TISSUE ENGINEERING: Part B
Volume 22, Number 4, 2016
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.teb.2015.0357

termis
Tissue Engineering
& Regenerative Medicine
International Society

REVIEW ARTICLE

A Review of the Clinical Side Effects of Bone Morphogenetic Protein-2

Aaron W. James, MD,[1–3,*] Gregory LaChaud, BS,[1–3,*] Jia Shen, PhD,[2] Greg Asatrian, BS,[2] Vi Nguyen, BS,[3] Xinli Zhang, MD, PhD,[2] Kang Ting, DMD, DMedSci,[2] and Chia Soo, MD, FACS[1,4]

Bone morphogenetic protein-2 (BMP-2) is currently the only Food and Drug Administration (FDA)-approved osteoinductive growth factor used as a bone graft substitute. However, with increasing clinical use of BMP-2, a growing and well-documented side effect profile has emerged. This includes postoperative inflammation and associated adverse effects, ectopic bone formation, osteoclast-mediated bone resorption, and inappropriate adipogenesis. Several large-scale studies have confirmed the relative frequency of adverse events associated with the clinical use of BMP-2, including life-threatening cervical spine swelling. In fact, the FDA has issued a warning of the potential life-threatening complications of BMP-2. This review summarizes the known adverse effects of BMP-2, including controversial areas such as tumorigenesis. Next, select animal models that replicate BMP-2's adverse clinical effects are discussed. Finally, potential molecules to mitigate the adverse effects of BMP-2 are reviewed. In summary, BMP-2 is a potent osteoinductive cytokine that has indeed revolutionized the bone graft substitute market; however, it simultaneously has accrued a worrisome side effect profile. Better understanding of these adverse effects among both translational scientists and clinicians will help determine the most appropriate and safe use of BMP-2 in the clinical setting.

Introduction

Perhaps no single protein has revolutionized the study of bone biology from bench to bedside as has bone morphogenetic protein-2 (BMP-2). In 1965, Marshall R. Urist discovered a substance in the extracellular bone matrix that had the ability in induce osteogenesis when implanted in extraskeletal soft tissue.[1] BMP-2 is a member of the transforming growth factor beta (TGF-β) superfamily, originally cloned by Wozney *et al.*[2] and is among more than 20 human BMPs described.[3] Although many BMPs have osteogenic properties,[4] recombinant human (rh)BMP-2 was the first and only to be introduced as a bone graft substitute.

First introduced in the United States in 2002 as approved by Food and Drug Administration (FDA) for single-level anterior lumbar interbody fusion (ALIF) within a specific threaded titanium tapered cage,[5,6] BMP-2 was then approved for tibial nonunions as an alternative to autograft in 2004, and for oral maxillofacial reconstructions in 2007.[7,8] In the United States alone, during the time period of 2002 to 2006, the use of BMP-2 dramatically increased from 0.7% to 24.9% of all spine fusion procedures performed, including 36.6% of all spine fusion revisions.[9] By 2007, use of BMP-2 rose to greater than 50% of all primary ALIF, and off-label use of BMP-2 rose correspondingly. For example, BMP-2 was used in 43% of posterior lumbar interbody fusion (PLIF)/transforaminal lumbar interbody fusion (TLIF), 30% in posterolateral lumbar fusion (PLF), and 20% of all anterior cervical discectomy and fusion (ACDF) procedures.[10] Indeed, in many instances, BMP-2 has been championed as a much needed clinical alternative to autograft bone. For example, a retrospective analysis of combined data from seven clinical trials demonstrated that BMP-2 is more effective than autogenous bone graft for radiographic spinal

[1]Department of Orthopaedic Surgery and the Orthopaedic Hospital Research Center, UCLA and Orthopaedic Hospital, University of California, Los Angeles, Los Angeles, California.
[2]Section of Orthodontics, Division of Growth and Development, School of Dentistry, University of California, Los Angeles, Los Angeles, California.
[3]Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California.
[4]Division of Plastic and Reconstructive Surgery, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California.
*The authors share equal responsibility for the work presented herein.

Table 1. The Adverse Effects of BMP-2 in Clinical and Preclinical Models

Clinical side effects associated with BMP-2

Side effect	*Main findings*	*References*
Inflammatory complications	Cervical spine swelling	65,69,145–148
	Seroma formation	65,66,149–154
Radiculopathy	Nerve root compression/postoperative radiculitis	48,55,67,152,155–158
Ectopic bone	Neuroforaminal and soft tissue ectopic bone and bone cyst formation	48,158–163
Osteoclast activation, osteolysis, and subsidence	Vertebral and nonvertebral bone resorption	41,48,55,60,61,156,164–170
	Graft subsidence	41,60,61,166,171
Urogenital events	Retrograde ejaculation	76,172–175
	Bladder retention	172,175
Wound complications	Hematoma	9,79,80,146,152,176,177
	Wound dehiscence	9,13,79,152,176
	Infection	9,48,79,80,151,152,16

*Preclinical side effects with BMP-2 (*in vitro *and* in vivo*)*

Side effect	*Main findings*	*References*
Induction of inflammation	Increase in IL-1β, IL-6, IL-10, IL-17, IL-18, TNF-α	102,105,123,178–180
Osteoclast activation	Potentiation of nuclear factor-kβ ligand and induction of RANKL	104,181–184
	Upregulation of PPARγ pathway and repression of Wnt signaling pathways	185
Induction of adipogenesis and bone cyst formation	Dose-dependent induction of PPARγ expression	64,102,178,186–188

BMP, bone morphogenetic protein-2; IL, interleukin; PPARγ, peroxisome proliferator-activated receptor gamma; RANKL, receptor activator of nuclear kappa-B ligand; TNF-α, tumor necrosis factor-α.

fusion in patients with single-level degenerative disc disease.[11] Compared to use of autograft alone, use of BMP-2 was correlated with reduction in both duration of operation time (25 min) and length of hospital stay (0.75 days).[11] Likewise, the efficacy of BMP-2 use has been elucidated in the treatment of open tibial fractures. The rate of secondary interventions is reduced 1.55-fold with BMP-2, from 27.1% in control patients to 17.5% in rhBMP-2 patients.[12] Furthermore, treatment failure rate of the control group was significantly higher than that of the rhBMP-2 treatment group (34.3% vs. 21.4%).[12]

The exponential rise in BMP-2 use has been associated with an increasing side effect profile. Despite preliminary human trials heralding adverse events with BMP-2 use in lumbar fusion,[13,14] initial industry-sponsored trials examining the use of BMP-2 showed no significant side effects in various common surgical procedures, including ALIF,[5,6,13,15] PLIF,[16]ACDF,[17,18] and PLF.[14,19–21] Remarkably, the combined reported risk from these initial industry-sponsored trials of the early 2000s accumulated to less than 0.5%.[22]

After FDA approval, BMP-2 was believed to be ''near-perfect'' in achieving bone induction without harmful adverse events. However, subsequent independent assessments of the original industry-sponsored publications and independent assessment of original FDA data revealed that BMP-2-associated complications were not nominal and infrequent, but rather ranged from 20% to 70% of cases, and were potentially life threatening because of significant cervical swelling and corresponding dyspnea and dysphagia, requiring intubation.[22] Systematic reviews through investigations from the FDA reports, Yale University of Open Data Access Project, and Medtronic internal reports examined the safety and efficacy of BMP-2. These reviews concluded that there was evidence of reporting bias in the initial industry-sponsored publications and no clear advantages of BMP-2 use as compared with iliac crest bone graft (ICBG).[23,24] Moreover, adverse events associated with BMP-2 were not only frequent but were also occasionally catastrophic especially in anterior cervical spine fusions.[24] These included cervical and soft tissue swelling, airway compromise, and need for reoperation. These new insights prompted the FDA to issue a Public Health Notification about BMP-2 use in 2008.[25]

The current review seeks to summarize the known clinical adverse effects of BMP-2 by presenting the relevant cellular effects of BMP-2, reviewing animal models previously shown to replicate these adverse events, and highlighting preclinical attempts to augment BMP-2 to improve its safety profile. Table 1 provides a summary of complications associated with BMP-2.

Review of BMP-2 Signaling

Canonical BMP signaling transduction begins when BMPs bind to BMP receptor types I and II whose signal is regulated by the phosphorylation of receptor-regulated R-Smad (typically Smad 1, 5, and 8).[26] Phosphorylated R-Smads perpetuate the signal by forming a heterodimer complex with Co-Smad (typically Smad 4) to translocate into the nucleus to direct transcriptional response.[27,28] Among other factors, BMP-2 induces the master osteogenic transcription factor runt-related transcription factor 2 (Runx2) in osteoblasts to induce osteogenic programming[29–31] (see Chen *et al.*[32] for a more complete review of BMP-2 signaling).

However, the cellular effects of BMP-2 a far more pleiotropic than this would suggest. Indeed, among many contexts, BMP-2 activates peroxisome proliferator-activated receptor gamma (PPARγ) signaling,[33] leading to adipogenesis differentiation and fat formation.[34] In addition, BMP-2 induces expression of numerous inflammatory cytokines and chemokines including interleukins (ILs) and tumor necrosis factor (TNF)-α.[35] Moreover, BMP-2 is well known to activate osteoclast through receptor activator of nuclear kappa-B ligand (RANKL).[36] With disparate effects on adipogenesis, inflammation, and osteoclast activation, it is understandable that BMP-2 has been found to elicit a range of clinically relevant side effects.

Clinical Side Effects Associated with BMP-2 Use

BMP-2 has marked species-specific concentration requirements for osteogenesis,[37,38] whereby the BMP-2 concentration necessary for inducing consistent bone formation is substantially higher in nonhuman primates (0.75–2.0 mg/mL) than in rodents (0.02–0.4 mg/mL)[37,38] (reviewed in Boden *et al.*[14]). The current FDA-approved 1.5 mg/mL BMP-2 concentration for human use was determined through nonhuman primate efficacy testing.[37,39] The dose-dependent efficacy in humans has been observed in studies of fracture healing, where the median time to fracture healing was reduced with 1.50 mg/mL but not 0.75 mg/mL BMP-2 (median time to healing was 184, 187, and 145 days in the control, 0.75 mg/mL, and 1.50 mg/mL BMP-2 groups, respectively).[40]

As effective bone healing in humans requires high amounts of proteins, the incidence of side effects is concomitantly increased. In fact, excessive concentrations of BMP-2 may be the most important contributory factor to the majority of adverse events, and increasing doses of BMP-2 do not necessarily result in higher fusion rates in spine procedures and long bone nonunions.[41,42] Various clinical trials are aimed toward elucidating the optimal dosage of BMP-2.[43] Currently, a phase-I clinical trial is evaluating a single percutaneous injection at varying doses of rhBMP-2 in patients with closed diaphyseal fractures.[44] Another clinical study examined the dose efficacy of rhBMP-2 in patients requiring extraction socket augmentation and found statistically significant differences between the 0.75 and 1.50 mg/mL groups. At 25% extraction socket length, 25% of implant positions demonstrated adequate bone formation in the 0.75 mg/mL group compared with 56.25% in the 1.50 mg/mL group.[45] Likewise, a feasibility sinus floor augmentation study showed that although 0.43 mg/mL rhBMP-2 was effective at inducing bone formation, 1.5 mg/mL rhBMP-2 was identified as the most effective concentration for inducing *de novo* bone.[46,47]

Ectopic bone formation with BMP-2

The most recognized adverse event related to BMP-2 use is ectopic bone formation, associated with BMP-2 leakage outside the implant site. Ectopic bone formation is estimated to occur at a rate of nearly six times more than control patients when BMP-2 is added into spinal canal/foramen; CT scan evaluation showed ectopic bone formation in 70.1% of patients who were administered rhBMP-2 compared with 12.9% of patients who were not administered rhBMP-2.[22] Leakage can occur into the epidural space and lead to root compression of nerves by ectopic bone impingement, and the incidence rate of postoperative radiculitis was 14.0% with rhBMP-2 treatment (in comparison with 3.0% in control groups).[48] Biologically, the occurrence of ectopic bone formation with BMP-2 is not surprising, as multiple nonosteoblastic cells undergo osteogenic programming when exposed to BMP-2, including myoblasts,[49] adipocytes,[50] and fibroblasts[51] among others.

Ectopic bone formation presumably could occur because of premature leakage of BMP-2 during manual manipulation of the absorbable collagen sponge into the cage[52,53] while preparing for insertion during an interbody fusion. While comparing the overall sizes in different segments of the spinal column, because of the narrower dimensions of the cervical spine, any leakage during graft placement of the BMP-soaked sponge would be in proximity to the spinal canal or foramina than the larger diameter of the other sections of the spine, ultimately leading to increased susceptibility to cord compression.[54] In summary, the implantation of BMP-2, delivered on the absorbable collagen sponge, increases the potential of BMP-2 leakage, resulting in subsequent neural impingement, secondary to ectopic bone formation. Of note, efforts have been made to determine mechanisms to reduce ectopic bone formation with BMP-2 on a collagen sponge carrier. Recent emphasis centers on the exact placement of the sponge. For example, placement of the rhBMP-2 sponge anterior to and within the interbody cage rather than in the posterior half of the interbody space minimizes the risk of ectopic bone formation in the spinal canal and intervertebral foramen.[55] In addition, the manufacturer of rhBMP-2 recommends a few cautions in the manipulation of the rhBMP-2/sponge product to minimized BMP-2 extravasation. According to the package insert and instructions for preparation of the Medtronic rhBMP-2 product, it is crucial to avoid ''use of irrigation or suction near the implanted device'' and ''excessive squeezing of the wetted sponge.''[56]

Osteoclast activation, osteolysis, and subsidence with BMP-2

Osteolysis and subsidence are serious adverse events of spinal surgeries that are also observed at an increased rate with BMP-2 treatment. As previously noted, application of BMP-2 is well known to enhance osteoclastic activity in a cancellous bone environment.[57] Three studies have examined the rates of subsidence and related complications in spinal procedures with BMP-2.[5,58,59] Postoperative radiological depiction of the surgical area discovered collapse of the disc space, large osteolytic cystic lesions, implant displacement or loosening, and subsidence all in increased frequency in the BMP-2 groups as compared with the control group in a randomized controled trial (RCT) of ALIF procedures.[5,58,59] Occurrence rates of these events summed to 9.6% in groups with BMP-2 use, nearly a 1.5-fold increase when compared with ICBG control group especially during the immediate postsurgical period. In another study, McClellan *et al.* found a 68% and 74% incidence of vertebral bone resorption and graft subsidence, respectively, in patients treated with rhBMP-2.[60] Similarly, Vaidya *et al.* found an 82% rate of lumbar end plate resorption correlated with use of rhBMP-2.[61] In summary, in keeping with the known osteoclastogenic effects of BMP-2, a significant increase in osteolysis and subsidence is observed with BMP-2 use.

Bone cyst formation with BMP-2

The eventual fate of mesenchymal stem cells (MSCs) is hypothesized to be controlled by the antagonistic balance between RUNX2 and PPARγ.[62] PPARγ promotes adipogenesis upon activation while suppressing osteogenesis by downregulating the expression and transactivation ability of Runx2.[33] Although there is no clinical summary of bone cyst formation at the patient level, it has been previously reported in *in vivo* preclinical studies.[63] Although bone volume is significantly increased with the use of BMP-2, the quality of bone mineral density has been reported to be poor. Previous studies have demonstrated an increased spacing in trabecular bone formed in proximity to BMP-2 implantation, with interspersed lipid deposition, resulting in cystic bone formation.[63,64] Thus, despite the robust proosteogenic effect BMP-2 elicits, the proadipogenic effects on MSCs decrease the overall quality of bone formed.

Inflammatory complications with BMP-2

Previous studies have also noted a significant host of side effects associated with induction of local inflammation secondary to BMP-2 implantation. These range from benign seroma formations to life-threatening effects, such as cervical spine swelling. As issued in 2008, the FDA released a black box warning for BMP-2 use owing to the recognized risk of cervical spine swelling and death.[25] Each inflammatory side effect of BMP-2 will be discussed sequentially hereunder.

Seroma formation is a common side effect of BMP-2, encountered most commonly in the first week postoperatively, as demonstrated by several case studies.[65,66] Similarly, Rihn *et al.* found that lumbar seroma occurred in 1.2% of rhBMP-2 patients compared with 0% of control patients.[48] Frequently seroma formations are subclinical until impingement on nearby organs by mass effect, causing pain or dysesthesias.[54] For example, Robin *et al.* described a postoperative seroma formation with BMP-2 use in the cervical region that led to bilateral paresthesia of the upper extremities until subsequent drainage.[66] In fact, investigators have found elevated levels of inflammatory cytokines in BMP-2-induced seromas, including $\sim$3000-, 5000-, and 34-fold levels of IL-6, IL-8, and TNF-α, respectively.[66]

Similarly, the incidence of radiculitis has been reported to be increased among BMP-2-treated patients. The incidence of radiculitis in the context of BMP-2 treatment is highest among PLIF and TLIF procedures.[67] Rihn *et al.* reported rates of radiculitis among BMP-2-treated patients as high as 14.0% in comparison with control groups (3.0%).[48] These clinical findings were independent of nerve root compression from ectopic bone formation and appear to be mediated by BMP-2-induced inflammation of the nerve root.[68] However, not all studies have reported a significant difference in leg pain scores between rhBMP-2 and control patient groups. Interestingly, the 2010 FDA Executive Summary of the Dimar *et al.* trial[20] found that postoperative leg pain scores in both the ICBG control and rhBMP-2 matrix groups improved in an almost identical manner over all time intervals. In addition, Boden *et al.* showed that patients in both the BMP-2 and autograft control groups reported similar degrees of leg pain in the early postoperative period, as indicated by their Oswestry Disability Index scores of 13.0 and 12.0, respectively.[14]

Most worrisome, however, is the well-described increased incidence in cervical spine swelling—a potentially life-threatening side effect of BMP-2, resulting in a FDA black box warning.[25] Inflammation of the cervical spine was first described as a side effect by Smucker *et al.* in 2006, and since six fatalities have been reported.[69] Overall, the FDA received 38 reports of complications because of ''off-label'' use of BMP-2 in anterior cervical surgery in 2008. Patients having swelling of the neck and throat tissues experienced compression of the airway dysphagia, and difficulty breathing or speaking.[70] In an attempt to define appropriate use of BMP-2, in 2014 the North American Spine Society released coverage policy recommendations stating: ''Based on the available evidence, BMP[-2] is indicated as an adjunct to spinal fusion in cases in which other alternatives are either not available or are not likely to lead to effective fusion.''[71] Furthermore, BMP-2 is not indicated in the following scenarios: ''Routine anterior and posterior cervical fusion procedures, single level posterior/posterolateral fusion in healthy adults, and routine pediatric spine fusion procedures.''[71] To the authors' knowledge, there have not been reports of major complications of BMP-2 use in cervical spinal surgeries since this time, which most likely reflects change in practices to avoid BMP-2 in this anatomic region.

Urogenital events

BMP-2 has also been observed to elicit urogenital complications in numerous clinical reports, including retrograde ejaculation and persistent bladder retention. Retrograde ejaculation after anterior lumbar surgery occurs at a rate less than 1%;[72–74] however, the adverse event of retrograde ejaculation associated with the use of BMP-2 with the LT-cage was reassessed by the FDA Summary of Safety and Effectiveness Data[59] using the same cohort and found to be significantly increased in the group receiving BMP-2.[75] This was confirmed by an RCT that observed higher rates of retrograde ejaculations in male patients who received BMP-2 than those who received an ICBG. The rate of retrograde ejaculation after anterior lumbar surgery with the use of BMP-2 increases the frequency of retrograde ejaculation by at least sixfold, depending on the surgical procedure specifics.[76,77] Specifically, Carragee *et al.* found a 7.2% rate of retrograde ejaculation in the rhBMP-2 groups (as compared with 0.6% rate among the control group).[76]

Bladder retention is also a concern with any invasive procedure, whether it be from anatomical obstruction (lysis of the ureter) or damage to the nervous system (obliteration of the parasympathetic system). Early bladder retention was studied by the FDA Summary of Safety and Effectiveness Data and found to be twice as common in patients receiving BMP-2. The frequency of early bladder retention in the control group saw a rate of 3.6%, whereas in those receiving BMP-2, a rate of 7.9% was observed; an increase that proved to be statistically significant. Thus, increased incidence of both retrograde ejaculation and persistent bladder retention has been observed in multiple independent reports related to BMP-2 use.

Wound complications with BMP-2

Wound complications entail a variety of adverse events including epidural hematoma, wound dehiscence, postoperative

fever, and hemorrhage, to name a few. A reassessment of the pilot study of Boden *et al.* found wound complication rates to be 10% in the BMP-2 group, significantly higher than the control group without BMP-2 use in PLF.[78] Likewise, Chan *et al.* found significantly higher wound complication rates among the rhBMP-2 treatment group (31%) than among the control group (18%).[79] Further analysis of complications in posterior fusion surgeries illustrates a fivefold increase (2.1% vs. 0.4%) in wound complications associated with BMP-2 use during a posterior approach.[80]

Infections, both local and systemic, are known complications of all invasive procedures. Early wound infections (less than 6 weeks postoperatively) were equivalent in BMP-2 and ICBG groups in lumbar fusion surgeries (9.4%); however, an increase was observed in delayed infections (>6 weeks postoperatively), whereby BMP-2 led to a threefold increase (4.2% in BMP-2 group vs. 1.4% in ICBG group).[59] Moreover, deep wound infections were found at a fivefold greater rate (2.1% vs. 0.4%) in patients receiving BMP-2 in anterior/posterior surgeries.[80] The mechanisms behind increased rates of infection with BMP-2 are not fully understood.

Risk of tumor formation with BMP-2

The role of BMP-2 in carcinogenesis is a controversial topic, for which the linkage (if any truly exists) is tenuous. However, it is clear that deviation from normal physiologic expression of BMP-2 is associated with multiple tumor types, involving diverse organs including prostate, breast, oral mucosa, pleura, and bone.[81–85] Although variation by organ system is seen, BMP-2 is generally upregulated in diverse tumors and is associated with tumor cell proliferation, invasion, and at times a poor clinical prognosis. The basic biologic importance of BMPs in diverse cancers raises potential concerns about its clinical use.[86] Data from a multicenter, RCT of patients undergoing posterolateral fusions with BMP-2 did show a higher incidence of carcinoma among patients with BMP-2 treatment than those with autograft control.[87] Of note, multiple investigators have studied the link between BMP-2 and the incidence of cancer in large-scale studies and have not been able to replicate these findings.[88–91] Of note, most studies are limited by long-term follow-up. Perhaps the most comprehensive study was recently published by Kelly *et al.* who examined the records of 467,916 Medicare patients undergoing spinal arthrodesis with or without BMP-2.[92] Their study that had an average of 2.9-year follow-up period showed that BMP-2 exposure actually reduced the risk of developing cancer (relative risk: 0.938; 95% CI: 0.913–0.964). Thus, the largest clinical studies to date have not been able to find a link between BMP-2 application and carcinogenesis.

Nevertheless, basic biologic studies have shown upregulated BMP ligand expression in breast carcinoma,[82,93] lung,[94] pancreatic,[95] and oral squamous cell carcinoma (SCC),[96] to name a few. BMP-2 increases proliferation within multiple carcinoma types, including breast,[97,98] lung,[94] and some pancreatic tumor cell lines.[95] Moreover, BMP-2 has been shown to increase invasiveness of prostate,[99] lung,[100] oral SCC,[96] and breast carcinoma,[97] potentially by activating Tenascin-W[101] and increasing CCL5 expression.[96] Moreover, aberrant BMP signaling has been proposed to be of biologic importance in bone metastases of breast[82] and prostate carcinoma.[99] Thus, the oncologic concerns of clinical BMP-2 use are largely rooted in the basic biology of BMP signaling, with clinical studies predominantly not able to find a cause for concern.

Bone-Related Side Effects with BMP-2 Use in Animal Studies

Induction of inflammation

In association with BMP-2 use, *in vitro*, studies have shown increased concentrations of numerous cytokines and chemokines including IL-1α, IL-1β, IL-6, IL-10, IL-18, TNF-α, macrophage inflammatory protein 1 α (MIP-1 α), and monocyte chemotactic protein 1 (MCP-1) in animals treated with BMP-2. Supra-physiologic levels of BMP-2 can alter the balance between proinflammatory and anti-inflammatory cytokines tipping it in favor of inflammation.

Recent studies by Zara *et al.* have extended these findings, showing that high-dose BMP-2 induces inflammation in an orthotopic model that results in structurally abnormal bone formation *in vivo*.[102] Here, BMP-2 (4 mg/mL) was applied to rats in a femoral-only model, assessing inflammation at early timepoints (3–14 days).[102] Compared with control-treated rats, BMP-2-treated animals exhibited significant local inflammation, including increased presence of monomorphonuclear and polymorphonuclear cells and calcitonin receptor (CTR)-positive osteoclast-like cells.[102] When extended to a femoral bone defect model, a similar induction of inflammation was noted along with a dose-dependent induction of structurally abnormal bone cysts. Of note, the time course of inflammation in these rodent experiments mimics that of published clinical reports of BMP-2-induced cervical swelling.[61]

Interestingly, Yamazaki *et al.* also determined that TNF-α represses BMP-2 signaling by interfering with the DNA binding of Smads through the activation of nuclear factor-kβ.[103] TNF-α inhibits both osteoblast differentiation and bone formation, as pretreatment with TNF-α abolished BMP-2-induced ALP and Id1-luciferase activity.[103] Thus, the increased levels of TNF-α associated with BMP-2 use result in not only induction of inflammation but also an unwanted inhibition of osteoblastogenesis.

Interferon-γ is one of the major proinflammatory cytokines that leads to significant inflammatory changes by elevating TNF-α expression, which is one of the principle cytokines that activates the inflammatory cascade. Acting as chemokines, IL-8 and MCP-1 recruit neutrophils and monocytes to their location and trigger the firm adhesion of monocytes to the local vascular endothelium.

Osteoclast activation

Multiple lines of evidence suggest that BMP-2 induces osteoclastic activity both *in vitro* and *in vivo*, through direct and indirect mechanisms. BMP-2 directly stimulates osteoclastogenesis by potentiating receptor activator of nuclear factor-kβ ligand. In addition, BMP-2 enhances RANKL-induced osteoclast differentiation.[104] In addition, numerous inflammatory cytokines induced by BMP-2 secondarily induce osteoclastic activity, including IL-6.[105] Indirectly, BMP-2 affects osteoclast activity by upregulation of the PPARγ pathway and repression of the Wnt signaling pathway. Also, this increases osteoclast quantity by upregulating c-fos expression.

In recent novel studies, Tasca *et al.* determined that expression of Smad1/5 and Smad4, elements of the canonical

BMP pathway, is integral to osteoclast differentiation.[106] Specifically, loss of *Smad1/5* or *Smad4* expression was induced in osteoclasts precursors through infection with adenoviruses expressing Cre recombinase (Ad-CRE).[106] Compared with control, Ad-CRE cells exhibited decreased osteoclast differentiation, as indicated by the presence of fewer and smaller multinuclear cells.[106] Moreover, expression of osteoclast-enriched genes *Dc-stamp* and *Cathepsin K* was reduced in Ad-CRE cells.[106] To further assess the effect of BMP-2 on osteoclast activity, mature osteoclasts were treated with either BMP-2 or dorsomorphin, a BMP pathway inhibitor.[106] Whereas BMP-2 stimulated osteoclast resorption, dorsomorphin blocked this activity.[106] Thus, both BMP-2 and elements of the canonical BMP pathway, including Smad1/5 and Smad4, are crucial to osteoclast differentiation and osteoclast-mediated resorption.

Induction of adipogenesis

Many pro-osteogenic cytokines show converse anti-adipogenic properties—however, BMP-2 is a notable exception. BMP-2 dose dependently induces PPARγ expression, the master transcriptional regulator of adipogenesis. Notably, Takada *et al.* recently described epigenetic crosstalk between BMP-2 and PPARγ.[107] After pretreatment with the PPARγ ligand troglitazone, BMP-2 upregulated mRNA levels of the adipocyte marker genes *Fabp4 and Gpd1* in ST2 stromal cells by enhancing the levels of active histone markers on the promoter of the PPARγ target gene *Fabp4*.[107] Thus, BMP-2 not only affects pleiotropic MSCs by inducing both osteogenic and chondrogenic differentiation through the RUNX2 pathway[108] but can also promote adipogenic differentiation.

Of note, recent studies have shown that BMP receptor type dictates whether adipogenesis or osteogenesis is induced. Whereas signaling through BMPR-IA exerts adipogenic effects, BMPR-IB exerts osteogenic effects.[49,109,110] For example, expression of constitutively active BMPR-IA induces adipogenic differentiation, whereas overexpression of inactive BMPR-IA inhibits adipogenic differentiation.[49] The converse effects were obtained by manipulation of BMPR-IB expression. Namely, constitutive BMPR-IB activation induces osteogenic differentiation, whereas inactive BMPR-IB inhibited osteogenic differentiation.[49] In another study, McArdle *et al.* evaluated the osteogenic potential of BMPR-IB(+) versus BMPR-IB(−) human adipose-derived stromal cells (ASCs).[109] Compared with BMPR-IB(−) ASCs, BMPR-IB(+) ASCs significantly enhanced osteogenesis *in vitro*, as evidenced by increased alkaline phosphatase staining, extracellular matrix mineralization, and osteocalcin expression.[109] *In vivo*, BMPR-IB(+) cells resulted in a twofold increase in bone regeneration in murine critical-sized calvarial defects.[109] However, conflicting data do exist regarding the specificity of BMPRs for lineage differentiation. For example, osteoblast-selective interference of BMPR-IA demonstrated antiosteogenic effects, including irregular calcification and decreased bone mass.[110]

The presence of adipogenesis occurring during BMP-2-mediated osteogenesis results in formation of cyst-like bone voids filled with fatty marrow instead of the standard trabecular bone structure.[102] Despite complete bone union, the increased concentrations of BMP-2 (150–600 μg/mL) that are required for human osteogenesis lead to inconsistent bone formation and decreased overall bone quality through activation of PPARγ and downregulation of the Wnt signaling pathway.[102] Moreover, the formation of cystic bone voids from high-dosage BMP-2 can be repressed with the coapplication of Wnt signaling pathway activators.[102]

Methods to Improve BMP-2 Use

With an increased knowledge secondary to clinical experience with BMP-2, numerous potential adverse events of BMP-2 have been elucidated, and a significant effort by investigators has been made to find a safer, cheaper, and more efficient pharmaceutical replacement for BMP-2. To date, no suitable alternative has been identified that would have similar or superior efficacy in inducing bone formation without the adverse effects that come with BMP-2.[22]

Thus, the appropriate dosing, carriers, and location of BMP application pose as pharmacologic variables potentially resulting in such adverse events,[111] and as such, investigators have been subject to numerous studies. Several studies, highlighted hereunder, have sought to investigate alterations in dosage, scaffold, and the implementation of supplemental proteins or growth factors to mediate the nonspecific action of BMP-2.

Addition of vascular endothelial growth factor

Effective healing of bone fractures depends on not only osteogenesis but also angiogenesis to reestablish the inflow of oxygen and nutrients necessary to produce healthy bone. Vascular endothelial growth factor (VEGF) is a protein produced by endothelial cells that induces new vascular formation after injury.[112–114] VEGF has been shown to be essential for endochondral and intramembranous bone formation.[115] Recent evidence suggests that not only does VEGF produce angiogenesis but there is also cross-talk between VEGF and the BMP signaling pathway that plays a role in osteoblastic differentiation of MSCs.[116]

Unlike BMP-2, the use of VEGF alone in bone fractures is not sufficient enough to lead to complete healing;[117] however, when combined with BMP-2, VEGF increases the rate of complete union healing in defects in a synergistic manner in rat models with critical size defects.[117] It must be noted, however, that although combination treatment with BMP-2 and VEGF possesses superior efficacy of bone formation, coimplantation and corresponding angiogenesis may result in aberrant migration of BMP-2 to unwanted areas and potentiate higher rates of ectopic bone formation.[118]

Addition of bisphosphonates

One of the many concerns with BMP-2 use is late osteoclast activation potential due to the effect BMP-2 has on both the number and functionality of osteoclasts. One budding solution to this increased activity of osteoclasts is to inhibit osteoclast function or number, thus effectively eliminating that particular side effect.[119] Bisphosphonates are a class of drugs directed at preventing loss of bone mass and are clinically relevant by treating osteoporosis.[120–122]

Use of bisphosphonates in conjunction with BMP-2 proved to significantly decrease the number of osteoclasts per bone surface area. In an experiment conducted on piglets with Legg–Calvé–Perthes disease, BMP-2 administered with bisphosphonate

decreased bone resorption while maintaining increased new bone formation compared with BMP-2 alone.[119] With this decrease, extreme bone loss associated with BMP-2 that could lead to osteolysis and subsidence can be avoided. Conversely, with a decreased quantity of osteoclasts, osteoblastic differentiation and functionality are much less regulated, and side effects such as heterotopic ossification are found much more frequently than with BMP-2 use alone.[119]

Addition of anti-inflammatory drugs

Supraphysiological dosing of BMP-2 causes a large inflammatory response as emphasized in the inflammation section. Major cytokines IL-6, IL-10, and TNF-α as well as the formation of inflammatory exudates, highlight the proinflammatory state that BMP-2 induces locally.[123] The administration of adjunctive corticosteroids along with BMP-2 is directed at decreasing the inflammatory reaction induced by BMP-2.[124]

To curb such an inflammatory response, low-dose dexamethasone has been studied and shown to significantly reduce soft-tissue inflammation and cellular invasion.[124] As demonstrated by high-powered microscopy, the inflammatory region in a rat model after administration of rhBMP-2 or the combination of rhBMP-2 with different steroid doses had a significantly larger mean area solely in the rhBMP-2-only group than that in the control.[124] This suppression of the chemokines avoids the infiltration of monomorphic and polymorphic nucleated cells that are attracted to the local connective tissues as well as the edema that impedes the healing process.[125,126]

Alternative carriers for BMP-2

Although an absorbable collagen sponge has been approved and used in humans, the optimal scaffold for BMP-2 delivery in humans has not been established. Of note, numerous organic and synthetic scaffolds have been tested for BMP-2 release, and a complete listing is not feasible here (see Haidar *et al*.[127,128] for a further discussion).

The four major categories of BMP-2 carriers include natural-origin polymers, inorganic materials, synthetic biodegradable polymers, and composites.[127] In addition to the previously discussed collagen sponge, natural-origin polymers include hyaluronic acid (HA) and chitosan (CH). Compared with collagen sponges, HA-based delivery vehicles possess greater BMP-2 retention capacity[129] and result in improved bone formation in mandibular defects.[130] Similarly, rhBMP-2-loaded CH has been reported to accelerate osteogenesis in a rat critical-sized mandibular defect.[131]

Inorganic BMP-2 carriers include hydroxyapatite (HAP) calcium phosphate-based cements, ceramics, and coatings (CPCs), and bioactive glasses (BGs). Thus far, only porous HAP has been evaluated as a scaffold for BMP-2; however, it is inefficient as a controlled release carrier because of high BMP affinity and lack of HAP resorption.[132] Interestingly, studies have shown that CPCs require lower doses of BMPs than other carriers.[133] In addition, various studies have demonstrated that BGs enhance the effects of BMP and induce osteoblast differentiation.[134,135]

Synthetic biodegradable polymers have also been deemed as favorable BMP-2 carriers because of lack of immunogenicity or disease transmission.[127] The most common polymers include polylactic acid (PLA), polyglycolide (PLG), and their copolymer, PLGA.[127] PLA, PGA, and PLGA have been effectively used as rhBMP-2 carriers to repair diverse bone defects, including mandibular defects in a rat model, and periodontal, maxillary alveolar cleft, and segmental ulnar long-bone defects in a canine model, among others.[136–139]

Lastly, composites are derived from combinations of the carriers discussed previously. In comparison to carriers composed of solely HAP, composites consisting of HAP, tricalcium phosphates, and collagen have been found to result in improved local BMP delivery and orthotopic bone formation.[140,141]

In summary, although there are many suitable delivery systems available, the ideal BMP-2 carrier remains elusive.

Summary and Conclusions

The proper use of BMP-2 in the treatment of some patients with impaired fusion capacity can be an immense medical benefit that can clinically alter the overall long-term outcome of a surgical procedure. Thus, circumstantial use of BMP-2 has the capability to decrease prevalence of repeated surgical re-entry, trauma, complications, and additional medical cost.[142] However, with this benefit comes a set of risks due to the supraphysiological dosing requirements and off-label use for inappropriate indication or anatomical location.

Once BMP-2 was declared to be FDA approved for one particular surgical procedure coupled with reporting ''near perfect'' safety,[143] this caused an extensive escalation of use in a wide range of orthopaedic procedures. Larger clinical trials were able to unearth more clinically relevant adverse events previously unknown. Still unclear is the tentative link between BMP-2 and an increased risk of cancer development.

The ideal candidate and best practices for BMP-2 use are difficult to determine and are dependent on the source consulted. Based on the available evidence, BMP-2 is indicated as a second-line adjunct to spinal fusion in cases in which other alternatives are either not available or are not likely to lead to effective fusion. Indications for application in the pediatric population are more difficult to define, as guidelines for age, weight, and level-dependent dosage of rhBMP-2 have yet to be elucidated.[144] Application in specific anatomic locations is correlated with distinctive adverse events. Although use in the lumbar spine is efficacious, ALIF is associated with increased risk for implant displacement, subsidence, infection, and urogenital events. Likewise, PLIF is associated with radiculitis, ectopic bone formation, and osteolysis.[22] Application in the cervical spine is associated with rare life-threatening complications and is contraindicated.[22,24]

In conclusion, the application of BMP-2 from bench to bedside has revolutionized the study of bone biology. Although BMP-2 is associated with a concerning side effect profile, it is currently the only osteoinductive growth factor to receive FDA approval for use as a bone graft substitute. Clearly there is an appropriate clinical context for rhBMP-2 use, especially when alternative bone graft substitutes are lacking. Despite the multitudes of studies over the past decades that have already been performed, further study of BMP-2 will better assess long-term results, examine alternative biologics or scaffolds to be included with BMP-2, and examine the cost–benefit of BMP-2 to the healthcare system.

Acknowledgments

This work was supported by the NIH/NIAMS (grants R01 AR061399, R01 AR066782, and K08 AR068316), the UCLA

Department of Pathology and Laboratory Medicine, the Translational Research Fund, the UCLA Daljit S. and Elaine Sarkaria Fellowship award, and the Orthopaedic Research and Education Foundation with funding provided by the Musculoskeletal Transplant Foundation.

Disclosure Statement

No competing financial interests exist.

References

1. Urist, M.R. Bone: formation by autoinduction. Science **150,** 893, 1965.
2. Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Kriz, R.W., Hewick, R.M., and Wang, E.A. Novel regulators of bone formation: molecular clones and activities. Science **242,** 1528, 1988.
3. Bragdon, B., Moseychuk, O., Saldanha, S., King, D., Julian, J., and Nohe, A. Bone morphogenetic proteins: a critical review. Cell Signal **23,** 609, 2011.
4. Marie, P.J., Debiais, F., and Hay, E. Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling. Histol Histopathol **17,** 877, 2002.
5. Burkus, J.K., Transfeldt, E.E., Kitchel, S.H., Watkins, R.G., and Balderston, R.A. Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976) **27,** 2396, 2002.
6. Burkus, J.K., Gornet, M.F., Dickman, C.A., and Zdeblick, T.A. Anterior lumbar interbody fusion using rhBMP-2 with tapered interbody cages. J Spinal Disord Tech **15,** 337, 2002.
7. Rengachary, S.S. Bone morphogenetic proteins: basic concepts. Neurosurg Focus **13,** e2, 2002.
8. Summary of Safety and Effectiveness Data. Available at www.fda.gov/cdrh/pdf5/p050053b.pdf (accessed October 28, 2014).
9. Cahill, K.S., Chi, J.H., Day, A., and Claus, E.B. Prevalence, complications, and hospital charges associated with use of bone-morphogenetic proteins in spinal fusion procedures. JAMA **302,** 58, 2009.
10. Ong, K.L., Villarraga, M.L., Lau, E., Carreon, L.Y., Kurtz, S.M., and Glassman, S.D. Off-label use of bone morphogenetic proteins in the United States using administrative data. Spine **35,** 1794, 2010.
11. Garrison, K.R., Donell, S., Ryder, J., Shemilt, I., Mugford, M., Harvey, I., and Song, F. Clinical effectiveness and cost-effectiveness of bone morphogenetic proteins in the non-healing of fractures and spinal fusion: a systematic review. Health Technol Assess **11,** 1, 2007.
12. Wei, S., Cai, X., Huang, J., Xu, F., Liu, X., and Wang, Q. Recombinant human BMP-2 for the treatment of open tibial fractures. Orthopedics **35,** e847, 2012.
13. Boden, S.D., Zdeblick, T.A., Sandhu, H.S., and Heim, S.E. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine (Phila Pa 1976) **25,** 376, 2000.
14. Boden, S.D., Kang, J., Sandhu, H., and Heller, J.G. Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: a prospective, randomized clinical pilot trial: 2002 Volvo Award in clinical studies. Spine **27,** 2662, 2002.
15. Burkus, J.K., Heim, S.E., Gornet, M.F., and Zdeblick, T.A. Is INFUSE bone graft superior to autograft bone? An integrated analysis of clinical trials using the LT-CAGE lumbar tapered fusion device. J Spinal Disord Tech **16,** 113, 2003.
16. Haid, R.W., Jr., Branch, C.L., Jr., Alexander, J.T., and Burkus, J.K. Posterior lumbar interbody fusion using recombinant human bone morphogenetic protein type 2 with cylindrical interbody cages. Spine J **4,** 527, 2004.
17. Baskin, D.S., Ryan, P., Sonntag, V., Westmark, R., and Widmayer, M.A. 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 **28,** 1219, 2003.
18. Boakye, M., Mummaneni, P.V., Garrett, M., Rodts, G., and Haid, R. Anterior cervical discectomy and fusion involving a polyetheretherketone spacer and bone morphogenetic protein. J Neurosurg Spine **2,** 521, 2005.
19. Glassman, S.D., Dimar, J.R., 3rd, Burkus, K., Hardacker, J.W., Pryor, P.W., Boden, S.D., and Carreon, L.Y. The efficacy of rhBMP-2 for posterolateral lumbar fusion in smokers. Spine **32,** 1693, 2007.
20. Dimar, J.R., 2nd, Glassman, S.D., Burkus, J.K., Pryor, P.W., Hardacker, J.W., and Carreon, L.Y. Clinical and radiographic analysis of an optimized rhBMP-2 formulation as an autograft replacement in posterolateral lumbar spine arthrodesis. J Bone Joint Surg Am Vol **91,** 1377, 2009.
21. Dawson, E., Bae, H.W., Burkus, J.K., Stambough, J.L., and Glassman, S.D. Recombinant human bone morphogenetic protein-2 on an absorbable collagen sponge with an osteoconductive bulking agent in posterolateral arthrodesis with instrumentation. A prospective randomized trial. J Bone Joint Surg Am Vol **91,** 1604, 2009.
22. Carragee, E.J., Hurwitz, E.L., and Weiner, B.K. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. Spine J **11,** 471, 2011.
23. Simmonds, M.C., Brown, J.V., Heirs, M.K., Higgins, J.P., Mannion, R.J., Rodgers, M.A., and Stewart, L.A. Safety and effectiveness of recombinant human bone morphogenetic protein-2 for spinal fusion: a meta-analysis of individual-participant data. Ann Int Med **158,** 877, 2013.
24. Fu, R., Selph, S., McDonagh, M., Peterson, K., Tiwari, A., Chou, R., and Helfand, M. Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: a systematic review and meta-analysis. Ann Intern Med **158,** 890, 2013.
25. Center for Devices and Radiological Health. Public Health Notifications (Medical devices)—FDA Public Health Notification: life-threatening complications associated with recombinant human bone morphogenetic protein in cervical spine fusion. Available at wwwfda.gov/MedicalDevices/Safety/AlertsandNotices/PublicHealthNotifications/ucm062000.htm (accessed October 23, 2014).
26. Shi, Y., and Massague, J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell **113,** 685, 2003.
27. ten Dijke, P., Korchynskyi, O., Valdimarsdottir, G., and Goumans, M.J. Controlling cell fate by bone morphogenetic protein receptors. Mol Cell Endocrinol **211,** 105, 2003.
28. Yi, J.J., Barnes, A.P., Hand, R., Polleux, F., and Ehlers, M.D. TGF-beta signaling specifies axons during brain development. Cell **142,** 144, 2010.
29. Lee, K.S., Kim, H.J., Li, Q.L., Chi, X.Z., Ueta, C., Komori, T., Wozney, J.M., Kim, E.G., Choi, J.Y., Ryoo, H.M., and Bae, S.C. Runx2 is a common target of transforming growth factor beta1 and bone morphogenetic protein 2, and

cooperation between Runx2 and Smad5 induces osteoblast-specific gene expression in the pluripotent mesenchymal precursor cell line C2C12. Mol Cell Biol **20,** 8783, 2000.
30. Takazawa, Y., Tsuji, K., Nifuji, A., Kurosawa, H., Ito, Y., and Noda, M. An osteogenesis-related transcription factor, core-binding factor A1, is constitutively expressed in the chondrocytic cell line TC6, and its expression is upregulated by bone morphogenetic protein-2. J Endocrinol **165,** 579, 2000.
31. Banerjee, C., Javed, A., Choi, J.Y., Green, J., Rosen, V., van Wijnen, A.J., Stein, J.L., Lian, J.B., and Stein, G.S. Differential regulation of the two principal Runx2/Cbfa1 n-terminal isoforms in response to bone morphogenetic protein-2 during development of the osteoblast phenotype. Endocrinology **142,** 4026, 2001.
32. Chen, D., Zhao, M., and Mundy, G.R. Bone morphogenetic proteins. Growth Factors **22,** 233, 2004.
33. Jeon, M.J., Kim, J.A., Kwon, S.H., Kim, S.W., Park, K.S., Park, S.W., Kim, S.Y., and Shin, C.S. Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem **278,** 23270, 2003.
34. Liu, J., Chen, L., Zhou, Y., Liu, X., and Tang, K. Insulin-like growth factor-1 and bone morphogenetic protein-2 jointly mediate prostaglandin E2-induced adipogenic differentiation of rat tendon stem cells. PLoS One **9,** e85469, 2014.
35. Suda, T., Takahashi, N., Udagawa, N., Jimi, E., Gillespie, M.T., and Martin, T.J. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev **20,** 345, 1999.
36. Okamoto, M., Murai, J., Yoshikawa, H., and Tsumaki, N. Bone morphogenetic proteins in bone stimulate osteoclasts and osteoblasts during bone development. J Bone Miner Res **21,** 1022, 2006.
37. McKay, W. Science-based assessment: accelerating product development of combination medical devices. *NMAB Roundtable on Biomedical Engineering Materials and Applications*. Washington, DC, 2003.
38. Bagaria, V., and Prasada, V. Bone morphogenic protein: current state of field and the road ahead. J Orthop **2,** e3, 2005.
39. FDA. InFUSE™ Bone Graft/LT-CAGE™ Lumbar Tapered Fusion Device. Summary of Safety and Effectiveness Data Premarket Approval Application P000058.
40. Govender, S., Csimma, C., Genant, H.K., Valentin-Opran, A., Amit, Y., Arbel, R., Aro, H., Atar, D., Bishay, M., Borner, M.G., Chiron, P., Choong, P., Cinats, J., Courtenay, B., Feibel, R., Geulette, B., Gravel, C., Haas, N., Raschke, M., Hammacher, E., van der Velde, D., Hardy, P., Holt, M., Josten, C., Ketterl, R.L., Lindeque, B., Lob, G., Mathevon, H., McCoy, G., Marsh, D., Miller, R., Munting, E., Oevre, S., Nordsletten, L., Patel, A., Pohl, A., Rennie, W., Reynders, P., Rommens, P.M., Rondia, J., Rossouw, W.C., Daneel, P.J., Ruff, S., Ruter, A., Santavirta, S., Schildhauer, T.A., Gekle, C., Schnettler, R., Segal, D., Seiler, H., Snowdowne, R.B., Stapert, J., Taglang, G., Verdonk, R., Vogels, L., Weckbach, A., Wentzensen, A., Wisniewski, T., and BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) Study Group. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J Bone Joint Surg Am **84-A,** 2123, 2002.
41. Pradhan, B.B., Bae, H.W., Dawson, E.G., Patel, V.V., and Delamarter, R.B. Graft resorption with the use of bone morphogenetic protein: lessons from anterior lumbar interbody fusion using femoral ring allografts and recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976) **31,** E277, 2006.
42. Lee, K.B., Johnson, J.S., Song, K.J., Taghavi, C.E., and Wang, J.C. Use of autogenous bone graft compared with RhBMP in high-risk patients: a comparison of fusion rates and time to fusion. J Spinal Disord Tech **26,** 233, 2013.
43. McKay, W.F., Peckham, S.M., and Badura, J.M. A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). Int Orthop **31,** 729, 2007.
44. Bishop, G.B., and Einhorn, T.A. Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery. Int Orthop **31,** 721, 2007.
45. Fiorellini, J.P., Howell, T.H., Cochran, D., Malmquist, J., Lilly, L.C., Spagnoli, D., Toljanic, J., Jones, A., and Nevins, M. Randomized study evaluating recombinant human bone morphogenetic protein-2 for extraction socket augmentation. J Periodontol **76,** 605, 2005.
46. Boyne, P.J., Marx, R.E., Nevins, M., Triplett, G., Lazaro, E., Lilly, L.C., Alder, M., and Nummikoski, P. A feasibility study evaluating rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. Int J Periodontics Restorative Dent **17,** 11, 1997.
47. Boyne, P.J., Lilly, L.C., Marx, R.E., Moy, P.K., Nevins, M., Spagnoli, D.B., and Triplett, R.G. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. J Oral Maxillofac Surg **63,** 1693, 2005.
48. Rihn, J.A., Patel, R., Makda, J., Hong, J., Anderson, D.G., Vaccaro, A.R., Hilibrand, A.S., and Albert, T.J. Complications associated with single-level transforaminal lumbar interbody fusion. Spine J **9,** 623, 2009.
49. Chen, D., Ji, X., Harris, M.A., Feng, J.Q., Karsenty, G., Celeste, A.J., Rosen, V., Mundy, G.R., and Harris, S.E. Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol **142,** 295, 1998.
50. Fraser, J.K., Wulur, I., Alfonso, Z., and Hedrick, M.H. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol **24,** 150, 2006.
51. Ishihara, A., Weisbrode, S.E., and Bertone, A.L. Autologous implantation of BMP2-expressing dermal fibroblasts to improve bone mineral density and architecture in rabbit long bones. J Orthop Res **33,** 1455, 2015.
52. Spector, M., Zanella, J.M., Peckham, S.M., and Sanduh, H.S. Release of recombinant human bone morphogenetic protein-2 from absorbable collagen during the mechanical compression associated with its use for spinal fusion. *Poster session present at 50th Annual Meeting of the Orthopaedic Research Society*, 2004.
53. Pradhan, B.B., Bae, H.W., Kropf, M.A, Patel, V.V., Zhao, L., Wong, P., and Delamarter R.B. Leakage of rhBMP-2 from absorbable collagen sponges during use in anterior cervical discectomy and fusion: quantification by assay and radiographic follow-up. *Poster presented at 51st Orthopaedic Research Society*, 2005.
54. Benglis, D., Wang, M.Y., and Levi, A.D. A comprehensive review of the safety profile of bone morphogenetic protein in spine surgery. Neurosurgery **62,** ONS423, 2008.
55. Rihn, J.A., Makda, J., Hong, J., Patel, R., Hilibrand, A.S., Anderson, D.G., Vaccaro, A.R., and Albert, T.J. The use

of RhBMP-2 in single-level transforaminal lumbar interbody fusion: a clinical and radiographic analysis. Eur Spine J **18,** 1629, 2009.
56. Zou, X., Shen, J., Chen, F., Ting, K., Zheng, Z., Pang, S., Zara, J.N., Adams, J.S., Soo, C., and Zhang, X. NELL-1 binds to APR3 affecting human osteoblast proliferation and differentiation. FEBS Lett **585,** 2410, 2011.
57. Burkus, J.K., Sandhu, H.S., and Gornet, M.F. Influence of rhBMP-2 on the healing patterns associated with allograft interbody constructs in comparison with autograft. Spine **31,** 775, 2006.
58. Smoljanovic, T., and Pecina, M. Re: Burkus J K, Sandhu H S, Gornet M F. Influence of rhBMP-2 on the healing patterns associated with allograft interbody constructs in comparison with autograft. Spine **31,** 775, 2006. Spine (Phila Pa 1976) **33,** 226, 2008.
59. United States Food and Drug Administration, Department of Health and Human Services, Center for Devices and Radiological Health. InFUSE bone Graft/LT-CAGE? Lumbar tapered fusion Devices—P000058. 2002. Available at www.accessdata.fda.gov/scripts/cdrh/cfdocs/cftopic/pma/pma.cfm?num5P000058 (accessed October 10, 2014).
60. McClellan, J.W., Mulconrey, D.S., Forbes, R.J., and Fullmer, N. Vertebral bone resorption after transforaminal lumbar interbody fusion with bone morphogenetic protein (rhBMP-2). J Spinal Disord Tech **19,** 483, 2006.
61. Vaidya, R., Sethi, A., Bartol, S., Jacobson, M., Coe, C., and Craig, J.G. Complications in the use of rhBMP-2 in PEEK cages for interbody spinal fusions. J Spinal Disord Tech **21,** 557, 2008.
62. Zhang, X., Guo, J., Zhou, Y., and Wu, G. The roles of bone morphogenetic proteins and their signaling in the osteogenesis of adipose-derived stem cells. Tissue Eng Part B Rev **20,** 84, 2014.
63. James, A.W., Zara, J.N., Zhang, X., Askarinam, A., Goyal, R., Chiang, M., Yuan, W., Chang, L., Corselli, M., Shen, J., Pang, S., Stoker, D., Wu, B., Ting, K., Peault, B., and Soo, C. Perivascular stem cells: a prospectively purified mesenchymal stem cell population for bone tissue engineering. Stem Cells Transl Med **1,** 510, 2012.
64. Aghaloo, T., Jiang, X., Soo, C., Zhang, Z., Zhang, X., Hu, J., Pan, H., Hsu, T., Wu, B., Ting, K., and Zhang, X. A study of the role of nell-1 gene modified goat bone marrow stromal cells in promoting new bone formation. Mol Ther **15,** 1872, 2007.
65. Shahlaie, K., and Kim, K.D. Occipitocervical fusion using recombinant human bone morphogenetic protein-2: adverse effects due to tissue swelling and seroma. Spine (Phila Pa 1976) **33,** 2361, 2008.
66. Robin, B.N., Chaput, C.D., Zeitouni, S., Rahm, M.D., Zerris, V.A., and Sampson, H.W. 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) **35,** E1350, 2010.
67. Mindea, S.A., Shih, P., and Song, J.K. Recombinant human bone morphogenetic protein-2-induced radiculitis in elective minimally invasive transforaminal lumbar interbody fusions: a series review. Spine (Phila Pa 1976) **34,** 1480, 2009.
68. Tannoury, C.A., and An, H.S. Complications with the use of bone morphogenetic protein 2 (BMP-2) in spine surgery. Spine J **14,** 552, 2014.
69. Smucker, J.D., Rhee, J.M., Singh, K., Yoon, S.T., and Heller, J.G. Increased swelling complications associated with off-label usage of rhBMP-2 in the anterior cervical spine. Spine (Phila Pa 1976) **31,** 2813, 2006.
70. Epstein, N.E. Complications due to the use of BMP/INFUSE in spine surgery: the evidence continues to mount. Surg Neurol Int **4,** S343, 2013.
71. North American Spine Society (NASS) Coverage Committee and Bono, C. Recombinant human bone morphogenetic protein-2: defining appropriate coverage positions. In: N.A.S.S., ed. *NASS Coverage Policy Recommendations*, 2014, p 1.
72. Sasso, R.C., Best, N.M., Mummaneni, P.V., Reilly, T.M., and Hussain, S.M. Analysis of operative complications in a series of 471 anterior lumbar interbody fusion procedures. Spine **30,** 670, 2005.
73. Sasso, R.C., Kitchel, S.H., and Dawson, E.G. A prospective, randomized controlled clinical trial of anterior lumbar interbody fusion using a titanium cylindrical threaded fusion device. Spine **29,** 113, 2004.
74. Kang, B.U., Choi, W.C., Lee, S.H., Jeon, S.H., Park, J.D., Maeng, D.H., and Choi, Y.G. An analysis of general surgery-related complications in a series of 412 mini-laparotomic anterior lumbosacral procedures. J Neurosurg Spine **10,** 60, 2009.
75. Smoljanovic, T., Siric, F., and Bojanic, I. Six-year outcomes of anterior lumbar interbody arthrodesis with use of interbody fusion cages and recombinant human bone morphogenetic protein-2. J Bone Joint Surg Am Vol **92,** 2614, 2010.
76. Carragee, E.J., Mitsunaga, K.A., Hurwitz, E.L., and Scuderi, G.J. Retrograde ejaculation after anterior lumbar interbody fusion using rhBMP-2: a cohort controlled study. Spine J **11,** 511, 2011.
77. Jarrett, C.D., Heller, J.G., and Tsai, L. Anterior exposure of the lumbar spine with and without an ''access surgeon'': morbidity analysis of 265 consecutive cases. J Spinal Disord Tech **22,** 559, 2009.
78. Parker, L.M., Murrell, S.E., Boden, S.D., and Horton, W.C. The outcome of posterolateral fusion in highly selected patients with discogenic low back pain. Spine **21,** 1909, 1996.
79. Chan, D.S., Garland, J., Infante, A., Sanders, R.W., and Sagi, H.C. Wound complications associated with bone morphogenetic protein-2 in orthopaedic trauma surgery. J Orthop Trauma **28,** 599, 2014.
80. Williams, B.J., Smith, J.S., Fu, K.M., Hamilton, D.K., Polly, D.W., Jr., Ames, C.P., Berven, S.H., Perra, J.H., Knapp, D.R., Jr., McCarthy, R.E., Shaffrey, C.I., Scoliosis Research Society, M., and Mortality, C. Does bone morphogenetic protein increase the incidence of perioperative complications in spinal fusion? A comparison of 55,862 cases of spinal fusion with and without bone morphogenetic protein. Spine (Phila Pa 1976) **36,** 1685, 2011.
81. Ye, L., Lewis-Russell, J.M., Kyanaston, H.G., and Jiang, W.G. Bone morphogenetic proteins and their receptor signaling in prostate cancer. Histol Histopathol **22,** 1129, 2007.
82. Ye, L., Bokobza, S.M., and Jiang, W.G. Bone morphogenetic proteins in development and progression of breast cancer and therapeutic potential (review). Int J Mol Med **24,** 591, 2009.
83. Yoshikawa, H., Nakase, T., Myoui, A., and Ueda, T. Bone morphogenetic proteins in bone tumors. J Orthop Sci **9,** 334, 2004.

84. Jin, Y., Tipoe, G.L., Liong, E.C., Lau, T.Y., Fung, P.C., and Leung, K.M. Overexpression of BMP-2/4, −5 and BMPR-IA associated with malignancy of oral epithelium. Oral Oncol **37,** 225, 2001.
85. Kiyozuka, Y., Miyazaki, H., Yoshizawa, K., Senzaki, H., Yamamoto, D., Inoue, K., Bessho, K., Okubo, Y., Kusumoto, K., and Tsubura, A. An autopsy case of malignant mesothelioma with osseous and cartilaginous differentiation: bone morphogenetic protein-2 in mesothelial cells and its tumor. Dig Dis Sci **44,** 1626, 1999.
86. Epstein, N.E. Basic science and spine literature document bone morphogenetic protein increases cancer risk. Surg Neurol Int **5,** S552, 2014.
87. Carragee, E.J., Chu, G., Rohatgi, R., Hurwitz, E.L., Weiner, B.K., Yoon, S.T., Comer, G., and Kopjar, B. Cancer risk after use of recombinant bone morphogenetic protein-2 for spinal arthrodesis. J Bone Joint Surg Am **95,** 1537, 2013.
88. Lad, S.P., Bagley, J.H., Karikari, I.O., Babu, R., Ugiliweneza, B., Kong, M., Isaacs, R.E., Bagley, C.A., Gottfried, O.N., Patil, C.G., and Boakye, M. Cancer after spinal fusion: the role of bone morphogenetic protein. Neurosurgery **73,** 440, 2013.
89. Cooper, G.S., and Kou, T.D. Risk of cancer after lumbar fusion surgery with recombinant human bone morphogenic protein-2 (rh-BMP-2). Spine (Phila Pa 1976) **38,** 1862, 2013.
90. Veeravagu, A., Cole, T.S., Jiang, B., Ratliff, J.K., and Gidwani, R.A. The use of bone morphogenetic protein in thoracolumbar spine procedures: analysis of the MarketScan longitudinal database. Spine J **14,** 2929, 2014.
91. Mines, D., Gu, Y., Kou, T.D., and Cooper, G.S. Recombinant human bone morphogenetic protein-2 and pancreatic cancer: a retrospective cohort study. Pharmacoepidemiol Drug Saf **20,** 111, 2011.
92. Kelly, M.P., Savage, J.W., Bentzen, S.M., Hsu, W.K., Ellison, S.A., and Anderson, P.A. Cancer risk from bone morphogenetic protein exposure in spinal arthrodesis. J Bone Joint Surg Am **96,** 1417, 2014.
93. Alarmo, E.L., and Kallioniemi, A. Bone morphogenetic proteins in breast cancer: dual role in tumourigenesis? Endocr Relat Cancer **17,** R123, 2010.
94. Langenfeld, E.M., Kong, Y., and Langenfeld, J. Bone morphogenetic protein 2 stimulation of tumor growth involves the activation of Smad-1/5. Oncogene **25,** 685, 2006.
95. Kleeff, J., Maruyama, H., Ishiwata, T., Sawhney, H., Friess, H., Buchler, M.W., and Korc, M. Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterology **116,** 1202, 1999.
96. Kim, M.J., Kim, K.M., Kim, J., and Kim, K.N. BMP-2 promotes oral squamous carcinoma cell invasion by inducing CCL5 release. PLoS One **9,** e108170, 2014.
97. Jin, H., Pi, J., Huang, X., Huang, F., Shao, W., Li, S., Chen, Y., and Cai, J. BMP2 promotes migration and invasion of breast cancer cells via cytoskeletal reorganization and adhesion decrease: an AFM investigation. Appl Microbiol Biotechnol **93,** 1715, 2012.
98. Clement, J.H., Raida, M., Sanger, J., Bicknell, R., Liu, J., Naumann, A., Geyer, A., Waldau, A., Hortschansky, P., Schmidt, A., Hoffken, K., Wolft, S., and Harris, A.L. Bone morphogenetic protein 2 (BMP-2) induces in vitro invasion and in vivo hormone independent growth of breast carcinoma cells. Int J Oncol **27,** 401, 2005.
99. Feeley, B.T., Gamradt, S.C., Hsu, W.K., Liu, N., Krenek, L., Robbins, P., Huard, J., and Lieberman, J.R. Influence of BMPs on the formation of osteoblastic lesions in metastatic prostate cancer. J Bone Miner Res **20,** 2189, 2005.
100. Langenfeld, E.M., Calvano, S.E., Abou-Nukta, F., Lowry, S.F., Amenta, P., and Langenfeld, J. The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. Carcinogenesis **24,** 1445, 2003.
101. Scherberich, A., Tucker, R.P., Degen, M., Brown-Luedi, M., Andres, A.C., and Chiquet-Ehrismann, R. Tenascin-W is found in malignant mammary tumors, promotes alpha8 integrin-dependent motility and requires p38MAPK activity for BMP-2 and TNF-alpha induced expression in vitro. Oncogene **24,** 1525, 2005.
102. Zara, J.N., Siu, R.K., Zhang, X., Shen, J., Ngo, R., Lee, M., Li, W., Chiang, M., Chung, J., Kwak, J., Wu, B.M., Ting, K., and Soo, C. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. Tissue Eng Part A **17,** 1389, 2011.
103. Yamazaki, M., Fukushima, H., Shin, M., Katagiri, T., Doi, T., Takahashi, T., and Jimi, E. Tumor necrosis factor alpha represses bone morphogenetic protein (BMP) signaling by interfering with the DNA binding of Smads through the activation of NF-kappaB. J Biol Chem **284,** 35987, 2009.
104. Itoh, K., Udagawa, N., Katagiri, T., Iemura, S., Ueno, N., Yasuda, H., Higashio, K., Quinn, J.M., Gillespie, M.T., Martin, T.J., Suda, T., and Takahashi, N. Bone morphogenetic protein 2 stimulates osteoclast differentiation and survival supported by receptor activator of nuclear factor-kappaB ligand. Endocrinology **142,** 3656, 2001.
105. Hori, M., Sawai, H., Tsuji, Y., Okamura, H., and Koyama, K. Bone morphogenetic protein-2 counterregulates interleukin-18 mRNA and protein in MC3T3-E1 mouse osteoblastic cells. Connect Tissue Res **47,** 124, 2006.
106. Tasca, A., Stemig, M., Broege, A., Huang, B., Davydova, J., Zwijsen, A., Umans, L., Jensen, E.D., Gopalakrishnan, R., and Mansky, K.C. Smad1/5 and Smad4 expression are important for osteoclast differentiation. J Cell Biochem **116,** 1350, 2015.
107. Takada, I., Yogiashi, Y., and Kato, S. Signaling crosstalk between PPARγ and BMP2 in mesenchymal stem cells. PPAR Res **2012,** 2012.
108. Liao, X., Wu, L., Fu, M., He, D., Gu, Y., Chen, W., and Yin, M. [Chondrogenic phenotype differentiation of bone marrow mesenchymal stem cells induced by bone morphogenetic protein 2 under hypoxic microenvironment in vitro]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi [Chin J Rep Reconstruct Surg] **26,** 743, 2012.
109. McArdle, A., Chung, M.T., Paik, K.J., Duldulao, C., Chan, C., Rennert, R., Walmsley, G.G., Senarath-Yapa, K., Hu, M., Seo, E., Lee, M., Wan, D.C., and Longaker, M.T. Positive selection for bone morphogenetic protein receptor type-IB promotes differentiation and specification of human adipose-derived stromal cells toward an osteogenic lineage. Tissue Eng Part A **20,** 3031, 2014.
110. Mishina, Y., Starbuck, M.W., Gentile, M.A., Fukuda, T., Kasparcova, V., Seedor, J.G., Hanks, M.C., Amling, M., Pinero, G.J., Harada, S., and Behringer, R.R. Bone morphogenetic protein type IA receptor signaling regulates postnatal osteoblast function and bone remodeling. J Biol Chem **279,** 27560, 2004.

111. Martin, G.J., Jr., Boden, S.D., Marone, M.A., Marone, M.A., and Moskovitz, P.A. Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman primate: important lessons learned regarding dose, carrier, and safety. J Spinal Disord **12,** 179, 1999.
112. Tatsuyama, K., Maezawa, Y., Baba, H., Imamura, Y., and Fukuda, M. Expression of various growth factors for cell proliferation and cytodifferentiation during fracture repair of bone. Eur J Histochem **44,** 269, 2000.
113. Detsch, R., Stoor, P., Grunewald, A., Roether, J.A., Lindfors, N.C., and Boccaccini, A.R. Increase in VEGF secretion from human fibroblast cells by bioactive glass S53P4 to stimulate angiogenesis in bone. J Biomed Mater Res Part A **102,** 4055, 2014.
114. Hoffmann, B.R., Wagner, J.R., Prisco, A.R., Janiak, A., and Greene, A.S. Vascular endothelial growth factor-A signaling in bone marrow-derived endothelial progenitor cells exposed to hypoxic stress. Physiol Genomics **45,** 1021, 2013.
115. Street, J., Bao, M., deGuzman, L., Bunting, S., Peale, F.V., Jr., Ferrara, N., Steinmetz, H., Hoeffel, J., Cleland, J.L., Daugherty, A., van Bruggen, N., Redmond, H.P., Carano, R.A., and Filvaroff, E.H. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci U S A **99,** 9656, 2002.
116. Aryal, R., Chen, X.P., Fang, C., and Hu, Y.C. Bone morphogenetic protein-2 and vascular endothelial growth factor in bone tissue regeneration: new insight and perspectives. Orthop Surg **6,** 171, 2014.
117. Patel, Z.S., Young, S., Tabata, Y., Jansen, J.A., Wong, M.E., and Mikos, A.G. Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. Bone **43,** 931, 2008.
118. Kempen, D.H., Lu, L., Heijink, A., Hefferan, T.E., Creemers, L.B., Maran, A., Yaszemski, M.J., and Dhert, W.J. Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. Biomaterials **30,** 2816, 2009.
119. Kim, H.K., Aruwajoye, O., Du, J., and Kamiya, N. Local administration of bone morphogenetic protein-2 and bisphosphonate during non-weight-bearing treatment of ischemic osteonecrosis of the femoral head: an experimental investigation in immature pigs. J Bone Joint Surg Am Vol **96,** 1515, 2014.
120. Michael, W.R., King, W.R., and Francis, M.D. Effectiveness of diphosphonates in preventing ''osteoporosis'' of disuse in the rat. Clin Orthop Relat Res **78,** 271, 1971.
121. Saville, P.D., and Heaney, R. Treatment of osteoporosis with diphosphonates. Semin Drug Treat **2,** 47, 1972.
122. Jowsey, J., and Holley, K.E. Influence of diphosphonates on progress of experimentally induced osteoporosis. J Lab Clin Med **82,** 567, 1973.
123. Lee, K.B., Taghavi, C.E., Song, K.J., Sintuu, C., Yoo, J.H., Keorochana, G., Tzeng, S.T., Fei, Z., Liao, J.C., and Wang, J.C. Inflammatory characteristics of rhBMP-2 in vitro and in an in vivo rodent model. Spine **36,** E149, 2011.
124. Xiong, C., Daubs, M.D., Montgomery, S.R., Aghdasi, B., Inoue, H., Tian, H., Suzuki, A., Tan, Y., Hayashi, T., Ruangchainikom, M., Chai, T., Corey, M., and Wang, J.C. BMP-2 adverse reactions treated with human dose equivalent dexamethasone in a rodent model of soft-tissue inflammation. Spine **38,** 1640, 2013.
125. Weber, C.R., and Griffin, J.M. Evaluation of dexamethasone for reducing postoperative edema and inflammatory response after orthognathic surgery. J Oral Maxillofac Surg **52,** 35, 1994.
126. Baxendale, B.R., Vater, M., and Lavery, K.M. Dexamethasone reduces pain and swelling following extraction of third molar teeth. Anaesthesia **48,** 961, 1993.
127. Haidar, Z.S., Hamdy, R.C., and Tabrizian, M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: delivery systems for BMPs in orthopaedic and craniofacial tissue engineering. Biotechnol Lett **31,** 1825, 2009.
128. Haidar, Z.S., Hamdy, R.C., and Tabrizian, M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: current challenges in BMP delivery. Biotechnol Lett **31,** 1817, 2009.
129. Kim, H.D., and Valentini, R.F. Retention and activity of BMP-2 in hyaluronic acid-based scaffolds in vitro. J Biomed Mater Res **59,** 573, 2002.
130. Arosarena, O.A., and Collins, W.L. Bone regeneration in the rat mandible with bone morphogenetic protein-2: a comparison of two carriers. Otolaryngol Head Neck Surg **132,** 592, 2005.
131. Issa, J.P., do Nascimento, C., Bentley, M.V., Del Bel, E.A., Iyomasa, M.M., Sebald, W., and de Albuquerque, R.F., Jr. Bone repair in rat mandible by rhBMP-2 associated with two carriers. Micron **39,** 373, 2008.
132. Noshi, T., Yoshikawa, T., Dohi, Y., Ikeuchi, M., Horiuchi, K., Ichijima, K., Sugimura, M., Yonemasu, K., and Ohgushi, H. Recombinant human bone morphogenetic protein-2 potentiates the in vivo osteogenic ability of marrow/hydroxyapatite composites. Artif Organs **25,** 201, 2001.
133. Ginebra, M.P., Traykova, T., and Planell, J.A. Calcium phosphate cements as bone drug delivery systems: a review. J Control Release **113,** 102, 2006.
134. Valimaki, V.V., and Aro, H.T. Molecular basis for action of bioactive glasses as bone graft substitute. Scand J Surg **95,** 95, 2006.
135. Moore, W.R., Graves, S.E., and Bain, G.I. Synthetic bone graft substitutes. ANZ J Surg **71,** 354, 2001.
136. Zellin, G., and Linde, A. Importance of delivery systems for growth-stimulatory factors in combination with osteopromotive membranes. An experimental study using rhBMP-2 in rat mandibular defects. J Biomed Mater Res **35,** 181, 1997.
137. Sigurdsson, T.J., Nygaard, L., Tatakis, D.N., Fu, E., Turek, T.J., Jin, L., Wozney, J.M., and Wikesjo, U.M. Periodontal repair in dogs: evaluation of rhBMP-2 carriers. Int J Periodontics Restorative Dent **16,** 524, 1996.
138. Mayer, M., Hollinger, J., Ron, E., and Wozney, J. Maxillary alveolar cleft repair in dogs using recombinant human bone morphogenetic protein-2 and a polymer carrier. Plast Reconstr Surg **98,** 247, 1996.
139. Itoh, T., Mochizuki, M., Nishimura, R., Matsunaga, S., Kadosawa, T., Kokubo, S., Yokota, S., and Sasaki, N. Repair of ulnar segmental defect by recombinant human bone morphogenetic protein-2 in dogs. J Vet Med Sci **60,** 451, 1998.
140. Schopper, C., Moser, D., Spassova, E., Goriwoda, W., Lagogiannis, G., Hoering, B., Ewers, R., and Redl, H. Bone regeneration using a naturally grown HA/TCP carrier loaded with rh BMP-2 is independent of barrier-membrane effects. J Biomed Mater Res A **85,** 954, 2008.

141. Kim, S.S., Gwak, S.J., and Kim, B.S. Orthotopic bone formation by implantation of apatite-coated poly(lactide-co-glycolide)/hydroxyapatite composite particulates and bone morphogenetic protein-2. J Biomed Mater Res A **87,** 245, 2008.
142. Cahill, K.S., Chi, J.H., Groff, M.W., McGuire, K., Afendulis, C.C., and Claus, E.B. Outcomes for single-level lumbar fusion: the role of bone morphogenetic protein. Spine **36,** 2354, 2011.
143. Smoljanovic, T., Cimic, M., and Bojanic, I. Aggressive end plate decortication as a cause of osteolysis after rhBMP-2 use in cervical spine interbody fusion. Spine J **10,** 187, 2010.
144. Mladenov, K.V., Kunkel, P., and Stuecker, R. The use of recombinant human BMP-2 as a salvage procedure in the pediatric spine: a report on 3 cases. Eur Spine J **19 Suppl 2,** S135, 2010.
145. Perri, B., Cooper, M., Lauryssen, C., and Anand, N. Adverse swelling associated with use of rh-BMP-2 in anterior cervical discectomy and fusion: a case study. Spine J **7,** 235, 2007.
146. Shields, L.B., Raque, G.H., Glassman, S.D., Campbell, M., Vitaz, T., Harpring, J., and Shields, C.B. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. Spine (Phila Pa 1976) **31,** 542, 2006.
147. Vaidya, R., Carp, J., Sethi, A., Bartol, S., Craig, J., and Les, C.M. Complications of anterior cervical discectomy and fusion using recombinant human bone morphogenetic protein-2. Eur Spine J **16,** 1257, 2007.
148. Yaremchuk, K.L., Toma, M.S., Somers, M.L., and Peterson, E. Acute airway obstruction in cervical spinal procedures with bone morphogenetic proteins. Laryngoscope **120,** 1954, 2010.
149. Anderson, D.W., Burton, D.C., and Jackson, R.S. Postoperative cervical myelopathy and cord compression associated with the use of recombinant bone morphogenetic protein-2 in posterior cervical decompression, instrumentation, and arthrodesis: a report of two cases. Spine (Phila Pa 1976) **36,** E682, 2011.
150. Garrett, M.P., Kakarla, U.K., Porter, R.W., and Sonntag, V.K. Formation of painful seroma and edema after the use of recombinant human bone morphogenetic protein-2 in posterolateral lumbar spine fusions. Neurosurgery **66,** 1044, 2010.
151. Hoffmann, M.F., Jones, C.B., and Sietsema, D.L. Complications of rhBMP-2 utilization for posterolateral lumbar fusions requiring reoperation: a single practice, retrospective case series report. Spine J **13,** 1244, 2013.
152. Owens, K., Glassman, S.D., Howard, J.M., Djurasovic, M., Witten, J.L., and Carreon, L.Y. Perioperative complications with rhBMP-2 in transforaminal lumbar interbody fusion. Eur Spine J **20,** 612, 2011.
153. Taher, F., Lebl, D.R., Hughes, A.P., and Girardi, F.P. Contralateral psoas seroma after transpsoas lumbar interbody fusion with bone morphogenetic protein-2 implantation. Spine J **13,** e1, 2013.
154. Scheer, J.K., Dahdaleh, N.S., and Smith, Z.A. Seroma observed 6 months after anterior lumbar interbody fusion that included use of recombinant bone morphogenetic protein 2. Spine J **15,** e33, 2015.
155. Muchow, R.D., Hsu, W.K., and Anderson, P.A. Histopathologic inflammatory response induced by recombinant bone morphogenetic protein-2 causing radiculopathy after transforaminal lumbar interbody fusion. Spine J **10,** e1, 2010.
156. Singh, K., Nandyala, S.V., Marquez-Lara, A., Cha, T.D., Khan, S.N., Fineberg, S.J., and Pelton, M.A. Clinical sequelae after rhBMP-2 use in a minimally invasive transforaminal lumbar interbody fusion. Spine J **13,** 1118, 2013.
157. Rowan, F.E., O'Malley, N., and Poynton, A. RhBMP-2 use in lumbar fusion surgery is associated with transient immediate post-operative leg pain. Eur Spine J **21,** 1331, 2012.
158. Choudhry, O.J., Christiano, L.D., Singh, R., Golden, B.M., and Liu, J.K. Bone morphogenetic protein-induced inflammatory cyst formation after lumbar fusion causing nerve root compression. J Neurosurg Spine **16,** 296, 2012.
159. Chen, N.F., Smith, Z.A., Stiner, E., Armin, S., Sheikh, H., and Khoo, L.T. Symptomatic ectopic bone formation after off-label use of recombinant human bone morphogenetic protein-2 in transforaminal lumbar interbody fusion. J Neurosurg Spine **12,** 40, 2010.
160. Deutsch, H. High-dose bone morphogenetic protein-induced ectopic abdomen bone growth. Spine J **10,** e1, 2010.
161. Wong, D.A., Kumar, A., Jatana, S., Ghiselli, G., and 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 **8,** 1011, 2008.
162. Christensen, T.J., Annis, P., Hohl, J.B., and Patel, A.A. Neuroforaminal chondrocyte metaplasia and clustering associated with recombinant bone morphogenetic protein-2 usage in transforaminal lumbar interbody fusion. Spine J **14,** e23, 2014.
163. Than, K.D., Rahman, S.U., McKeever, P.E., Wang, A.C., La Marca, F., and Park, P. Symptomatic calcified perineural cyst after use of bone morphogenetic protein in transforaminal lumbar interbody fusion: a case report. Spine J **13,** e31, 2013.
164. Hansen, S.M., and Sasso, R.C. Resorptive response of rhBMP2 simulating infection in an anterior lumbar interbody fusion with a femoral ring. J Spinal Disord Tech **19,** 130, 2006.
165. Woo, E.J. Adverse events after recombinant human BMP2 in nonspinal orthopaedic procedures. Clin Orthop Relat Res **471,** 1707, 2013.
166. Knox, J.B., Dai, J.M., 3rd, and Orchowski, J. Osteolysis in transforaminal lumbar interbody fusion with bone morphogenetic protein-2. Spine (Phila Pa 1976) **36,** 672, 2011.
167. Lewandrowski, K.U., Nanson, C., and Calderon, R. Vertebral osteolysis after posterior interbody lumbar fusion with recombinant human bone morphogenetic protein 2: a report of five cases. Spine J **7,** 609, 2007.
168. Balseiro, S., and Nottmeier, E.W. Vertebral osteolysis originating from subchondral cyst end plate defects in transforaminal lumbar interbody fusion using rhBMP-2. Report of two cases. Spine J **10,** e6, 2010.
169. Helgeson, M.D., Lehman, R.A., Jr., Patzkowski, J.C., Dmitriev, A.E., Rosner, M.K., and Mack, A.W. Adjacent vertebral body osteolysis with bone morphogenetic protein use in transforaminal lumbar interbody fusion. Spine J **11,** 507, 2011.
170. Meisel, H.J., Schnoring, M., Hohaus, C., Minkus, Y., Beier, A., Ganey, T., and Mansmann, U. Posterior lumbar interbody fusion using rhBMP-2. Eur Spine J **17,** 1735, 2008.

171. Vaidya, R., Weir, R., Sethi, A., Meisterling, S., Hakeos, W., and Wybo, C.D. Interbody fusion with allograft and rhBMP-2 leads to consistent fusion but early subsidence. J Bone Joint Surg Br **89,** 342, 2007.
172. Comer, G.C., Smith, M.W., Hurwitz, E.L., Mitsunaga, K.A., Kessler, R., and Carragee, E.J. Retrograde ejaculation after anterior lumbar interbody fusion with and without bone morphogenetic protein-2 augmentation: a 10-year cohort controlled study. Spine J **12,** 881, 2012.
173. Burkus, J.K., Dryer, R.F., and Peloza, J.H. Retrograde ejaculation following single-level anterior lumbar surgery with or without recombinant human bone morphogenetic protein-2 in 5 randomized controlled trials: clinical article. J Neurosurg Spine **18,** 112, 2013.
174. Lindley, E.M., McBeth, Z.L., Henry, S.E., Cooley, R., Burger, E.L., Cain, C.M., and Patel, V.V. Retrograde ejaculation after anterior lumbar spine surgery. Spine (Phila Pa 1976) **37,** 1785, 2012.
175. Lubelski, D., Abdullah, K.G., Nowacki, A.S., Alvin, M.D., Steinmetz, M.P., Chakka, S., Li, Y., Gajewski, N., Benzel, E.C., and Mroz, T.E. Urological complications following use of recombinant human bone morphogenetic protein-2 in anterior lumbar interbody fusion: presented at the 2012 Joint Spine Section Meeting: clinical article. J Neurosurg Spine **18,** 126, 2013.
176. Luhmann, S.J., Bridwell, K.H., Cheng, I., Imamura, T., Lenke, L.G., and Schootman, M. Use of bone morphogenetic protein-2 for adult spinal deformity. Spine (Phila Pa 1976) **30,** S110, 2005.
177. Tumialan, L.M., Pan, J., Rodts, G.E., and Mummaneni, P.V. The safety and efficacy of anterior cervical discectomy and fusion with polyetheretherketone spacer and recombinant human bone morphogenetic protein-2: a review of 200 patients. J Neurosurg Spine **8,** 529, 2008.
178. Shen, J., James, A.W., Zara, J.N., Asatrian, G., Khadarian, K., Zhang, J.B., Ho, S., Kim, H.J., Ting, K., and Soo, C. BMP2-induced inflammation can be suppressed by the osteoinductive growth factor NELL-1. Tissue Eng Part A **19,** 2390, 2013.
179. Lee, K.B., Murray, S.S., Taghavi, C.E., Song, K.J., Brochmann, E.J., Johnson, J.S., Keorochana, G., Liao, J.C., and Wang, J.C. Bone morphogenetic protein-binding peptide reduces the inflammatory response to recombinant human bone morphogenetic protein-2 and recombinant human bone morphogenetic protein-7 in a rodent model of soft-tissue inflammation. Spine J **11,** 568, 2011.
180. Lee, K.B., Taghavi, C.E., Murray, S.S., Song, K.J., Keorochana, G., and Wang, J.C. BMP induced inflammation: a comparison of rhBMP-7 and rhBMP-2. J Orthop Res **30,** 1985, 2012.
181. Blackwell, K.A., Hortschansky, P., Sanovic, S., Choudhary, S., Raisz, L.G., and Pilbeam, C.C. Bone morphogenetic protein 2 enhances PGE(2)-stimulated osteoclast formation in murine bone marrow cultures. Prostaglandins Other Lipid Mediat **90,** 76, 2009.
182. Tachi, K., Takami, M., Zhao, B., Mochizuki, A., Yamada, A., Miyamoto, Y., Inoue, T., Baba, K., and Kamijo, R. Bone morphogenetic protein 2 enhances mouse osteoclast differentiation via increased levels of receptor activator of NF-kappaB ligand expression in osteoblasts. Cell Tissue Res **342,** 213, 2010.
183. Jensen, E.D., Pham, L., Billington, C.J., Jr., Espe, K., Carlson, A.E., Westendorf, J.J., Petryk, A., Gopalakrishnan, R., and Mansky, K. Bone morphogenic protein 2 directly enhances differentiation of murine osteoclast precursors. J Cell Biochem **109,** 672, 2010.
184. Kanatani, M., Sugimoto, T., Kaji, H., Kobayashi, T., Nishiyama, K., Fukase, M., Kumegawa, M., and Chihara, K. Stimulatory effect of bone morphogenetic protein-2 on osteoclast-like cell formation and bone-resorbing activity. J Bone Miner Res **10,** 1681, 1995.
185. Pederson, L., Ruan, M., Westendorf, J.J., Khosla, S., and Oursler, M.J. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U S A **105,** 20764, 2008.
186. Kang, Q., Song, W.X., Luo, Q., Tang, N., Luo, J., Luo, X., Chen, J., Bi, Y., He, B.C., Park, J.K., Jiang, W., Tang, Y., Huang, J., Su, Y., Zhu, G.H., He, Y., Yin, H., Hu, Z., Wang, Y., Chen, L., Zuo, G.W., Pan, X., Shen, J., Vokes, T., Reid, R.R., Haydon, R.C., Luu, H.H., and He, T.C. A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor cells. Stem Cells Dev **18,** 545, 2009.
187. Sottile, V., and Seuwen, K. Bone morphogenetic protein-2 stimulates adipogenic differentiation of mesenchymal precursor cells in synergy with BRL 49653 (rosiglitazone). FEBS Lett **475,** 201, 2000.
188. James, A.W. Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. Scientifica (Cairo) **2013,** 684736, 2013.

Address correspondence to:
Kang Ting, DMD, DMedSci
Section of Orthodontics
Division of Growth and Development
School of Dentistry
University of California, Los Angeles
10833 Le Conte Avenue, CHS 30-117
Los Angeles, CA 90095

E-mail: kting@dentistry.ucla.edu

Chia Soo, MD, FACS
Division of Plastic and Reconstructive Surgery
Department of Surgery
David Geffen School of Medicine
University of California, Los Angeles
675 Charles E. Young Dr. South, MacDonald Research Laboratory Room 2641A
Los Angeles, CA 90095

E-mail: bsoo@ucla.edu

Received: July 31, 2015
Accepted: January 11, 2016
Online Publication Date: April 13, 2016