

# Clinical Validation of Allogeneic Morphogenetic Protein: Donor Intersubject Variability, Terminal Irradiation and Age of Product is not Clinically Relevant

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## Abstract

Donor to donor variation has long been a concern of the allograft industry. DBM and stem cell products have been particularly susceptible to intersubject variability between donors, regardless of the process used to manufacture these products. Manufacturers of allograft based products have often utilized *in vitro* or small animal models to help predict reliability, yet little data is available correlating preclinical outcomes with clinical efficacy.

OsteoAMP is a commercially available allograft-derived growth factor rich in osteoinductive, angiogenic, and mitogenic proteins. An analysis of radiographic results comparing fusion outcomes was conducted for 285 consecutive cervical and lumbar spinal fusion patients utilizing OsteoAMP bone grafts from 114 donors of varying ages. A blinded radiological fusion assessment, performed by an independent radiologist, showed all patients, except one, fused within 18 months (average time to fusion was 189.9 days). This evidentiary analysis shows that OsteoAMP fusion success did not show donor intersubject variability and that fusion rate/time is not dependent on donor age. In addition, the implant retained bioactivity over time and terminal sterilization via low-dose gamma irradiation did not impair the bioactivity of the grafts.

**Keywords:** Donor intersubject variability; Allograft; OsteoAMP; Spine fusion

## Introduction

Clinical efficacy between donors has been a challenge in the allograft industry for many decades [1,2]. Compounding the problem, research has shown that there is in fact more variability between donors than between products and the processes used to manufacture these products [3,4]. Variables contributing to these concerns include donor age and gender, processing techniques and sterilization methods [2,5]. According to the U.S. Department of Health & Human Services approximately 60% of deceased donors recovered in the US between 1988 and 2013 were male with the majority of all donors ranging in age between 35 and 64 years.

To try and predict efficacy of the tissue recovered, demineralized bone matrix (DBM) products and stem cell products often utilize *in vitro* or ectopic rat *in vivo* assays. There have been little data correlating preclinical outcomes with clinical efficacy, limiting the value of such testing [2]. Quite often, the donor material tested preclinically is at an intermediary processing step, prior to terminal sterilization and storage.

Sterilization is a critical process to ensure graft preservation [6]. There have been a number of product recalls in recent years for products that do not undergo terminal sterilization. In particular, allograft-derived stem cell products have been reported to the US Food and Drug Administration for contamination of hepatitis [7] and multiple strains of clostridium [8-10]. Terminal sterilization provides the surgeon with an additional safeguard against microbial

contaminates, although the perceived detrimental effects of irradiation have caused uncertainty in its overall clinical value [11]. Studies have shown that irradiation with 25 kGy reduces osteoblast differentiation and expression of BMP-7 when compared to non-irradiated human bone allograft implanted in a nude rat model [12]. Other studies have shown that irradiation used by bone banks did not influence the inductive properties of DBM [13,14]. In addition, adding a carrier like lecithin to a DBM could negatively influence biological activity after sterilization [11]. This study attempted to identify the effects of processing and donor selection used for a novel allograft growth factor by analyzing the fusion rates of patients that underwent spine surgery. It was hypothesized that the growth factors remained bioavailable and were not affected by the age of the donor, the age of the product or whether the product was irradiated.

## Methods and Materials

A retrospective radiographic analysis was conducted to evaluate the fusion success rate in patients receiving allograft material from donors of different genders, ages and preparations utilizing a commercially available allogeneic morphogenetic protein, OsteoAMP® (Advanced Biologics, Carlsbad, CA). The study involved two sites with three treating physicians total. The indications for surgery were symptomatic patients diagnosed with degenerative disc disease (DDD), stenosis, and/or spondylolisthesis.

OsteoAMP bone allograft was processed from human cadavers cleared for implantation utilizing a proprietary processing technique. OsteoAMP was selected as the biologic and used in conjunction with the centers' preferred spinal spacer and fixation system. All products were prepared per the instructions for use. No other biologic product

was used in combination with OsteoAMP other than autologous bone marrow aspirate or local autologous bone (if available).

**Patient Demographics**

Two hundred and eighty five consecutive patients underwent lumbar (n=166) or cervical (n=119) fusions. The mean patient age of

the lumbar group was 59.6 and the mean age of the cervical group was 52.6. The majority of lumbar patients underwent a transforaminal approach for fusion (74.7%) while the majority of cervical patients underwent an anterior approach for fusion (93.3%). Patient demographics are shown in Table 1.

Demographics	Lumbar (n=166)	Cervical (n=119)
Age, mean ± SD	59.6 ± 13.0	52.6 ± 10.4
Female, n (%)	90 (54%)	54 (45%)
Avg Levels/Case	1.6	1.7
Approach, n (%)		
Anterior	23 (13.9%)	111 (93.3%)
Posterior	19 (11.4%)	12 (10.1%)
Transforaminal	124 (74.7%)	0 (0.0%)
Format, n (%)		
Cervical spacer	0 (0.0%)	4 (3.4%)
Granules	105 (63.3%)	26 (21.8%)
Sponge	24 (14.5%)	89 (74.8%)
Granules & sponge	37 (22.3%)	0 (0.0%)

Table 1: Patient Characteristic

\*Note: Some cervical cases involved both an anterior and posterior approach with the same patients.

**Donors**

OsteoAMP used in these procedures was processed by two AATB accredited and FDA registered tissue banks.

	Female	Male
# donors	6	108
Donor age, mean ± SD	35.2 ± 20.0	55.4 ± 15.5
% donors used in cervical procedures	16.7%	38.0%
% donors used in lumbar procedures	83.3%	77.8%
# patients	9	395
Patient age, mean ± SD	56.7 ± 14.2	57.5 ± 12.2
Avg Levels/Case	2.1	1.9
Cervical anterior approach, # pt	1	134
Cervical posterior approach, # pt	0	14
Lumbar anterior approach, # pt	1	41
Lumbar posterior approach, # pt	6	251
Lumbar transforaminal/lateral approach, # pt	6	181
Irradiated		Aseptic

# donors	17	97
Donor age, mean ± SD	47.5 ± 17.5	55.5 ± 15.9
% donors used in cervical procedures	23.5%	39.2%
% donors used in lumbar procedures	88.2%	76.3%
# patients	28	376
Patient age, mean ± SD	62.7 ± 10.7	57.1 ± 12.2
Avg Levels/Case	2.8	1.8
Cervical anterior approach, # pt	5	130
Cervical posterior approach, # pt	2	12
Lumbar anterior approach, # pt	0	42
Lumbar posterior approach, # pt	19	239
Lumbar transforaminal/lateral approach, # pt	15	172

Table 2: Donor Information

\*Due to procedural overlap, the percent of donors and number of patients may not add up to 100%.

One hundred fourteen donors (488 allografts) were processed in various formats. Of the 114 donors, 108 were male and 6 were female with a mean age of 55.4 and 35.2, respectively. Female donors were 36% younger than male donors ( $p < 0.01$ ). Of these same 114 donors, 97 were processed aseptically while 17 donors were terminally irradiated

after processing (Table 2). Average aseptic donor age was 55.5 years. Average irradiated donor age was 47.5 years. Irradiated donors were 14% younger than aseptic donors but the difference was not statistically significant ( $p = 0.06$ ). Of the 488 allografts that were processed from the 114 donors, 445 were processed aseptically while 43 were terminally sterilized after processing. Product age was designated as the time from packaging to implant (Table 3).

	Irradiated	Aseptic
# allografts	43	445
# female	18	228
Patient age, mean ± SD	64.5 ± 9.8	56.8 ± 12.3
% allografts used in cervical procedures	20.9%	35.7%
% allografts used in lumbar procedures	79.1%	64.3%
Time to fusion, mean ± SD (days)	150.3 ± 41.5	193.2 ± 92.9
Allograft product age, mean ± SD (days)	91.1 ± 24.3	213.2 ± 137.9
Cervical anterior approach, # pt	7	153
Cervical posterior approach, # pt	2	14
Lumbar anterior approach, # pt	0	60
Lumbar posterior approach, # pt	16	36
Lumbar transforaminal/lateral approach, # pt	18	190

Table 3: Allograft Information

## Analysis

Patients had radiographs (x-ray and/or CT) at the post-operative follow-up timepoints, which were generally 3, 6, 12, 18 months post-surgical procedure. Fusion was defined as clearly bridging bone

between both endplates. The evaluation was conducted by a radiologist blinded to treating physician or other study variables and to ensure that the opacity of the biologic or local bone did not interfere with the evaluation. Fusion assessment results were used to analyze differences in clinical efficacy in both cervical and lumbar surgery. The analysis

attempted to simply identify any correlation between time to fusion and donor age, donor gender, irradiated product or the age of the product. Linear regression was used in an attempt to identify any correlation between variables. All other data points were analyzed with a one-way analysis of variance ( $p < 0.05$ ).

## Results

Representative lateral radiographs are shown in Figures 1 and 2. Fusion analysis time points are shown in Table 4.



**Figure 1:** 62 y/o Male, L4-5 at 6m Follow-Up



**Figure 2:** 60 y/o Male, C5-6 at 6m Follow-Up

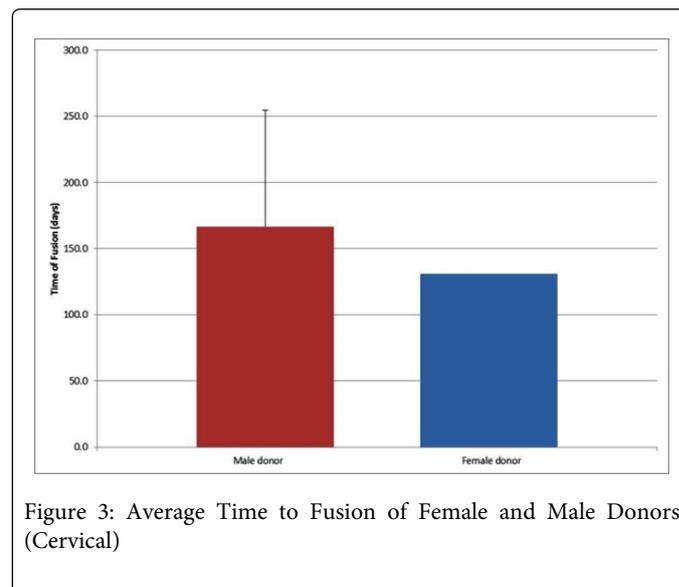
6m	113 (68.1%)	99 (83.2%)
12m	163 (98.2%)	117 (98.3%)
18m	165 (99.4%)	119 (100%)

**Table 4:** Fusion Analysis by Time Point

By the 12 month mark, 98.2% of lumbar patients and 98.3% of cervical patients had shown a solid bridging arthrodesis. The cervical group expectedly fused faster at 167.2 days (5.5 months) while the lumbar group fused at 206.2 days (6.8 months).

## Cervical Fusion Analysis

Average fusion time of patients who received OsteoAMP from male donors for cervical cases was 166.7 days. The average fusion time of patients who received OsteoAMP from female donors was 131.0 days. Although there were fewer female donors, there was no statistical difference in fusion times between male and female donors ( $p = 0.51$ ) (Figure 3). Average patient age for those receiving a female donor was 56.7 years. Average patient age for those receiving a male donor was 57.5 years. There was no statistical difference in patient age whether they received a male or female donor ( $p = 0.86$ ).



**Figure 3:** Average Time to Fusion of Female and Male Donors (Cervical)

Time Points (Months)	Lumbar n=166	Cervical n=119
3m	27 (16.3%)	38 (31.9%)

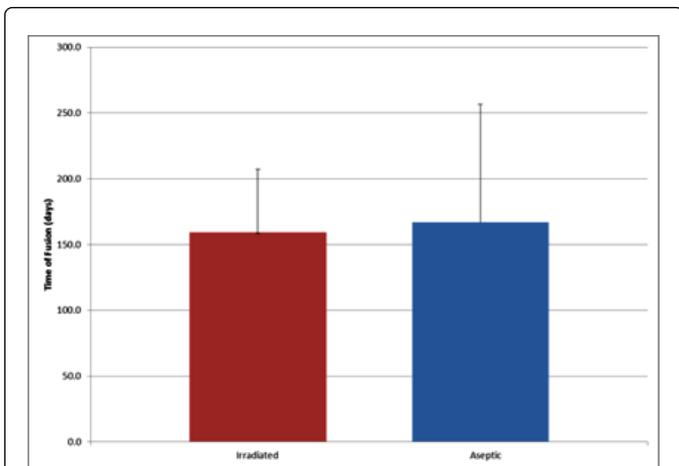


Figure 4: Average Time to Fusion of Terminally Irradiated and Aseptic Donors (Cervical)

Average fusion time of patients who received OsteoAMP from aseptically processed donors was 166.8 days. Average time of fusion of patients who received OsteoAMP from terminally irradiated donors was 159.3 days but this difference was not significant ( $p=0.71$ ) (Figure 4). Average patient age for those receiving an irradiated donor was 57.1 years. Average patient age for those receiving an aseptic donor was 52.8 years.

Among the fused patients in the cervical fusion analysis, no correlation was found between the time to fusion and OsteoAMP product age ( $R^2=0.0142$ ) (Figure 5).

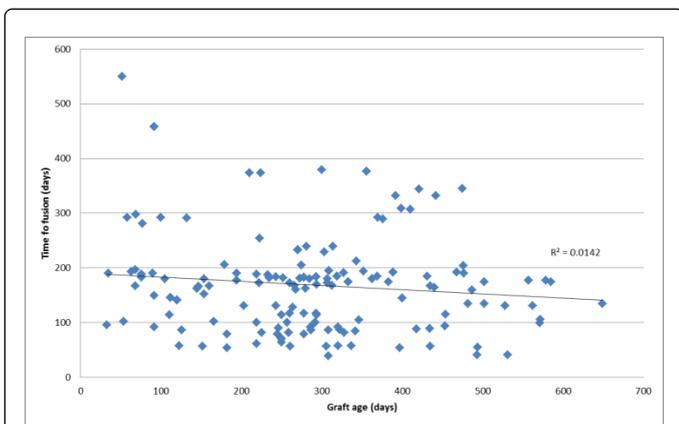


Figure 5: Average Time to Fusion versus Age of the Product in Cervical Procedures

Among the fused patients in the lumbar fusion analysis, no correlation was found between the time to fusion and OsteoAMP donor age ( $R^2=0.0088$ ) (Figure 6).

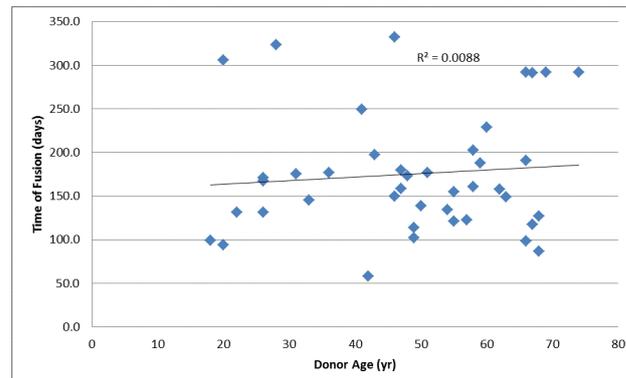


Figure 6: Time to Fusion versus OsteoAMP Donor Age (Cervical)

### Lumbar Fusion Analysis

Among the fused patients in the lumbar fusion analysis, no correlation was found between the time to fusion and OsteoAMP donor age ( $R^2 = 0.0031$ ) (Figure 7).

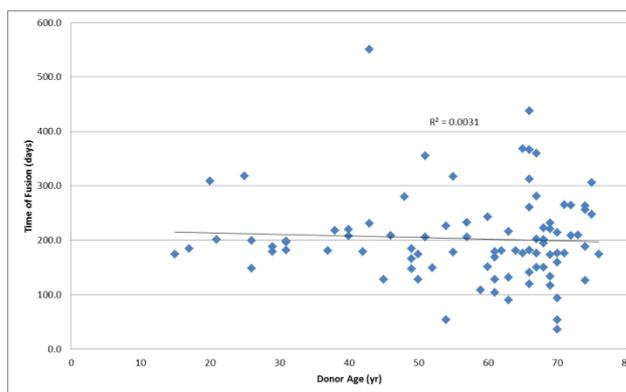


Figure 7: Time to Fusion versus OsteoAMP Donor Age (Lumbar)

Average fusion time of lumbar patients who received OsteoAMP from male donors was 203.6 days. The average fusion time of patients who received OsteoAMP from female donors was 211.0 days. There was no statistical difference between male and female donors and time to fusion ( $p=0.60$ ) (Figure 8). Average patient age for those receiving a female donor was 56.3 years. Average patient age for those receiving a male donor was 60 years. There was no statistical difference in patient age whether they received a male or female donor ( $p=0.51$ ).

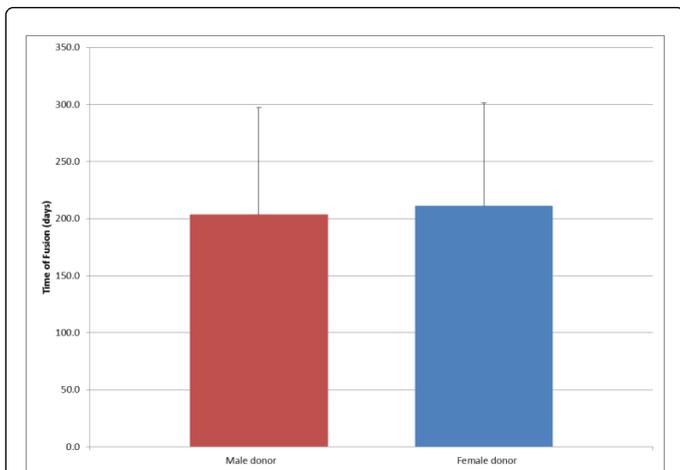


Figure 8: Average Time to Fusion of Female and Male Donors (Lumbar)

Average fusion time of patients who received OsteoAMP from aseptically processed donors was 208.7. Average time of fusion of patients who received OsteoAMP from terminally irradiated donors was 152.3 days. There was a statistical difference in fusion times between aseptically processed and terminally sterilized allografts ( $p > 0.001$ ) (Figure 9). Average patient age for those receiving an irradiated donor was 64.6 years. Average patient age for those receiving an aseptic donor was 59.5 years.

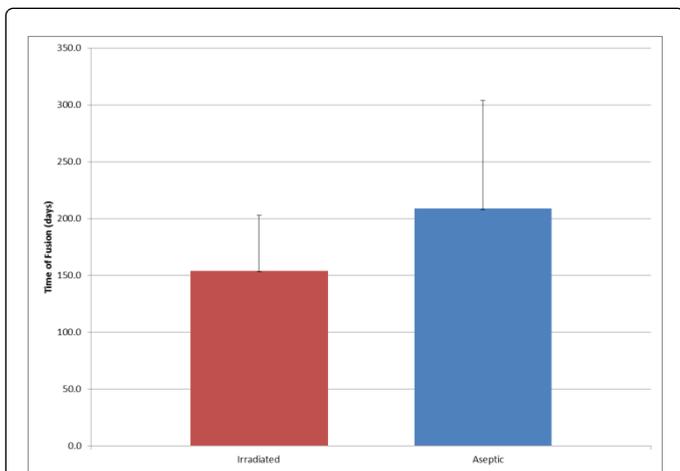


Figure 9: Average Time to Fusion of Terminally Irradiated and Aseptic Donors (Lumbar)

Among the fused patients in the lumbar fusion analysis, no correlation was found between the time to fusion and OsteoAMP product age ( $R^2 = 0.001$ ) (Figure 10).

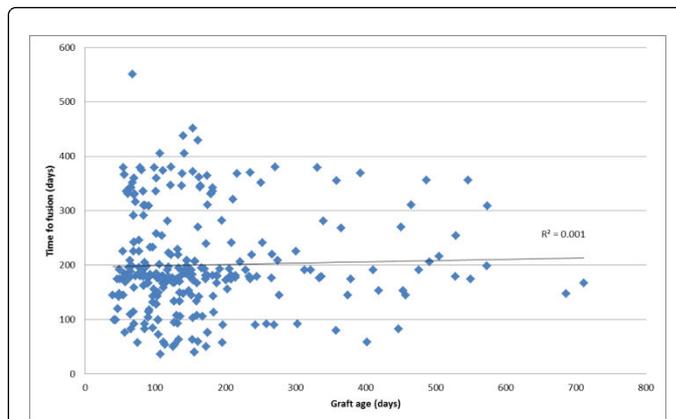


Figure 10: Average Time to Fusion versus Age of the Product in Lumbar Procedures

## Discussion

Literature reports success rates in cervical spine fusion between 70% to 98% depending on the number of levels involved [15,16]. In lumbar spine surgery, literature reports nonunion rates for a single level fusion to be between 10% and 20% and is greatly affected by surgical technique, fixation system and biologic used [17,18].

Demineralized allograft bone has been shown to contain limited amounts of BMP [4]. Raw material screening for donor comorbidities and age has become standard practice in the allograft industry. However, processing and sterilization methods vary and can affect active BMP content [19]. Many DBMs are tested for osteoinductivity *in vitro* but very few are tested *in vivo* to ensure a minimum level of osteoinductivity with the final sterile product. In addition, there is little data supporting that preclinical testing actually translates to clinical efficacy of these bone grafts [2].

Allograft stem cell products are classified as Human Cellular and Tissue Products (HCT/Ps) and therefore contain a biologically insignificant amount of bone forming cells (FDA 2006).

According to the US Food and Drug Administration, to be classified as HCT/P these minimally manipulated tissues cannot rely on the metabolic activity of living cells as their primary function. Stem cells undergo challenging processing techniques to maintain cell viability [20]. In addition, these cells require a signal to transform them into a bone forming cell relying on trace amounts of BMPs found in the demineralized bone tissue [4,21].

Data from the current study demonstrated over 98% fusion rates for both lumbar and cervical patients at 18 months. This confirms the results from previous studies using OsteoAMP where reported fusion results of 98.9% at 18 months in lumbar and 100% at 18 months in cervical cases when OsteoAMP was used as the biologic [22,23]. We found no biological adverse events, although one patient did develop a pseudarthrosis without a clear reason. When looking at complication rates with other biologics, in particular rh-BMP-2, there have been reports of dysphagia, inflammation and death in cervical cases while lumbar adverse events with rh-BMP 2 include infection, ectopic bone growth, retrograde ejaculation and cancer [24-26]. Having a biological alternative to rh-BMP 2 with equal or better fusion rates with an

improved safety profile would be clinically attractive but this requires further study.

Like with all retrospective studies, there were a few potential limitations. There was no control group used in the study, although the information on historical controls in the literature provides same basic comparison on fusion rates and complications. Clinical outcomes were not evaluated (VAS, ODI, etc.); however, these data will be captured in further studies utilizing a similar study design. Despite these limitations, the analysis demonstrated that the age of the product and donor intervariability were not clinically relevant to time to fusion. There were differences in donor gender and age, as well as differences in graft preparation and storage life but these represent the common variants that would potentially occur when using allograft at the time of surgery. Despite these differences, they had no effect on the successful fusion outcomes in patients with inherent variabilities themselves (smoking, BMI, etc). Future study is needed to further track any donor dependent gender based differences in the time to fusion.

## Consent

When necessary, written informed consent was obtained from the patient for the publication of this report and any accompanying images.

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