BMP and Beyond: A 25-Year Historical Review of Translational Spine Research at Emory University

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Abstract:

A high rate of symptomatic spinal pseudoarthrosis and a wide range of complications associated with the use of iliac crest bone graft (the gold standard) have prompted the spine surgery community to seek alternative options to promote spinal fusion. Emory University has been one of the global leaders in this endeavor. This invited review covers the last 25 years of Emory's contributions to translational spine research, focusing specifically on our work with bone morphogenetic proteins (BMP) and the BMP signaling pathway. As a result of this work, recombinant human BMP-2 is the only Food and Drug Administration approved biologic bone graft substitute. It has been shown to significantly increase spinal fusion rates across the spinal column because of its potent ability to stimulate local bone formation through the recruitment of mesenchymal stem cells. This review covers our development of animal models of spinal fusion, our body of work regarding the translation of BMP from the benchtop to the clinic, the discovery of LMP-1 and strategies to enhance cellular responsiveness to BMPs, and the design of various small molecule drugs that can enhance local bone formation.

Keywords:

bone morphogenetic protein, BMP, spinal fusion, pseudarthrosis, translational research, small molecules, drug design

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Since the introduction of spinal arthrodesis surgery in 1911, it has become a commonly used procedure for the treatment of multiple spinal conditions across the cervical, thoracic, and lumbar spine. Despite state-of-the-art spinal instrumentation and techniques, failure of spinal fusion (pseudarthrosis) is still unfortunately a common complication, with a rate in primary fusions as high as 20%[1]. In addition, almost one-quarter of all revision arthrodesis surgeries are performed for a pseudarthrosis[2]. Clinically, patients with a pseudarthrosis often report much poorer outcomes (23% positive outcome) compared with those in whom a solid fusion was achieved (81%)[3]. In addition, the high cost required to continue to treat spinal pseudarthrosis represents a major burden on the global healthcare system[4].

The current "gold standard" bone graft for spinal fusion remains autologous iliac crest bone graft (ICBG), but its harvest is associated with long-term donor site pain in up to 25% of patients[5,6]. Moreover, ICBG itself has a pseudarthrosis rate nearing 40% in the posterolateral spine in primary arthrodesis procedures and up to 60% in revisions[7,8]. Although a variety of alternative bone graft options are available (i.e., bone allograft and demineralized bone matrix), none have proven to be suitable and effective substitutes for ICBG[9].

The high rate of pseudarthrosis and the lack of viable alternatives for ICBG have motivated the scientific community to expand research efforts into biologic bone graft substitutes. Marshall Urist in 1965 first popularized the notion that bone matrix itself contains proteins capable of osteoinduction (ability to directly induce bone formation). Dr. Urist dedicated his entire research career to purifying, identifying, and characterizing these proteins, which he named bone morphogenetic proteins (BMP)[10,11]. Although over 20 types of BMP have since been described, only some of the BMPs are osteoinductive: BMP-2, BMP-6, and BMP-9[12]. The most widely studied recombinant growth factor for the use in spine surgery is recombinant human (rh) BMP-2. Since gaining Food and Drug Administration (FDA) approval in the United States for anterior lumbar interbody fusions (ALIFs) in 2002 (Infuse®, Medtronic), rhBMP-2 is widely considered a critical addition to the armamentarium that the surgeon has at their disposal to enhance spinal fusion rates.

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In fact, because multiple studies have indicated that patients receiving rhBMP-2 achieve solid fusions without major adverse events, it has been reported to be used in an "off-label" fashion in more than 85% of all spinal fusion cases across the entire spinal column.

Although many labs around the world have contributed significantly to our understanding of BMP in the setting of spinal fusion, Emory University has been at the forefront of translational spine fusion and BMP research for over 25 years. The purpose of this invited review is to highlight our work and experience in this exciting arena. We have divided this research into four discreet categories, each reviewed below.

I. Development of Animal Models for Spinal Fusion

In 1995, Dr. Scott Boden of Emory University developed and validated the first preclinical model of lumbar intertransverse process spinal fusion[13]. Up to this point, all previous animal models of spinal fusion had not replicated the surgical technique, the mechanical environment, the biology of graft incorporation, or the pseudarthrosis rate that is found in humans. Once the model was developed, it served as a much-needed tool that became critical in gaining a basic understanding of the biology of spinal fusion as well as allowing the testing of biologic fusion enhancement by entities such as cytokines, growth factors, and hormones. Although much was known at that point about the biology of fracture repair, surprisingly little was understood about the biology of posterolateral spinal fusions. It was reasoned that a more fundamental approach to solving the spinal pseudarthrosis problem would lie in experiments designed to illuminate the sequences of events, at a cellular level, involved in the spine fusion process. In other words, the problem had to first be understood in order to solve it.

Later that year, in a series of papers, Boden et al.[14,15] used their animal model of intertransverse spinal fusion for just that purpose. In the first of two papers[14], the authors characterized the healing sequence of a developing intertransverse spine fusion using autologous ICBG from a temporal and spatial standpoint using sequential histology. Three distinct phases of healing (inflammatory, reparative, and remodeling) were reported. Membranous bone formation, evident first at the ends of the fusion emanating from the decorticated transverse processes, was the predominant mechanism of healing. The central zone, however, was somewhat different in that there was a period of endochondral bone formation, where cartilage formed first but was subsequently converted to bone only in successful fusions. The authors concluded that the persistence of a central cartilage zone may possibly be related to some types of pseudarthroses.

Next, Boden and colleagues[15] determined the minimum effective dose of a bovine-derived osteoinductive bone protein extract (known to contain BMPs) for intertransverse process lumbar fusion in both rabbits and nonhuman primates. The authors reported that there was a dose-dependent response to the osteoinductive growth factors when used in rabbits and rhesus monkeys. The authors found that there was a dose threshold for locally delivered growth factors that must be overcome before bone formation is consistently induced.

A few years later, Morone et al.[16] followed up those studies with another set of experiments in rabbits in which the authors determined: (1) the temporal and spatial pattern of gene expression within the healing fusion mass following posterolateral intertransverse arthrodesis using ICBG and (2) how the addition of locally delivered rhBMP-2 to ICBG affected the gene expression pattern within the developing spine fusion mass. This study provided the first evidence that a reproducible temporal sequence and spatial pattern of gene expression exists in healing spine fusions. Interestingly, the authors found a temporal lag in gene expression in the central portion of the fusion mass that paralleled the lag in healing within the central zone that had previously been observed in histologic studies[14]. The addition of rhBMP-2 resulted in an increase in the early expression of BMP-6, which was subsequently associated with the expression of higher levels of type I collagen, osteocalcin, and other important bone-related genes. The authors concluded that rhBMP-2 may have the potential to decrease the likelihood of a pseudarthrosis by increasing the level of bone-related gene expression throughout the fusion mass and eliminating the delay in endochondral healing within the central zone.

These landmark studies represented an essential building block in our understanding of the biology of spinal fusion and showed the potential for the use of osteoinductive growth factors like rhBMP-2 to enhance posterolateral intertransverse process spinal fusions. For the first time, experiments could be designed in which spine fusions were either enhanced or retarded in order to elucidate the underlying mechanisms involved. This milestone was critical in that we now had the tools necessary to better understand how to effectively manipulate the spine fusion healing process.

II. Translation of BMP from Benchtop to Bedside

Once this groundwork was laid and the potential of BMP-2 to act as a biologic bone graft extender was realized, the next phase of BMP research at Emory focused on taking BMP-2 from the lab to the clinic. Using the now validated rabbit model of intertransverse process spinal fusion, Schimandle et al.[17] performed a series of experiments to determine the efficacy of rhBMP-2 as a potential substitute for ICBG. To do this, different dosages of rhBMP-2 loaded onto a type 1 collagen sponge were compared with the results obtained with ICBG. The authors reported that 100% of the rabbits implanted with rhBMP-2 achieved solid fusion, whereas only 42% of the autograft control fusions were solid (Fig. 1). In addition, fusions achieved with rhBMP-2 were biomechanically stronger and stiffer than fusions achieved using ICBG. These results were subsequently re-

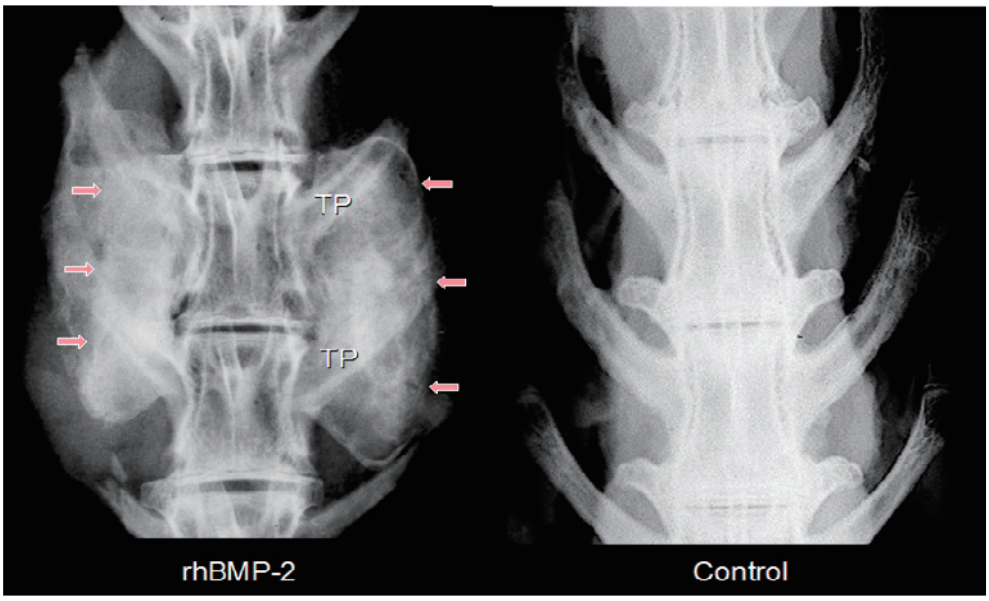

Figure 1. Radiographs of rabbit lumbar spines 5 weeks following implantation of 1.3 mg of rhBMP-2 on a collagen matrix in the posterolateral spine. A good fusion mass is seen on both sides of the spine (arrows), indicating a successful fusion in the rabbit that received rhBMP-2 (left panel). This is in contrast to the control animal (collagen alone).

confirmed in another set of experiments that were evaluated by computed tomography[18].

Since it is difficult to extrapolate results from lower vertebrates (i.e., rabbits) to humans, a critical step in determining the viability of an osteoinductive protein like BMP for use in humans is the demonstration of its success in nonhuman primates. This is important since there are several examples of osteoinductive substances that have demonstrated good efficacy in lower vertebrates but failed to induce bone in primates[19]. As such, Martin et al.[20] examined the issues of dose, carrier, and safety when using rhBMP-2 in a rhesus monkey posterolateral intertransverse process spinal arthrodesis with or without laminectomy. The rhesus monkey was specifically chosen over other nonhuman primate species because the successful BMP dose and healing pattern are most predictive of the human response[21]. This study was the first to show the ability of rhBMP-2 to successfully form bone in nonhuman primates. In addition, the most striking findings were as follows: (1) the presence of a laminectomy defect with exposed dura did not preclude the safe use of rhBMP-2 for posterolateral fusion as there was no evidence of bone overgrowth causing impingement of the neural elements; (2) soft tissue compression of the collagen sponge carrier prevented bone induction at previously successful BMP doses, presumably due to squeezing of the protein out of the sponge; and (3) mechanical protection of the carrier from soft tissue compression allowed more bone induction at lower doses of rhBMP-2.

This landmark study not only provided important proof of feasibility for the use of rhBMP-2 in spinal arthrodesis, but it also presented an unanticipated result. Although rhBMP-2 was able to influence the local biology to achieve successful fusion, it was the carrier that was now presenting a problem in larger bipedal animals like nonhuman primates. To address this, the Emory group next set out to optimize the carrier and make it more resistant to compression from the surrounding muscle, allowing the rhBMP-2 to stay locally longer so that it could exert its intended effect.

First, we performed a set of experiments in nonhuman primates in which the authors tested a biphasic calcium phosphate (BCP) granule carrier (60% hydroxyapatite) loaded with rhBMP-2[22]. Although 100% of the spines receiving rhBMP-2 fused posterolaterally at 24 weeks, the major limitation of the carrier was its slow resorption time resulting from the high hydroxyapatite content. This made radiographic detection of new bone formation difficult. To overcome this, the authors next tested collagen sponges loaded with BCP granules and only 15% hydroxyapatite[23]. This time, a 100% fusion rate in the posterolateral spine was achieved at 24 weeks in the nonhuman primate group receiving rhBMP-2 loaded onto the new compression-resistant collagen/BCP carrier. In addition, the carrier had significantly improved radiographic resorption properties that permitted easy radiographic visualization of new bone formation. In addition, a 50% lower dose of rhBMP-2 (3 mg/side) produced consistently successful fusions (the previously used dose with the plain collagen sponge was 6 mg/side). This 100% effective dose in the posterolateral spine of nonhuman primates was then further lowered to 2 mg/side when rhBMP-2 was loaded onto an absorbable collagen sponge (ACS) wrapped around a bulking agent consisting of a BCP/collagen composite[24].

Although the body of evidence from preclinical animal studies showed that rhBMP-2 had immense potential to enhance spinal fusion and potentially replace autologous ICBG, definitive evidence that it would work in humans was

Table 1. Rates of Successful Spinal Fusion in Each Location of the Spine Are Shown with and without the Use of RhBMP-2. The Numbers in the First and Second Row for Each Location Are the Fusion Rates Cited by Hofstetter et al.[30] and Galimberti et al.[31], Respectively. A Green Color in the "Indicated?" Column Means That Both Meta-analyses Agreed That BMP-2 Is Indicated in That Location. Red Indicates That Both Sets of Authors Agree That It Is Not Indicated, and Yellow Means That There Is a Disagreement between the Authors.

Meta-Analysis of BMP Fusions				
Location	Rate of Fusion		Delta	Indicated?
	Control	BMP-2		
ACDF (2+)	85.8	95.4	9.6%	Yes
ALIF	79.1	96.9	17.8%	?
	88.0	97.8	9.8%	
TLIF	93.0	95.0	2.0%	NO
	89.5	95.7	6.2%	
PLF	75.3	95.2	19.9%	YES
	83.1	93.6	10.5%	

still lacking. The first definitive evidence was provided by Boden et al.[25] in the form of a prospective randomized human clinical pilot trial in which patients underwent a single-level ALIF using a tapered cylindrical threaded cage filled with either rhBMP-2 on an ACS or autologous ICBG. High rates of radiographic fusion (100% rhBMP-2 groups vs. 66% fusion with ICBG) and a more rapid improvement in clinical outcome were reported. This was followed by a pilot study using rhBMP-2 for posterolateral spinal fusion. Twenty-five patients were randomized to instrumented posterior fusion supplemented with ICBG or rhBMP-2 or rhBMP-2 alone without instrumentation[26]. Follow-up at a minimum of 12 months found that fusion was achieved in only 40% of the patients who received autograft, whereas 100% of the patients who were treated with BMP-2 had achieved fusion.

In the years to follow, rhBMP-2/ACS has been shown to result in significantly larger and more consistent posterolateral fusion masses: (1) in patients with degenerative spondylolisthesis when used in combination with ICBG[27], (2) when local bone is substituted for ICBG in one- and two-level posterolateral fusions[28], and (3) in active cigarette smokers (95.2% successful fusion in rhBMP-2 group vs. 76.2% in ICBG group)[29]. More recently, two separate meta-analyses assessing the dose-dependent fusion rate when rhBMP-2/ACS is used in two or more level anterior cervical discectomy with fusions (ACDFs), ALIFs, transforaminal lumbar interbody fusions (TLIFs), and posterolateral fusions were performed[30,31]. Both studies concluded that rhBMP-2 is indicated in ACDFs with two or more levels and in posterolateral fusions, but not in TLIFs. The papers reached a different conclusion regarding ALIFs. These results are summarized in Table 1.

Despite these successes, local side effects have been reported. In a retrospective review, Cahill reported the following complications with the use of rhBMP-2 in the lumbar spine: vertebral osteolysis (44%), graft subsidence (27%) and graft migration (31%), ectopic/heterotopic bone formation (7%), and seroma/hematoma formation (3%)[32]. A concern about retrograde ejaculation has also been raised by the FDA with regard to the use of rhBMP-2 in ALIFs[33]. Similarly, systematic reviews looking at the complications associated with the use of rhBMP-2 in the cervical spine have also reported increased amounts of osteolysis and graft subsidence[32,34]. The occurrence of most of these side effects is proportional to the dose of rhBMP-2 used. Often, the doses used in the majority of the case reports of BMP-related side effects are much higher than the recommended doses.

Despite the reported side effects, rhBMP-2 remains one of the most powerful commercially available osteogenic agents. The surgeon should reserve its use for patients in whom the risks of failed spine surgery outweigh the risks of BMP use, including older patients with osteoporosis, chronic smokers at increased risk of pseudarthrosis/delayed union, and patients undergoing revision surgery for pseudarthrosis.

III. Discovery of LMP

Despite its immense potential and ability to potently induce an osteogenic response *in vivo*, widespread clinical use of rhBMP-2 has been limited because of the unexpectedly high dose required in humans for consistent bone formation[15]. A 15,000-fold higher concentration of BMP-2 is required to induce bone in humans (1.5 mg/mL) than in cell culture (100 ng/mL). This need for significantly higher concentrations is at least partially because primates have a slower influx of mesenchymal stem cells and thus require higher initial loading doses of rhBMP-2 to ensure enough BMP remains by the time stem cells arrive[35]. This larger human dose (12-40 mg for one spine level) has resulted in local side effects (swelling, bone resorption, and nerve inflammation) that were not seen in preclinical studies at lower doses[25,36,37].

As a result, investigations at Emory were initiated to explore mechanisms by which the cellular responsiveness to BMP could be improved. It was reasoned that by increasing a cell's responsiveness, the BMP dose needed to achieve a given osteoinductive effect could be lowered, thus improving safety and cost. Using differential display polymerase chain reaction in an effort to find novel genes expressed during early osteoblast differentiation, a novel osteoinductive protein, LMP-1, was discovered[38]. Although initially discovered by treating cells with glucocorticoid, LMP-1 expression is regulated by BMP-6, which was previously shown to be stimulated by glucocorticoid and to be one of the earliest BMPs expressed during osteoblast differentiation[39]. It was subsequently shown that blocking LMP-1 expression prevented osteoblast differentiation and bone nodule formation in calvarial osteoblast cultures[40]. When LMP-1 was overex-

pressed, however, osteoblast differentiation was initiated.

Unlike a BMP, which is a secreted protein that binds to a cell surface receptor to initiate a response, LMP-1 is an intracellular signaling molecule and must be located inside cells to exert its osteoinductive effect. Thus, any attempt at using LMP-1 to form bone must involve gene therapy techniques to deliver its complementary deoxyribonucleic acid (cDNA) inside the cell, resulting in the synthesis of LMP-1 protein *in situ*. Once the cDNA for LMP-1 was cloned and sequenced[40], it was demonstrated in both animal and *in vitro* studies that a potent and consistent osteoinductive effect could be produced by delivering the LMP-1 cDNA into cells using very low doses of adenoviral or plasmid vectors[41]. To test whether these results could be extended to promoting successful posterolateral spine fusions, a proof-of-feasibility study was performed in rats[42]. After undergoing a single-level posterior lumbar arthrodesis, the rats received bone graft material that was soaked in cells that were transfected with either the LMP-1 cDNA or the reverse copy of the cDNA that did not express any protein. Successful spine fusion was obtained in 100% of the arthrodesis sites that received the cells transfected with the active LMP-1 cDNA, whereas none of the sites that received the inactive cDNA formed bone (0% fusion).

Although these results were very encouraging, the exact mechanism by which LMP-1 worked was still unknown. Multiple mechanistic studies were next undertaken. It was found that LMP-1 transfection caused cells both *in vitro* and *in vivo* to express elevated levels of multiple BMPs, including BMP-2, BMP-6, and BMP-7, and that this resulted in the recruitment of host cells to differentiate and participate in direct membranous bone formation *in vivo*[43]. It was also found that the effects of LMP-1 reinforce the action of exogenously applied BMPs and that LMP activity is blocked by the BMP inhibitor noggin[44]. Although a clear link between LMP-1 and BMP signaling had been established, the question still remained: does LMP-1 exert its effect by somehow affecting the cellular responsiveness to extracellular BMP?

The answer to this question came in 2006 when Sangadala and colleagues[45] showed that specific binding occurs between LMP-1 and Smurf1, an E3 ligase that ubiquitinates many molecules in the BMP-2 signaling pathway, which leads to their degradation by proteasomes (Fig. 2). The authors demonstrated that LMP-1 works by competing with Smad1 and Smad5 for Smurf1 binding, thereby enhancing cellular responsiveness to exogenous BMP-2 by preventing the degradation of these key intracellular signaling molecules in the BMP pathway. The authors also showed that the LMP-1/Smurf1 interaction requires the Smurf1 WW2 domain, which is dependent on a specific motif in LMP-1, and that LMP-1's biologic effects on BMP responsiveness can be mimicked by a small peptide containing only that motif.

This was the first study to show that the cellular responsiveness to BMP could in fact be modulated via regulation of Smad degeneration. It was becoming clear that the use of LMP-1 was a potential viable strategy to reduce the BMP-2 dose needed in humans to achieve successful spine fusion in an attempt to limit the financial cost and local side effects that were being seen with the high doses being used clinically. The issue of delivery remained, however, since LMP-1 is only an intracellular protein with no extracellular receptors to interact with. Since gene therapy in humans is still not widely accepted due to the potential risks[46], alternative strategies were investigated. These included the engineering and cloning of a recombinant LMP-1 containing an N-terminal HIV-derived membrane transduction domain called TAT, which conveys the ability to cross cell membranes. In fact, it was demonstrated that fusing the TAT motif to LMP-1 indeed allowed LMP-1 to cross the cell membrane and exert its usual intracellular effects *in vitro*[47].

Although being able to deliver extracellular LMP-1 without gene therapy techniques was an exciting result[47], this strategy did not adequately address the restrictive high cost and large-scale manufacturing problems associated with recombinant proteins. Thus, the Emory group decided to build on everything that they had learned from their years of BMP and LMP-1 research and focus on designing osteoinductive small molecule drugs that could potentially activate the BMP signaling cascade.

IV. Small Molecule Design

In contrast to full-sized recombinant proteins like BMP-2 and LMP-1, small molecules have several important advantages: (1) they have significantly cheaper manufacturing cost, (2) greater stability and far superior shelf life, and (3) easier engineering and incorporation into carriers to achieve controlled spatial and temporal release. In addition, their relatively small size compared with recombinant proteins affords them immunoprivilege[48]. These factors significantly increase the attractiveness of using pharmacologic small molecules over recombinant protein strategies in the spine.

Although multiple negative regulators of the BMP signaling pathway have been identified, LMP-1 is unique in that it represents a rare positive regulator of BMP signaling and thus is an attractive target for small molecule drugs. Since it was already known that Smurf1 interaction with LMP-1 is based on the presence of a unique WW2 domain-interacting motif[45], site-directed mutagenesis and binding studies on purified recombinant proteins was performed[49] in order to find the specific WW-interacting motif within the osteogenic region of LMP-1 that binds to this WW2 domain on Smurf1. Computational, homology-based modeling of the LMP-1/Smurf1 interaction was performed *in silico*, and the key amino acids involved in their binding regions were identified (Fig. 3). This knowledge was paramount in the design of effective mimetic compounds that could either mimic or disrupt this specific interaction.

In order to screen large numbers of potential candidate compounds, the Emory group next developed and optimized a cell-based assay to use as a tool to identify reagents that

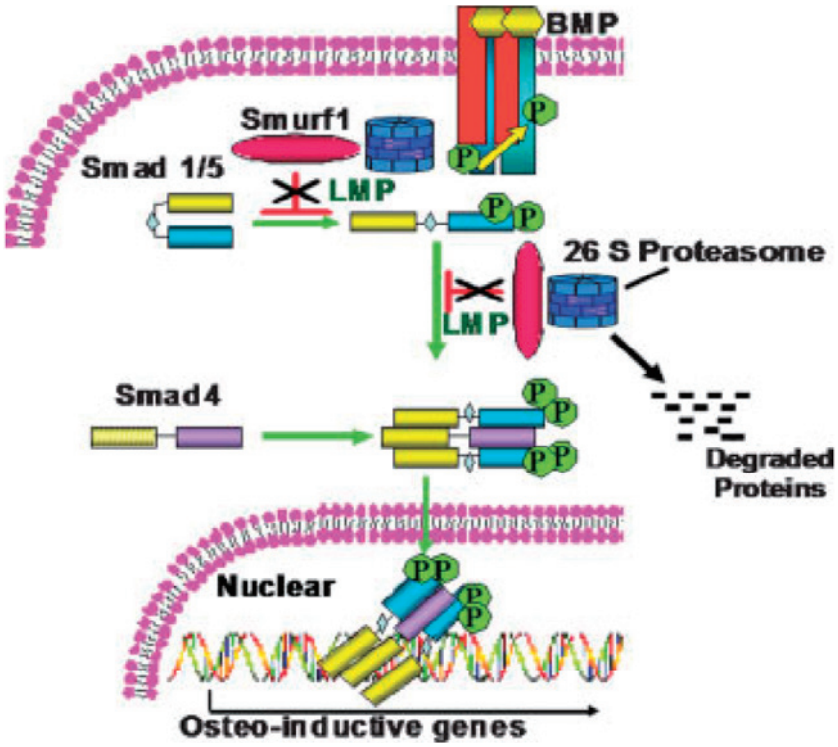

Figure 2. LMP-1 rescues Smads from Smurf1-mediated proteosomal degradation. LMP-1 regulates cellular responsiveness to BMP. Upon binding of BMP ligand to its specific cell surface receptor, intracellular signaling proteins Smad1/5 are phosphorylated. The activated Smad1/5 associates with Smad4. The oligomerized Smad complex then enters the nucleus to induce BMP-responsive genes in concert with other transcription factors. LMP-1 competitively binds to Smurf1 and rescues Smads1/5 from being targeted for Smurf1-dependent ubiquitin-mediated proteasomal degradation. Consequently, the rescued Smads1/5 leads to potentiation of the BMP pathway by enhancing the expression of BMP-induced genes such as alkaline phosphatase and osteocalcin. (Reproduced with permission from Okada M, et al. Development and optimization of a cell-based assay for the selection of synthetic compounds that potentiate bone morphogenetic protein-2 activity. Cell Biochem Funct. 2009; 27 (8): 526-34.)

potentiate intracellular BMP activity. Since traditional biochemical screening approaches (i.e., *in vitro* binding assays) are not a true representation of interactions that occur in living cells, Okada et al.[50] developed a new gene expression-based cell monitoring approach that selects reagents based on their real effects on cell physiology. This novel cell-based assay was successfully used to screen for active compounds from a select group of compounds that were identified by computational screenings as the most likely candidates for mimicking the function of LMP-1. One particular compound, SVAK-3, showed a dose-dependent potentiation of BMP-2 activity by inducing osteoblastic transdifferentiation of myoblastic C2C12 cells[50].

Although SVAK-3 at first seemed to be a great candidate for a clinically translatable small molecule drug, it was unfortunately found to be rather unstable and quickly lost its biologic activity *in vivo*. This warranted a new search for a more stable and efficacious compound. First, Kato and colleagues[51] performed extensive chemo-informatic *in silico* analyses of the Smurf1 WW2 domain and its interacting site with LMP-1 on over 70,000 small molecules with known drug-like capabilities (Fig. 4). The authors next tested those leading candidates in their cell-based screening assay using a Smad1-specific luciferase reporter. A unique compound called SVAK-12 was identified and was found to be more stable than SVAK-3 as well as promote the BMP-induced expression of phenotypic markers characteristic of a differentiated osteoblast in a dose-dependent manner.

The small synthetic molecule SVAK-12 was further tested for its ability to enhance bone formation *in vivo*[52]. Specifically, it was tested for its ability to enhance bone formation of a suboptimal dose of rhBMP-2 in a rodent ectopic model and to test whether a single percutaneous injection of SVAK-12 could accelerate callus formation in a rodent femoral fracture model. In the ectopic model, SVAK-12 produced a dose-dependent enhancement of rhBMP-2 activity (Fig. 5). SVAK-12 also resulted in significantly higher fracture healing rates in the femoral fracture model as well as

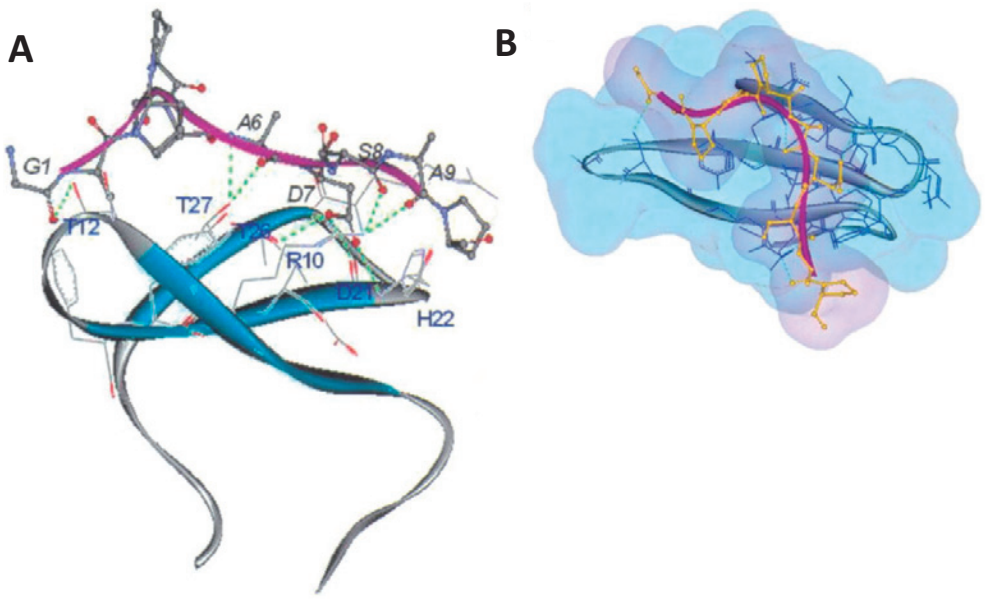

Figure 3. (A) Modeling of the WW2 domain and the LMP-1 peptide showing amino acid residues involved in hydrogen bonding interaction with the residues in peptide ligand (represented as ball and stick model). Intermolecular hydrogen bonds are represented as green dotted lines. The specific amino acid residues involved in the hydrogen bonding are labeled. (B) Molecular surface view of the WW2 domain and LMP-1 peptide docked complex showing the side chains of amino acids with the conservation index greater than 0.8. (Reproduced with permission from Sangadala S, et al. Modeling and analysis of molecular interaction between Smurf1-WW2 domain and various isoforms of LIM mineralization protein. Proteins. 2007; 68 (3): 690-701.)

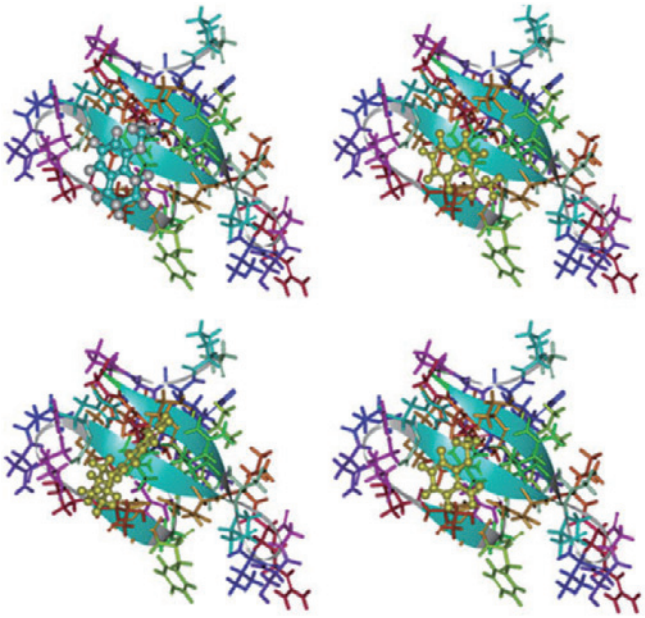

Figure 4. Binding poses are shown for four selected high-scoring low molecular weight molecules that were in the pool of 300 consensus high-scoring molecules used for virtual testing. The WW2 domain atoms are shown in stick form, whereas the compounds are shown in ball and stick form. (Reproduced with permission from Kato S, et al. A synthetic compound that potentiates bone morphogenetic protein-2-induced transdifferentiation of myoblasts into the osteoblastic phenotype. Mol Cell Biochem. 2011; 349 (1-2): 97-106.)

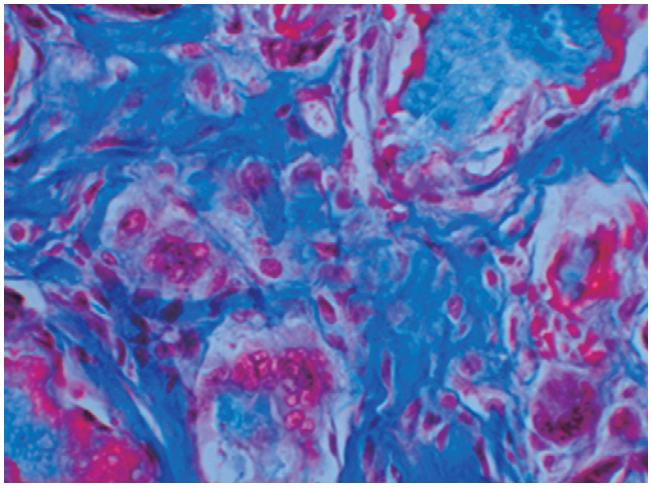

Figure 5. Representative histological image of a collagen disk containing rhBMP-2 and SVAK-12 four weeks after subcutaneous transplantation, showing ectopic trabecular bone formation and mineralized osteoid. This photomicrograph demonstrates that the bone formed has been deposited by osteoblasts and is not a chemical deposition of mineral (Gomori one-step trichrome stain, ×33). (Reproduced with permission from Wong E, et al. A novel low-molecular-weight compound enhances ectopic bone formation and fracture repair. J Bone Joint Surg Am. 2013; 95 (5): 454-61.)

better radiographic healing scores and biomechanical testing results (i.e., 43% stronger and 93% stiffer). This landmark study showed for the first time that a single dose of a small molecule drug administered subcutaneously could enhance bone healing by increasing the cellular responsiveness to both exogenous rhBMP-2 (ectopic model) and endogenous BMPs (femoral fracture model).

Since this study, the Emory group has continued to work on discovering, characterizing, and testing both novel and FDA-repurposed small molecule drugs to enhance bone formation. We have worked on producing small molecules that target Jab1[53], which targets a common Smad, Smad4, shared by both the BMP and transforming growth factor-β (TGF-β) pathways, for proteasomal degradation. Jab1 also binds to Smad4, Smad5, and Smad7, key intracellular signaling molecules of the TGF-β superfamily, and causes ubiquitination and/or degradation of these Smads as well. In addition to Jab1, our lab has also more recently developed small molecule drugs that inhibit the function of Noggin, a BMP antagonist, as well as a small molecule that binds directly to the sclerostin receptor, LRP-5/6. This sclerostin small molecule inhibitor has been shown in our lab to enhance both BMP and canonical Wnt signaling *in vitro* and *in vivo* (unpublished data).

Conclusion

Careful consideration must be given to the eventual biologic goal of a spinal arthrodesis: successful bone formation and fusion. Bone is the material that, for a lifetime, will bear the stresses that instrumentation can support only temporarily. Over 25 years of translational spine and BMP-related research here at Emory has been dedicated toward advancing our knowledge of how to use biologics to enhance bone formation and achieve consistently reliable spinal fusions. Our lab continues to work on testing multiple candidate small molecule drugs in translational models, and we remain confident that the local application of osteogenic small molecules has the potential to lower the required doses of rhBMP used clinically and might someday decrease their cost, improve their safety profile, and allow for their more widespread routine use.

Conflicts of Interest: Neither Dr. Presciutti nor any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article. Dr. Boden or an immediate family member has received royalties from Medtronic, serves as a paid consultant to SeaSpine, and has stock options in Regeneration Technology Inc. and Bone Biologics Corporation.

References

1. Kim YJ, Bridwell KH, Lenke LG, et al. Pseudarthrosis in primary fusions for adult idiopathic scoliosis: incidence, risk factors, and outcome analysis. Spine. 2005;30(4):468-74.
2. Martin BI, Mirza SK, Comstock BA, et al. Reoperation rates following lumbar spine surgery and the influence of spinal fusion procedures. Spine. 2007;32(3):382-7.
3. Kim SS, Michelsen CB. Revision surgery for failed back surgery syndrome. Spine. 1992;17(8):957-60.
4. Yeramaneni S, Robinson C, Hostin R. Impact of spine surgery complications on costs associated with management of adult spinal deformity. Curr Rev Musculoskelet Med. 2016;9(3):327-32.
5. Gupta AR, Shah NR, Patel TC, et al. Perioperative and long-term complications of iliac crest bone graft harvesting for spinal surgery: a quantitative review of the literature. Int Med J. 2001;8: 163-6.
6. Ahlmann E, Patzakis M, Roidis N, et al. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. J Bone Joint Surg Am. 2002;84A (5):716-20.
7. Albert TJ, Pinto M, Denis F. Management of symptomatic lumbar pseudarthrosis with anteroposterior fusion. A functional and radiographic outcome study. Spine. 2000;25(1):123-30.
8. Cheh G, Bridwell KH, Lenke LG, et al. Adjacent segment disease following lumbar/ thoracolumbar fusion with pedicle screw instrumentation: a minimum 5-year follow-up. Spine. 2007;32(20):2253-7.
9. Boden SD. Biology of lumbar spine fusion and use of bone graft substitutes: present, future, and next generation. Tissue Eng. 2000; 6(4):383-99.
10. Urist MR. Bone: Formation by autoinduction. Science. 1965;150 (3698):893-9.
11. Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop Relat Res. 1998;(346):26-37.
12. Cheng H, Jiang W, Phillips FM, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg Am. 2003;85-A(8):1544-52. Erratum in: (2004). J Bone Joint Surg Am;86-A:141.
13. Boden SD, Schimandle JH, Hutton WC. An experimental lumbar intertransverse process spine fusion model. Spine. 1995;20(4):412-20.
14. Boden SD, Schimandle JH, Hutton WC, et al. 1995 Volvo Award in basic sciences. The use of an osteoinductive growth factor for lumbar spinal fusion. Part I: Biology of spinal fusion. Spine (Phila Pa 1976). 1995;20(24):2626-32.
15. Boden SD, Schimandle JH, Hutton WC. 1995 Volvo Award in basic sciences. The use of an osteoinductive growth factor for lumbar spinal fusion. Part II: Study of dose, carrier, and species. Spine (Phila Pa 1976). 1995;20(24):2633-44.
16. Morone MA, Boden SD, Hair G, et al. The Marshall R. Urist Young Investigator Award. Gene expression during autograft lumbar spine fusion and the effect of bone morphogenetic protein 2. Clin Orthop Relat Res. 1998;(351):252-65.
17. Schimandle JH, Boden SD, Hutton WC. Experimental spinal fusion with recombinant human bone morphogenetic protein-2. Spine (Phila Pa 1976). 1995;20(12):1326-37.
18. Holliger EH, Trawick RH, Boden SD, et al. Morphology of the lumbar intertransverse process fusion mass in the rabbit model: a comparison between two bone graft materials--rhBMP-2 and autograft. J Spinal Disord. 1996;9(2):125-8.
19. Aspenberg P, Lohmander LS, Thorngren KG. Failure of bone induction by bone matrix in adult monkeys. J Bone Joint Surg Br. 1988;70(4):625-7.
20. Martin GJ Jr, Boden SD, Marone MA, et al. Posterolateral intertransverse process spinal arthrodesis with rhBMP-2 in a nonhuman

primate: important lessons learned regarding dose, carrier, and safety. J Spinal Disord. 1999;12(3):179-86.

21. Boden SD, Grob D, Damien C. Ne-Osteo bone growth factor for posterolateral lumbar spine fusion: results from a nonhuman primate study and a prospective human clinical pilot study. Spine (Phila Pa 1976). 2004;29(5):504-14.

22. Boden SD, Martin GJ, Morone MA, et al. Posterolateral lumbar intertransverse process spine arthrodesis with rhBMP-2/hydroxyapatite-tricalcium phosphate (HA-TCP) following laminectomy in the non-human primate. Spine. 1999;24(12):1179-85.

23. Akamaru T, Suh D, Boden SD, et al. Simple carrier matrix modifications can enhance delivery of recombinant human bone morphogenetic protein-2 for posterolateral spine fusion. Spine (Phila Pa 1976). 2003;28(5):429-34.

24. Barnes B, Boden SD, Louis-Ugbo J, et al. Lower dose of rhBMP-2 achieves spine fusion when combined with an osteoconductive bulking agent in non-human primates. Spine (Phila Pa 1976). 2005;30(10):1127-33.

25. Boden SD, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine. 2000;25(3):376-81.

26. Boden SD, Kang J, Sandhu H, Heller JG. 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 (Phila Pa 1976). 2002;27(23):2662-73.

27. Singh K, Smucker JD, Gill S, Boden SD. Use of recombinant human bone morphogenetic protein-2 as an adjunct in posterolateral lumbar spine fusion: a prospective CT-scan analysis at one and two years. J Spinal Disord Tech. 2006;19(6):416-23.

28. Park DK, Kim SS, Thakur N, Boden SD. Use of recombinant human bone morphogenetic protein-2 with local bone graft instead of iliac crest bone graft in posterolateral lumbar spine arthrodesis. Spine (Phila Pa 1976). 2013;38(12):E738-47.

29. Glassman SD, Dimar JR 3rd, Burkus K, et al. The efficacy of rhBMP-2 for posterolateral lumbar fusion in smokers. Spine (Phila Pa 1976). 2007;32(15):1693-8.

30. Hofstetter CP, Hofer AS, Levi AD. Exploratory meta-analysis on dose-related efficacy and morbidity of bone morphogenetic protein in spinal arthrodesis surgery. J Neurosurg Spine. 2016;24(3):457-75.

31. Galimberti F, Lubelski D, Healy AT, et al. A systematic review of lumbar fusion rates with and without the use of rhBMP-2. Spine (Phila Pa 1976). 2015;40(14):1132-9.

32. Cahill KS, Chi JH, Day A, et al. Prevalence, complications, and hospital charges associated with use of bone-morphogenetic proteins in spinal fusion procedures. JAMA. 2009;302(1):58-66.

33. 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. Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cftopic/pma/pma.cfm?num5P000058. 2002.

34. Mroz TE, Wang JC, Hashimoto R, et al. Complications related to osteobiologics use in spine surgery: a systematic review. Spine. 2010;35(9 Suppl):S86-104.

35. Martin GJ, Boden SD, Morone MA, et al. Posterolateral intertransverse process spinal fusion arthrodesis with rhBMP-2 in a non-human primate- Important lessons learned regarding dose, carrier, and safety. J Spinal Disord. 1999;12(3):179-86.

36. Ackerman SJ, Mafilios MS, Polly DW, Jr. Economic evaluation of bone morphogenetic protein versus autogenous iliac crest bone graft in single-level anterior lumbar fusion: an evidence based modeling approach. Spine. 2002;27(16 Suppl 1):S94-9.

37. Kusumoto K, Bessho K, Fujimura K, et al. Osteoinduction by recombinant human bone morphogenetic protein-2 in muscles of non-human primates. J Int Med Res. 2002;30(3):251-9.

38. Liu Y, Hair G, Titus L, et al. BMP-6 induces a novel LIM protein involved in bone mineralization and osteocalcin secretion. J Bone Min Res. 1997;12:Sl 15.

39. Boden SD, Hair G, Titus L, et al. Glucocorticoid-induced differentiation of fetal rat calvarial osteoblasts is mediated by bone morphogenetic protein-6. Endocrinology. 1997;138(7):2820-8.

40. Boden SD, Liu Y, Hair GA, et al. LMP-1, a LIM-domain protein, mediates BMP-6 effects on bone formation. Endocrinology. 1998;139(12):5125-34.

41. Viggeswarapu M, Boden SD, Liu Y, et al. Adenoviral delivery of LIM mineralization protein-1 induces new-bone formation in vitro and in vivo. J Bone Joint Surg Am. 2001;83-A(3):364-76.

42. Boden SD, Titus L, Hair G, et al. Lumbar spine fusion by local gene therapy with a cDNA encoding a novel osteoinductive protein (LMP-1). Spine (Phila Pa 1976). 1998;23(23):2486-92.

43. Minamide A, Boden SD, Viggeswarapu M, et al. Mechanism of bone formation with gene transfer of the cDNA encoding for the intracellular protein LMP-1. J Bone Joint Surg Am. 2003;85-A(6):1030-9.

44. Yoon ST, Park JS, Kim KS, et al. ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs in vitro and in vivo. Spine. 2004;29(23):2603-11.

45. Sangadala S, Boden SD, Viggeswarapu M, et al. LIM mineralization protein-1 potentiates bone morphogenetic protein responsiveness via a novel interaction with Smurf1 resulting in decreased ubiquitination of Smads. J Biol Chem. 2006;281(25):17212-9.

46. Noguchi P. Risks and benefits of gene therapy. N Engl J Med. 2003;348(3):193-4.

47. Sangadala S, Okada M, Liu Y, et al. Engineering, cloning, and functional characterization of recombinant LIM mineralization protein-1 containing an N-terminal HIV-derived membrane transduction domain. Protein Expr Purif. 2009;65(2):165-73.

48. Blaich G, Janssen B, Roth G, et al. Overview: differentiating issues in the development of macromolecules compared with small molecules. In Handbook of Pharmaceutical Biotechnology (Gad, S.C., ed. John Wiley & Sons).

49. Sangadala S, Boden SD, Metpally RP, et al. Modeling and analysis of molecular interaction between Smurf1-WW2 domain and various isoforms of LIM mineralization protein. Proteins. 2007;68(3):690-701.

50. Okada M, Sangadala S, Liu Y, et al. Development and optimization of a cell-based assay for the selection of synthetic compounds that potentiate bone morphogenetic protein-2 activity. Cell Biochem Funct. 2009;27(8):526-34.

51. Kato S, Sangadala S, Tomita K, et al. A synthetic compound that potentiates bone morphogenetic protein-2-induced transdifferentiation of myoblasts into the osteoblastic phenotype. Mol Cell Biochem. 2011;349(1-2):97-106.

52. Wong E, Sangadala S, Boden SD, et al. A novel low-molecular-weight compound enhances ectopic bone formation and fracture repair. J Bone Joint Surg Am. 2013;95(5):454-61.

53. Sangadala S, Yoshioka K, Enyo Y, et al. Characterization of a unique motif in LIM mineralization protein-1 that interacts with jun activation-domain-binding protein 1. Mol Cell Biochem. 2014;385(1-2):145-57.