At 8 weeks the rabbits were sacrificed and faxitron imaging for high resolution imaging of the extracted lumbar spines was used. Results showed that in group A 2 of 5 rabbits had unilateral fusion and one rabbit had bilateral fusion (40% fusion). In group B, 5 of 6 rabbits had bilateral fusion and 1 rabbit had unilateral fusion (91.7% fusion). In group C, 5 of 6 rabbits had

bilateral fusion and 1 rabbit had unilateral fusion (91.7%). In group D, 5 of 5 rabbits had bilateral fusion (100% fusion). Statistical analysis of the data using Fischer's test showed that all 3 experimental groups B, C, and D had significantly greater fusion rates than the control group A (A vs. B: p=0.020; A vs. C: p=0.020; A vs. D: p=0.011) (Figure 1, Table 1). Representative 8 week radiographs from 2 animals in each group are shown in Figure 2.

Manual Palpation

Excised spines from the control and experimental groups were assessed for fusion after removal of soft tissue by manual palpation as previously described[13-16]. In the control group, 0 of 5 rabbits had bilateral fusion (0% fusion) In group B, 5 of 6 rabbits had bilateral fusion (83.3% fusion); likewise, in group C, 5 of 6 rabbits had bilateral fusion (83.3% fusion). In group D, 4 of 5 rabbits had bilateral fusion (80% fusion rate) (Figure 3). Gross evaluation of specimens revealed obvious and significant fusion masses in the experimental groups, which were most prominent in group D. In contrast gross examination of fibrous tissue between the transverse processes in the control group revealed only remnants of ossimend and no obvious bone formation.

Micro Computed Tomography

The results of microCT at 8 weeks were analyzed for both fusion rate and bone quality. Fusion rate bilaterally for group A was 0%; group B's fusion rate was 67.0%, group C fusion rate was 91.7% and group D's fusion rate was 80%. Fusion rates in all three experimental groups were significantly greater than in the control group (A vs B: P= 0.012; A vs C: P=0.001; A vs D: P=0.001) (Figure 4).

MicroCT analysis of the bone quality was performed, although it was made challenging

given the similar radiodensity of the ossimend to bone. Tissue volume (TV) of the fusion mass was significantly increased for the experimental groups compared to the control group volume of 901.5 mm^3 +/- 185.7. The rhBMP2 TV was 1651.6 +/- 491.6; TV for low dose Oxy133 was 1943 +/- 325.0, and TV for high dose Oxy133 was 2149.6 +/- 800.3 (A vs B: P= 0.005; A vs C: P<0.001; A vs D: P=0.001). A trend towards increased TV in low dose Oxy133 group compared to BMP2 group and high dose Oxy133 was present but did not reach significance (Table 2 and Figure 5). Aside from TV, other variables analyzed were bone volume within fusion tissue mass, % bone volume of fusion mass (the ratio of volume of the trabecular bone compared to the volume of the total fusion mass) and trabecular thickness and bone volume in fusion mass. There was predictably significantly greater bone volume in all three experimental groups than in the control group (A vs B: P< 0.001; A vs C: P<0.001; A vs D: P=0.001) (Table 2). However, bone volume, % bone volume, trabecular separation and trabecular thickness were all difficult to interpret because the carrier ossimend appeared more radiodense than bone. Of note, the left side "fusion mass" of one control sample was excluded from CT data analysis as it had previously been judged to not have fused by all criteria, including manual palpation, radiographic analysis and microCT analysis and it clearly was an exceptionally dense carrier only. Percent bone volume for the excluded sample was more than 10 SD greater than the control group's mean.

Histologic Assesment

Review of sagittal histological specimens show no bridging cortical rim or trabecular bone in the control group. Examination of rhBMP2 specimens and Oxy133 20 ug and Oxy133 60 ug all showed cortical bone along the rim of the fusion mass connecting the transverse processes and bridging trabecular bone within the fusion mass. Interestingly inspection of the histology

11

slides suggested that low dose Oxy133 had more trabecular bone and less adipocytes then high dose Oxy133 (Fig 6).

Discussion

Nonunion of bone, or lack of bone fusion, is a challenging surgical problem that is encountered after treatment of fractures, surgical conditions of the jaw, and spinal surgery. In spinal surgery it is a major concern following treatment of large deformity or multilevel degenerative disc disease. Surgical treatment of non-unions is difficult as the postoperative local environment is often poorly vascularized; therefore every attempt is made at the time of surgery to maximize the likelihood of bone fusion. Prior to 2002 this was accomplished by harvesting autogenous bone graft from the iliac crest and implanting it in the surgical site, which ultimately stimulated new bone formation and fusion. This procedure, however, was associated with significant donor site morbidity and surgical complications and the volume of bone harvested from the iliac crest was often inadequate for the target site [3-5].

As a result of these issues there has been a large amount of research into biologics that possess osteoinductive properties, which ~~are~~ would serve as synthetic fusion adjuncts. To date, the only such biological compound on the market is rhBMP2 which was approved by the FDA for single level anterior lumbar interbody fusion in 2002 [6]. Early results with rhBMP2 showed great efficacy, and it was quickly adopted by surgeons for off label use for posterior and multilevel lumbar fusion, and for fusion surgeries in the cervical spine [17-19]. However, more recently there have been growing concerns about the quality of bone in the fusion masses of patients treated with rhBMP2, possibly related to its adipogenic potential on stem cells [20]. Moreover there are possible side effects, including soft tissue inflammation, airway edema, seroma formation and possible carcinogenicity [7-10, 21, 22]. Also rhBMP2 remains quite expensive

and hence a burden to patients as well as healthcare providers [23].

Our group has previously developed a small osteogenic oxysterol analogue, Oxy133, which has shown promising results for induction of osteogenesis both in vitro when applied to rodent, rabbit, and human mesenchymal stem cells, and in vivo when used in rat posterolateral lumbar fusion procedures (Parhami et al., unpublished data). The purpose of this study was to determine whether Oxy133 is able to stimulate osteogenesis in a larger animal model. To that end, we used a rabbit spinal fusion model and analyzed the fusion rate induced by Oxy133 compared to rhBMP2. Overall, results clearly demonstrated that Oxy133 induces spine fusion equivalent to the rhBMP2 dose used in this study, while the quality and amount of bone formed are at least equal to those induced by rhBMP2 (Tables 1, 2). This confirms and further validates our previous reports of Oxy133 induced bone formation and spinal fusion in rodents (Parhami et al. unpublished observations)[13].

In the present study we used 3 methods to assess and compare fusion among different groups. These methods were radiographic analysis at 4 weeks and 8 weeks, manual palpation of excised spines after 8 weeks, and microCT of the excised specimens. Although our final conclusion from the study takes into account all 3 assessments, it is important to note the specific aspects, strengths and limitations of each method used. Specifically, manual palpation is considered the gold standard for fusion measurement and probably has the greatest specificity but it also doesn't allow analysis of the volume and quality of bridging bone which may be possible with microCT and to a lesser extent radiography. Radiography has the advantage of being possible in a live specimen. These modalities were supplemented by qualitative examination of bone quality with histology.

We believe the slight decrease in fusion rates when assessed by manual palpation

compared to radiography is a function of the fact that manual palpation was considered fused only if the fusion is bilateral, whereas with radiography we considered unilateral fusion to be fusion, ie we used more stringent fusion criteria for manual palpation. Also the mineral collagen matrix of ossimend used as the delivery scaffold for the control vehicle, Oxy133, and rhBMP2 has a radiodensity similar to bone, which made the radiographs, especially at 4 weeks, difficult to interpret. Gross examination of the spines revealed significant fusion masses in the Oxy133 and rhBMP2 group, whereas in the control groups the mineral collagen matrix often seemed to have been incorporated into a fibrous nonunion between the transverse processes. The very similar radiodensity of ossimend to bone potentially caused misclassification of control specimens as fused via radiologic analysis. However, manual palpation, histology and microCT analysis made it clear that those samples were in fact not bony unions and instead represented fibrous nonunion. It is also noteworthy that the lack of any apparent differences between the rate of fusion induced by Oxy133 at 20 mg vs. 60 mg is interesting since we had anticipated a dose response. In our previous reports we had found a dose dependent response to spine fusion induced by Oxy133 in rats (Parhami et al. unpublished data). We speculate that at 20 mg the mechanism by which Oxy133 induces bone formation, i.e. Hedgehog signaling (Parhami et al. unpublished data) is fully activated, and hence higher doses of Oxy133 do not induce any further increases in fusion rate. Alternately, it is possible that some, as yet undetermined interaction, with the carrier or inability of the carrier to carry excess oxysterol may have negated the effect of extra oxysterol. It is also interesting that 20 mg appears to be the optimal dose for stimulating spinal fusion in the rat and in the rabbit model, suggesting a conserved phenomenon between the two species. It remains to be seen whether in larger animals and ultimately in humans a similar or a higher dose will be optimal for stimulation of spine fusion.

14

Our 0% fusion rate of the control group as assessed by manual palpation is consistent with other studies [24]. Our rhBMP2 fusion rate of 83.3% by manual palpation and 91.7% by radiography was similar to the 100% seen in earlier rabbit studies looking at rhBMP2's efficacy [25, 26]. The reason that we saw slightly lower fusion rates for rhBMP2 than previously published data is unclear, but may be related to the relatively lower dose of rhBMP2 and shorter time frame used in this study than some other studies. Itoi et al. did not show 100% fusion with their low dose rhBMP2 group (10 ug) until 12 weeks post op[26]. Another possible reason for the lower fusion rate of rhBMP2 in our study is that the ossimend carrier affected the effectiveness of the rhBMP2. The delivery mechanism of biologics is known to be essential to their effectiveness[12].

It is clear from the microCT analysis that Oxy133 leads to large fusion mass production. Unfortunately, beyond that it was difficult to interpret the microCT results as the ossimend carrier had radiodensity very similar to that of bone. We believe that the ossimend radiodensity was a significant confounding variable. The control group had the second greatest trabecular thickness and least trabecular separation on microCT analysis, but specimens clearly showed no fusion upon manual assessment, suggesting that the microCT results were due to the remaining unincorporated ossimend. We are currently unable to analyze with microCT the extent to which any of the experimental groups, which were considered fused, had incorporated the ossimend carrier and therefore we do not think any meaningful conclusions can be drawn from the analysis of bone volume, % bone volume, trabecular thickness and trabecular separation. There was a trend for the BMP2 and 20 mg dose of Oxy133 groups to have denser bone on microCT analysis than control or 60 mg dose Oxy133. Moreover, histologic analysis suggested that the high dose Oxy133 may in fact have more adipocytes within the fusion mass, where as the 20 mg Oxy133 group and rhBMP2 both appeared to have similarly dense trabecular bone. It remains possible

that this is related to incomplete time for resorbtion of remaining ossimend in the high does Oxy133 group. We are hopeful that a longer time frame in our future experiments will help clarify this issue.

In conclusion, Oxy133 appears to be significantly superior to control and at least as efficacious as rhBMP2 in a rabbit posterolateral lumbar fusion model. Given its antiadipogenic effect on human mesenchymal cells it also may lead to improved fusion mass bone quality though we have not seen that effect at higher doses of Oxy133 in this study. As it is also inexpensive and easy to produce we believe that it remains an excellent subject for further preclinical study as a possible rhBMP2 alternative in surgeries such fracture treatment, jaw surgery and spinal surgery in which biologic fusion adjuncts have been used.

References

1.   Schofferman J, Slosar P, Reynolds J, Goldthwaite N, Koestler M. A prospective randomized comparison of 270 degrees fusions to 360 degrees fusions (circumferential fusions). Spine (Phila Pa 1976) 2001;26:E207-12.
2.   Weinstein JN, Lurie JD, Olson PR, Bronner KK, Fisher ES. United States' trends and regional variations in lumbar spine surgery: 1992-2003. Spine (Phila Pa 1976) 2006;31:2707-14.
3.   Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. Clin Orthop Relat Res 1996:300-9.
4.   Vaccaro AR, Chiba K, Heller JG, et al. Bone grafting alternatives in spinal surgery. Spine J 2002;2:206-15.
5.   Rihn JA, Kirkpatrick K, Albert TJ. Graft options in posterolateral and posterior interbody lumbar fusion. Spine (Phila Pa 1976) 2010;35:1629-39.
6.   Mitka M. Questions about spine fusion product prompt a new process for reviewing data. JAMA 2011;306:1311-2.
7.   Lewandrowski KU, Nanson C, Calderon R. Vertebral osteolysis after posterior interbody lumbar fusion with recombinant human bone morphogenetic protein 2: a report of five cases. Spine J 2007;7:609-14.
8.   Wong DA, Kumar A, Jatana S, Ghiselli G, Wong K. Neurologic impairment from ectopic bone in the lumbar canal: a potential complication of off-label PLIF/TLIF use of bone morphogenetic protein-2 (BMP-2). Spine J 2008;8:1011-8.
9.   Smucker JD, Rhee JM, Singh K, Yoon ST, Heller JG. Increased swelling complications associated with off-label usage of rhBMP-2 in the anterior cervical spine. Spine (Phila Pa 1976) 2006;31:2813-9.
10.   Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. Spine J 2011;11:471-91.
11.   Kha HT, Basseri B, Shouhed D, et al. Oxysterols regulate differentiation of mesenchymal stem cells: pro-bone and anti-fat. J Bone Miner Res 2004;19:830-40.
12.   Minamide A, Kawakami M, Hashizume H, Sakata R, Tamaki T. Evaluation of carriers of bone morphogenetic protein for spinal fusion. Spine (Phila Pa 1976) 2001;26:933-9.
13.   Johnson JS, Meliton V, Kim WK, et al. Novel oxysterols have pro-osteogenic and anti-adipogenic effects in vitro and induce spinal fusion in vivo. J Cell Biochem 2011;112:1673-84.
14.   Sintuu C, Simon RJ, Miyazaki M, et al. Full-length spp24, but not its 18.5-kDa proteolytic fragment, inhibits bone-healing in a rodent model of spine fusion. J Bone Joint Surg Am 2011;93:1022-32.
15.   Miyazaki M, Morishita Y, He W, et al. A porcine collagen-derived matrix as a carrier for recombinant human bone morphogenetic protein-2 enhances spinal fusion in rats. Spine J 2009;9:22-30.
16.   Zhu W, Rawlins BA, Boachie-Adjei O, et al. Combined bone morphogenetic protein-2 and -7 gene transfer enhances osteoblastic differentiation and spine fusion in a rodent model. J Bone Miner Res 2004;19:2021-32.
17.   Shields LB, Raque GH, Glassman SD, et al. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. Spine (Phila Pa 1976) 2006;31:542-7.
18.   Baskin DS, Ryan P, Sonntag V, Westmark R, Widmayer MA. A prospective, randomized, controlled cervical fusion study using recombinant human bone morphogenetic protein-2 with the CORNERSTONE-SR allograft ring and the ATLANTIS anterior cervical plate. Spine (Phila Pa 1976) 2003;28:1219-24; discussion 25.

19.   Robin BN, Chaput CD, Zeitouni S, Rahm MD, Zerris VA, Sampson HW. Cytokine-mediated inflammatory reaction following posterior cervical decompression and fusion associated with recombinant human bone morphogenetic protein-2: a case study. Spine (Phila Pa 1976) 2010;35:E1350-4.
20.   Zara JN, Siu RK, Zhang X, et al. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. Tissue Eng Part A 2011;17:1389-99.
21.   Garrett MP, Kakarla UK, Porter RW, Sonntag VK. Formation of painful seroma and edema after the use of recombinant human bone morphogenetic protein-2 in posterolateral lumbar spine fusions. Neurosurgery 2010;66:1044-9; discussion 9.
22.   Stachniak JB, Diebner JD, Brunk ES, Speed SM. Analysis of prevertebral soft-tissue swelling and dysphagia in multilevel anterior cervical discectomy and fusion with recombinant human bone morphogenetic protein-2 in patients at risk for pseudarthrosis. J Neurosurg Spine 2011;14:244-9.
23.   Buttermann GR. Prospective nonrandomized comparison of an allograft with bone morphogenic protein versus an iliac-crest autograft in anterior cervical discectomy and fusion. Spine J 2008;8:426-35.
24.   Jenis LG, Wheeler D, Parazin SJ, Connolly RJ. The effect of osteogenic protein-1 in instrumented and noninstrumented posterolateral fusion in rabbits. Spine J 2002;2:173-8.
25.   Schimandle JH, Boden SD, Hutton WC. Experimental spinal fusion with recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976) 1995;20:1326-37.
26.   Itoh H, Ebara S, Kamimura M, et al. Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine (Phila Pa 1976) 1999;24:1402-5.

Figure Legends

Figure 1. Radiographic Assessment of Fusion at 4 and 8 Weeks After Surgery
At 4 weeks, both oxysterol groups (C and D) had greater fusion than the control group (A). The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). At 8 weeks groups B, C and D all had fusion greater than that seen in the control group. A vs D P=0.011; A vs B P= 0.020; A vs C 0.020; p value <0.05

Figure 2. Sample Radiographs at 8 Weeks.
Sample radiographs of two animals from each group are shown. The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). Bilateral fusion masses can be seen in the samples from groups B, C, and D and are indicated by arrows. Lack of fusion with unincorporated ossimend is seen in the samples from group A, highlighted by the arrowheads.

Figure 3. Fusion at 8 weeks by Manual Palpation
Animals were sacrificed and the spines surgically excised and the soft tissue cleared away. Manual palpation was performed as described previously. The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). All experimental groups B, C, and D had significantly greater fusion than the control group A. A vs B P= 0.015; A vs C P=0.015; A vs D P=0.048. Significance was set at p<0.05.

Figure 4. Fusion at 8 weeks by microCT
Excised spines were analyzed by microCT as described in the Methods section. The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). All experimental groups had significantly greater fusion than the control group's fusion rate of 0%. A vs B P= 0.012; A vs C P=0.001; A vs D P=0.001

Figure 5. Sample microCT images at 8 Weeks.
Sample microCT images of two animals from each group are shown. The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). Bilateral fusion masses can be seen in the samples from groups B, C, and D and are indicated by arrows. Lack of fusion with unincorporated ossimend is seen in the samples from group A highlighted by the arrowheads

Figure 6. Sample histology images at 8 Weeks.
Sample sagittal H&E histology images of two animals from each group are shown. The groups were as follows: control with saline (A), 30 ug rhBMP2 (B), 20 mg Oxy133 (C), and 60 mg Oxy133 (D). Bridging rim cortical bone and trabecular bone can be seen in the samples from groups B, C, and D. Lack of rim cortical bone or bridging trabecular bone with unincorporated ossimend is seen in the samples from group A.