Journal of Orthopaedics 60 (2025) 159–166

Contents lists available at ScienceDirect

Journal of Orthopaedics

journal homepage: www.elsevier.com/locate/jor

Use of osteogenic bone matrix in patients with traumatic long bone defects: An open label, single center study

Marshall Murdoch [a,*], Craig Wittstock [a], George Psaras [a], Alan Widgerow [b], Mkhululi Lukhele [c], Mmampapatla Thomas Ramokgopa [c], Jacques Snyman [d], Jane Hutchings [e], Elizabeth Marcos [c], Anna Grisillo Biscardi [c], Duncan Cromarty [f], Xu Zheng [g], Nicolaas Duneas [g], Shunmugam Govender [h]

[a] Division of Plastic and Reconstructive Surgery, University of the Witwatersrand, South Africa
[b] Center for Tissue Engineering, Plastic Surgery Department, University of California, Irvine, USA
[c] Department of Orthopaedic Surgery, University of the Witwatersrand, South Africa
[d] Isimo Health (Pty) Ltd, South Africa
[e] Medical Consultant, South Africa
[f] Department of Pharmacology, University of Pretoria, South Africa
[g] Department of Oral Medicine and Periodontology, University of the Witwatersrand, South Africa
[h] Department of Orthopaedics, University of KwaZulu Natal, South Africa

ARTICLE INFO

Keywords:
Bone graft
Demineralized bone matrix
Tissue engineering
Bone morphogenetic proteins
Open long bone fractures

ABSTRACT

Background: Osteogenic Bone Matrix (Altis™ OBM) is a tissue-engineered, porcine-derived demineralized bone matrix prepared using a humanization processing technology that confers biocompatibility and improved osteoinductivity. The objective of this study was to determine the safety and efficacy of OBM in patients with traumatic long bone defects in an open-label, non-randomized single-center study.

Methods: Diagnosis and main criteria for inclusion were open long bone fractures graded as Gustilo-Anderson Grade II, IIIA or IIIB. 24 participants were enrolled from one center, of which 17 were assigned to the investigational group (OBM) and 7 to the standard of care (SOC) group. Participants were followed at intervals of one, two, six, and 13 weeks to undergo physical examinations and record adverse events, vital signs, electrocardiograms, hematology, blood biochemistry and circulating humoral antibodies against human and porcine Type I and II collagens. Efficacy of treatment over six months post-surgery was assessed by a panel of blinded radiologists to determine the proportion of subjects with radiographic bridging of fractures in both the OBM efficacy group and the SOC group. Limb function, weight-bearing, pain and mobility at the fracture site were assessed by the investigator. Patient satisfaction with the treatment and quality of life were assessed using the SF 36 quality of life questionnaire.

Results: 14 OBM patients and five SOC patients completed the first three months of the safety investigation. 10 OBM patients and four SOC patients completed the full six months of the efficacy investigation. Biochemical and hematological parameters were within normal ranges. The efficacy evaluation at six months indicated that 70 % of participants in the OBM group had bridging of the bone defect and 80 % were weight-bearing versus 50 % in the SOC group. The quality of life study demonstrated an increased level of satisfaction as compared with the baseline. Histological analysis of a single biopsy specimen at three months revealed bone regeneration activity within the implanted OBM.

Conclusions: The study showed that treatment with OBM was well tolerated in participants and there was no evidence of clinically relevant toxicity or immunological, biochemical, hematological or adverse reaction due to

* Corresponding author.
E-mail addresses: Marshall@drmjmurdoch.co.za (M. Murdoch), Craig.Wittstock@wits.ac.za (C. Wittstock), psarasg@cytanet.cy (G. Psaras), awidgero@uci.edu (A. Widgerow), Christeleen.Ontlong@wits.ac.za (M. Lukhele), jacques.snyman@isimo.co.za (J. Snyman), j.hutchings@mweb.co.za (J. Hutchings), emhealth531@gmail.com (E. Marcos), anna.grisillo@gmail.com (A. Grisillo Biscardi), Duncan.Cromarty@up.ac.za (D. Cromarty), gene.zheng@gmail.com (X. Zheng), nic@altisbiologics.com (N. Duneas), profgovender@gmail.com (S. Govender).

https://doi.org/10.1016/j.jor.2024.08.018
Received 5 August 2024; Received in revised form 28 August 2024; Accepted 29 August 2024
Available online 4 September 2024

the use of OBM. There was better bridging in the OBM group *versus* SOC. Pharmacoeconomic analysis showed OBM to be cost-effective versus standard of care.
Trial registration: Medicines *Control* Council of South Africa (MCC number N2/19/8/2).

1. Introduction

The tissue engineering approach to restoration and regeneration of traumatic bone loss and non-healing defects has been a central pursuit of skeletal reconstructionists in recent decades. Fractures account for the largest total lifetime cost (24 %) associated with any one injury type, with over $99 billion in estimated medical costs and productivity loss in the USA.[1] It is estimated that approximately 10 % of human bone fractures fail to unite successfully.[2,3] (see Fig. 1) (Fig. 2)

Open long bone fractures are generally difficult to treat, and a significant proportion will have complications such as infection and non-union.[4] There is a high cost associated with failed fracture treatment, stemming from repeated surgical interventions, patient disability and the inability to work. Open long bone fractures occur with a frequency of about 11.5 per 100,000 persons per year, the majority being open tibial diaphyseal fractures of which about 60 percent are classed as Gustilo-Anderson type III.[4] A prospective evaluation of patients with open tibial fractures in the Lower Extremity Assessment Project (LEAP) study showed that at 2 years most had poor outcomes, with only half returning to work.[5]

Approximately 1.5 million bone-grafting operations are performed annually in the United States[6] of which 250,000 are autograft or allograft procedures performed by orthopedic surgeons to treat segmental bone defects.[7] Autogenous bone grafting has been considered to be the gold standard because of its known osteogenic properties and complement of live cells and has been widely used in the treatment of problematic fractures. However, clinical studies have demonstrated up to a thirty percent rate of unsatisfactory results after autogenous grafting of segmental bone defects caused by complicated fractures.[8] Although highly effective, the autogenous grafting technique nevertheless is associated with certain risks. Approximately thirty percent of patients that undergo autogenous iliac crest grafting will suffer complications that include increased infection risk, symptoms of pain, hypersensitivity, buttocks anesthesia, extra hospital stay and costs, and late-onset orthopedic complications.[3,9] There is a need therefore for the development of a suitable bone graft substitute biomaterial that can match or supersede the efficacy of the autogenous graft.

The discovery that the demineralized matrix of bone (DBM) is osteoinductive in allogeneic implantation experiments[10] by its content of the bone morphogenetic proteins (BMPs)[11,12] led to the cloning,[13] and clinical validation of the use of 2 recombinantly produced BMP protagonists in human skeletal therapeutics.[14,15] The commercial introduction of 2 recombinant bone morphogenetic proteins (OP-1/BMP-7, Stryker Biotech; BMP-2 InductOS Wyeth Biotech) and a human bone-derived morphogenetic protein allograft (OsteoAMP, Pioneer) has consequently offered surgeons biologic alternatives to autogenous bone grafts.[15,16] Enhanced bone allografts such as OsteoAmp have shown superiority in clinical studies versus recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) in lumbar interbody fusion.[17] OsteoAMP has boosted levels of endogenously sourced morphogens and growth factors that are known inducers of bone formation aimed to

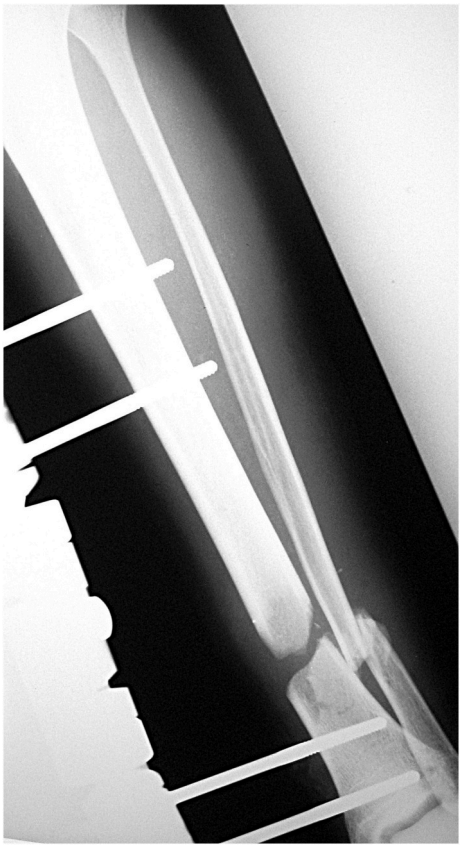

Fig. 1. Gustilo-Anderson grade III fracture with extensive loss of tibial bone andsurrounding soft tissue requiring flap surgery.

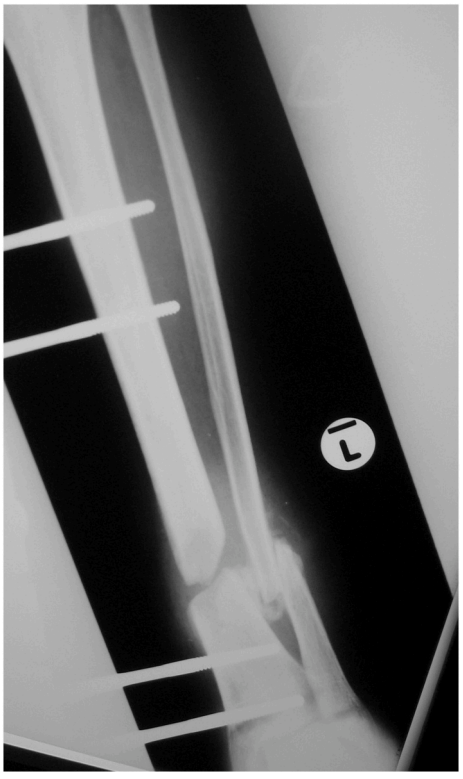

Fig. 2. Bone briding and extensive mature callus formation on all cortices of the tibia and fibula evidenced at 6 weeks. The surgeon opted to shorten the tibia leading to the overlap of the fibula. Soft tissue healing was excellent.

encourage superior osteoinductivity with a high safety profile. However, the paucity of human donors from which to manufacture enhanced bone allografts may limit their global availability. Tissue-banked bone grafts such as demineralized bone matrix (DBM), have shown an important role in orthopedics, but are less effective than autogenous grafting[18] and possess a degree of immunogenicity that persists following extensive processing.[19] Other potential disadvantages of allografts may include the potential for transmission of infectious disease,[20] immunogenicity and rejection,[21] decreased osteoinductivity after processing and sterilization[22] and intra-product and inter-product variability concerning BMP activity.[23]

The limitations due to the paucity of allografts may be overcome by sourcing bone tissue from abundant slaughterhouse animals such as porcine, bovine, and equine species, for the manufacture of enhanced bone graft biomaterials. Xenogeneic bone however presents its challenges, and in its native state, is a poor osteoinductor in cross-species studies,[24] ascribed to the host's immunological reaction against the animal-derived extracellular bone matrix (ECM).[25] Overcoming the osteogenesis inhibition seen in native xenogeneic DBM is an important first step in the successful development of animal-derived bone grafts and specific processing steps must be applied to overcome this inhibition issue.[24] Traditional humanization techniques applied to collagenous biomaterials that rely upon pepsin digestion to produce a biocompatible atelocollagen biomaterial[25] would also digest endogenous morphogens in DBM that are responsible for DBM's osteoinductivity. In the case of the investigational OBM, this obstacle has been overcome through the process of separation of the bone morphogenetic protein-complex (BMP-complex) from the ECM of DBM, followed by the extensive processing of the isolated ECM to render it biocompatible (humanized). Following ECM humanization, the isolated BMP complex is reconstituted with the biocompatible ECM to restore osteoinductivity of the final re-assembled OBM. This OBM process results in a higher abundance of endogenous BMP-2 in the final reconstituted OBM when compared to human DBM (Table 4).[26,27] We define BMP-complex as the consortium of non-collagenous proteins responsible for the osteoinductivity of DBM and comprise numerous proteins of the TGF-β superfamily including BMP-2, BMP-7, and TGF-β1.[10,11] This manipulation procedure provides a human-compatible, enhanced, homologous xenogeneic DBM with the desired levels of biologic activity, prepared from a single source of animal bone as starting material.

The central objectives of the open-label study were to establish whether Altis™ OBM (Altis Biologics Pty Ltd, Pretoria, South Africa) is safe and effective for use as a bone graft substitute in the treatment of traumatic long bone defects in human subjects. An evaluation was conducted of the quality of life of the OBM recipients relative to their baseline, assessed using the patient-reported outcome questionnaire. A pharmaco-economic study was conducted to determine the feasibility of OBM for clinical practice.

Table 1
Patient baseline demographic data of per-protocol safety population.

Parameter	OBM group (n = 14)	SOC group (n = 5)
Age, mean ± SD	31.1 ± 7.3	29.6 ± 4.6
Range (years)	20–50	24–34
Coeff. of variation	23.5	15.5
Male, (%)	100 %	100 %
Race (black)	100 %	100 %
Gustilo-Anderson Class		
II	8	5
IIIA	1	
IIIB	5	
Treated bone		
tibia	6	1
tibia & fibula	6	1
femur		1
femur & tibia	–	1
radius & ulna		1
metacarpals	2	

Table 2
Changes in IgG antibody titers for anti-human collagen antibodies, pre-surgery and at endpoint of three months.

	anti-human Type I		anti-human Type II	
	pre-surgery	at endpoint	pre-surgery	at endpoint
Mean antibody titer U/L, mean ± SD	135 ± 107	216 ± 191	422 ± 690	521 ± 826
P value (2-tailed)[a]		0.16		0.22
Range of antibody titer U/L	36–369	41–678	22–2394	20–2612
Coefficient of variation	0.79	0.89	1.64	1.59
F statistic[b]		0.31		0.70
No. of patients with high antibody titer at endpoint		2		1

[a] Two-tailed paired *t*-test.
[b] Two sample F Test for variances. Endpoint was three months.

Table 3
Changes in IgG antibody titers for anti-porcine collagen antibodies, pre-surgery and at endpoint of three months.

	anti-porcine Type I		anti-porcine Type II	
	pre-surgery	at endpoint	pre-surgery	at endpoint
Mean antibody titer U/L, mean ± SD	219 ± 425	472 ± 782	413 ± 785	709 ± 1054
P value (2-tailed)[a]		0.20		0.11
Range of antibody titer U/L	18–1479	26–2464	8–2634	48–2804
Coefficient of variation	1.94	1.66	1.90	1.49
F statistic[b]		0.28		0.43
No. of patients with high antibody titer at endpoint		1		1

[a] Two-tailed paired *t*-test.
[b] Two sample F Test for variances. Endpoint was three months.

Table 4
BMP-2 content of OBM, human bone matrix and commercial preparations of human DBM.

BMP-2 content ng/g DBM		
Altis™ OBM	human bone matrix[a]	commercial human DBM[b]
1400	21.4 ± 12	46.3 ± 23.9

a 2.
b 28.

2. Methods

2.1. Study design

The trial was an open-label, non-randomized study over six months. There was a standard of care (SOC) group that received standard of care treatment only without trial interventions. The SOC consisted of autograft procedure following Open-Reduction Internal Fixation (ORIF) and was followed solely to obtain comparative radiographic data for a small cohort of patients that had also suffered open long bone fractures.

The OBM was used in conjunction with the investigators' preferred instrumentation which included intramedullary nailing, plates, screws and external fixators. Assessment of quality of life was conducted using the SF-36 quality of life questionnaire.[29,28] Non-returning participants were not replaced and were excluded from the analysis, hence the per-protocol data set was used in the analysis. Participants were followed for 6 months, with follow-up assessments conducted generally at one, two, six, 13 and 26 weeks after treatment. A subpopulation of

participants that registered high circulating antibody titer for human and porcine collagen Type I and Type II at the study endpoint, were followed for a further 18-month period to determine if there were any clinical changes related to the OBM implant.

2.2. Patient inclusion criteria

Participants had to be adults over 18 years of age diagnosed with open long-bone fractures graded as Gustilo-Anderson Grade II, IIIA or IIIB. The Gustilo-Anderson classification defines the severity of the soft tissue injury associated with open fractures.[22,30,31] The bone fracture had to be less than 5 cm linear length in size, or require an approximate minimum total volume of 3.3 cm^3 and an approximate maximum total volume of 9.9 cm^3 of OBM to be used to treat the fracture. Females that were pregnant or breastfeeding were excluded. Participants were excluded if they had known or suspected allergies to components of the investigational product, systemic diseases such as liver or kidney disease, diabetes, immune-related disorders and connective tissue disorders such as rheumatoid arthritis, systemic lupus erythematosus or any other medical condition that might interfere with the evaluation of the investigational product. Concomitant medication such as immune modifiers, non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, steroids and chronic antimicrobial drugs were not permitted. Analgesic drugs including tramadol, aspirin and paracetamol were permitted. Altis™ OBM was prepared for use according to the manufacturer's instructions, which requires reconstitution with water for injection to form an injectable paste. The OBM is delivered into the bone defect using the injection device.

Primary Efficacy Analyses: radiographic outcome parameters.

Evaluation of fusion or bridging of the bone fracture postoperatively for the OBM group and the SOC group was done using plain radiographs for the anterior-posterior, lateral and oblique radiographic aspects of the fracture site. Interpretations of the radiographs were conducted by a radiologist and an orthopedic surgeon, both of whom were blinded to the intervention and product information, and who scored individually. Bridging was assessed using a standard 2-point bridging scale (0 = not bridged, 1 = bridged). Clinical union was defined as pain-free, full weight-bearing requiring no further surgical intervention whilst radiological union as evidence of new bone bridging of the fracture site on more than one radiographic aspect.[32]

The primary efficacy analyses aimed to determine the following:

1. If treatment with Altis™ OBM induced fusion or bridging of the bone deformity based on fracture site radiographs.
2. If treatment with Altis™ OBM improved weight-bearing, reduced the tenderness, and improved mobility at the fracture site.

2.3. Secondary efficacy analyses

The OBM participants rated their overall response at day zero and week two, 13, and 26 post-surgery by using the patient-reported outcome questionnaire SF-36[29,28] containing 36 questions across 11 categories and additional three supplementary questions related to limb function in one supplementary category. Weight-bearing is a parameter that represents the limb functional recovery after treatment. Assessment of pain was done by participants and investigators using a zero to 10 numeric pain intensity scale which ranged from "no pain" to "severe pain".

2.4. Safety outcome parameters for the OBM group

The safety population was defined as all participants who had received OBM and had undergone subsequent safety assessments. There were no concomitant diseases present in the participants except one who had asthma. Data for vital signs (blood pressure, heart rate, respiratory rate) at each visit were recorded in addition to the changes from baseline values.

Hematological evaluations were done for hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration; absolute and differential counts were done for neutrophils, monocytes, lymphocytes, eosinophils and basophils, and absolute counts were done for white blood cells, red blood cells and platelets. Biochemistry evaluations were done for alkaline phosphatase, alanine transaminase, aspartate transaminase, gamma-glutamyl transferase, lactate dehydrogenase, and creatine phosphokinase. Sodium, potassium, chloride, calcium, carbon dioxide, phosphorus, creatinine, uric acid, urea, total protein, albumin, total bilirubin, bilirubin conjugated, triglycerides, total cholesterol, p-glucose fasting, p-glucose random, anion gap, were determined.

Antibody determinations were conducted for circulating immunoglobulin (IgG) antibodies against Type I and Type II porcine and human collagens by ELISA immunosorbent assay kits (Chondrex, USA). The laboratory values were classified by comparing baseline and post-baseline values. Participants that developed titer values that were greater than the 75th percentile accompanied by titer increases that were fourfold greater than baseline values were followed for a further 18 months and tested for rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (aCCPs), markers of autoimmune disease.[33]

2.5. Statistical methodology for laboratory data

Data analysis was performed using Statistical Analysis Software (ver 9.1, NC, USA) and spreadsheet software (Microsoft® Excel® 2016). Shift tables were used to evaluate categorical changes (below, within or above normal range) in hematological and biochemical laboratory parameters by examining the proportion of participants whose laboratory values were outside the specific ranges at their final visit or changed from screening to the final assessment. All shifts in values were examined on a case-by-case basis whilst any changes from baseline were reviewed to investigate changes of clinical significance.

2.6. Analysis of adverse events

Adverse event (AE) evaluations were performed at screening and each visit of the study on weeks one, two, six and 13. The analysis of treatment-emergent adverse events was done overall and for related adverse events (with suspected or probable relationship to investigational product). The treatment-emergent adverse events were summarized by preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA version 10.0) and by body system. The World Health Organisation (WHO) drug dictionary was used to code treatments, investigations and medical procedures. Drugs were classified as per their anatomic-therapeutic classification. Overall incidences of adverse events including 95 % confidence intervals were calculated. Changes in results of vital signs from day 0 to the last visit at 13 weeks or early discontinuation were described.

3. Results

24 participants were enrolled, of which 17 were assigned to the OBM group and seven to the SOC group. 14 participants in the OBM group and five participants in the SOC group completed the three-month safety study and represent the per-protocol safety subgroup, whose data were included for baseline versus follow-up analyses (Table 1). 10 participants in the OBM group and four participants in the SOC group completed the efficacy study of six months and represent the efficacy subgroup.

3.1. Safety results

3.1.1. Overview of adverse events (AEs)

12 AEs were reported by seven participants, out of which three were

Table 5
Primary outcome parameters at 6 months per Gustilo-Anderson classification.

	OBM Group				SOC group
	total OBM group	Type II	Type IIIA	Type IIIB	Type II
No. of patients	10	7	1	2	4
Bridged n (%)	7 (70 %)	6 (86 %)	1 (100 %)	–	2 (50 %)
Weight bearing n (%)	8 (80 %)	6 (86 %)	1 (100 %)	1 (50 %)	n/a

severe adverse events (SAEs), four were moderate and five were mild events. The common AEs noted were increased blood immunoglobulin (three events) and pyrexia (two events). The injury, poisoning and procedural complications registered four events. All the other AEs had a single occurrence. Two participants experienced serious adverse events (SAE) which were post-surgical and related to wound complications, both cases occurring in the OBM group, but were found to be unrelated to the use of OBM. No patient died during this study and no adverse events led to the premature withdrawal of any patient from the study. There were no obvious differences between the two treatment groups in mean values or mean change from baseline for systolic and diastolic blood pressure, respiratory rate and heart rate.

3.1.2. Laboratory parameters

All laboratory parameters in the OBM group remained on average within normal reference values. There was a trend of increase in alkaline phosphatase (ALP) and red cell distribution width over time, which was detected in three participants (23 %). The observed increase in ALP activity over time in fracture patients has previously been reported after trauma[34] and our data corroborate these results. There was an increase in differential lymphocytes and a decrease in mean corpuscular hemoglobin and mean corpuscular volume in two (15 %) participants with values remaining within the normal reference range.

Table 6
Proportion of patients in SOC and OBM group with successful fusion at each time point.

Time point(weeks)	SOC group	OBM group
	n (%)	n (%)
0	0/5 (0 %)	0/14 (0 %)
6	0/5 (0 %)	0/14 (0 %)
13	1/4 (25 %)	1/13 (8 %)
26	2/4 (50 %)	7/10 (70 %)

Table 7
Primary efficacy parameters outcomes at each time point for OBM group.

Weeks	Proportion fused	Proportion weight bearing	Median pain score	
	n (%)	n (%)	(investigator's assessment)	(patient's assessment)
0	0/14 (0 %)	1/17 (6 %)	7.4	7.5
1	–	0/13 (0 %)	4.6	3.5
2	–	0/14 (0 %)	2.1	2
6	0/14 (0 %)	3/14 (21 %)	0.6	0.85
13	1/13 (8 %)	9/11 (82 %)	0.2	0.2
26	7/10 (70 %)	8/10 (80 %)	0.45	0.75

3.1.3. Antibody study

It was observed that about 30 % of the population had high (75th percentile) pre-existing (pre-surgery) baseline antibody titers for at least one of the four collagen species that were investigated. On week 13, three participants recorded a greater than four-fold increase in antibody titers from baseline, accompanied by an absolute titer value above the 75th percentile (Table 2; Table 3). One participant developed anti-porcine Type I and Type II collagen antibodies, but did not develop any anti-human collagen antibodies. Two participants developed increases in anti-human Type I collagen antibodies but lacked antibodies against porcine collagen, and this finding could thus not be associated with the porcine OBM implant. No participants developed anti-porcine collagen antibodies in conjunction with anti-human Type I and Type II collagen antibodies, a result that corroborates previous findings of an intradermal bovine collagen study.[35] The participants that recorded greater than four-fold increases in their antibody titers were followed for a further 18 months by an independent rheumatologist to monitor changes in their rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (aCCPs).[33] Tests for RF and aCCPs were negative throughout the follow-up period of 18 months. There were no adverse clinical manifestations or changes in RF and aCCPs markers as a result of the OBM exposure in the follow-up period.

3.2. Primary efficacy results

Based on the two-point radiographic assessment scale, seven (70 %) out of 10 subjects who received Altis™ OBM had evidence of cortical bridging on at least three of the four cortices, and eight (80 %) were weight-bearing at week 26. Two (50 %) out of four subjects at week 26 of the SOC group had evidence of cortical bridging on at least three of the four cortices (Table 5). Table 6 shows the proportion of participants in the SOC and OBM groups with successful fusion at each time point. The pain scores and weight-bearing scores for the OBM group at each time point are presented in Table 7.

3.3. Secondary efficacy results

Satisfaction of OBM treatment was assessed by the patient-reported outcomes questionnaire (SF-36 Quality of Life questionnaire[36]) at

Table 8
Summary of SF-36 patient reported outcomes at endpoint of 6 months.

Parameter	Min value	Max Value	Mean	Standard Alpha[a]
Physical Functioning	0	100	51.41 ± 33.25	0.95
Role Functioning/physical	0	100	31.25 ± 40.59	0.90
Functioning/emotional	0	100	44.68 ± 45.72	0.89
Energy/fatigue	10	85	46.96 ± 14.38	−0.28
Emotional well-being	0	85	50.92 ± 17.61	0.47
Social functioning	0	100	54.52 ± 32.18	0.81
Pain	0	100	64.31 ± 32.61	0.91
General Health	25	87.5	55.19 ± 14.35	0.15

[a] Cronbachs'a alpha statistic was used to test reliability of Likert scales in SF-36 questionnaire.

baseline and weeks two, 13 and 26. Mean positive changes were seen for all the parameters at six months post-surgery (Table 8).

3.3.1. Cost-effectiveness analysis and pharmaco-economic-based price determination for OBM in long-bone fractures

The study included a literature survey of standard of care utilization and delayed and non-union of fractures.[37–39] The cost-effectiveness assessment of Altis™ OBM was done by calculating the incremental cost-effectiveness ratio (ICER), which is calculated as the incremental costs associated with the introduction of OBM divided by the incremental economic effect achieved by such introduction. ICER facilitated a calculation of the cost per additional non-union/delayed union after the introduction of OBM. South African private sector hospital data were sourced from two independent healthcare funders, which provided a cost base for fracture management and delayed/nonunion of fractures. The costs associated with the management of delayed and non-union of tibia fractures in the South African context were estimated to be ZAR 31, 801 (US$ 2355), as compared to published figures of US$11,333[35] in the US, €13,899 in Europe and £7338 in the United Kingdom.[37] It was determined that an acquisition and administration cost of up to ZAR 85, 860 could be considered cost-effective. The cost of the OBM product in South Africa is ZAR 13,800. The survey showed that in South Africa autogenous grafting is performed with an annual frequency of 40,000 at an additional economic cost estimated at ZAR 300 million (USD 20 million) per annum.

4. Discussion

The discovery and demonstration of synergistic bone induction from binary applications of transforming growth factor-β1 (TGF-β1) and BMP-7, two bone matrix morphogens, has alluded to the deployment by nature of multiple morphogen combinations during bone induction and regeneration processes.[12,30] The extracellular matrix (ECM) of bone is home to a large complement of morphogens, mitogens and osteopromotive proteins that cooperate synergistically in the course of bone induction as exemplified by studies in non-human primates *(Papio ursinus)* models.[26,40] Implantation of bovine ECM impregnated with rhBMP-7 and porcine-derived TGF-β1, yielded faster bone regeneration in critical-sized cranial defects of *Papio ursinus* when compared to single morphogen applications.[26] This knowledge has been an important catalyst in the innovation of Altis™ Osteogenic Bone Matrix, a new class of novel bone graft substitutes that exert their action through the combination osteogenesis principle. In this respect, the processing strategy of OBM from porcine DBM achieves the preservation of the natural complex of synergistically interacting bone morphogens that belong to the TGF-β1 superfamily of bone inducers and bone promoters, which includes bone morphogenetic proteins-2 and TGF-β1.[26,35,40] Additionally, the enzymatic treatment[25] of the ECM component of OBM under acidic conditions, eliminates non-collagenous proteins, reduces collagen telopeptides, and greatly improves the osteoinductivity and biocompatibility of the tissue-engineered DBM, as demonstrated previously by xenotransplantation experiments in rodents.[26]

The commercial introduction of advanced bone graft substitute biomaterials based upon the bone induction principle[10] which include the recombinant BMPs (OP-1, Stryker; BMP-2 Medtronic) and naturally derived morphogen complexes (OsteoAMP, Advanced Biologics Carlsbad CA, USA) has offered surgeons effective alternatives to autogenous grafting and its associated disadvantages. The first clinical reports on the use of bone inductive protein extracts from human demineralized bone matrix to treat femoral and tibial defects were published in the 1980s.[21,41] There are however advantages and disadvantages with these biomaterials. The recombinant BMPs, although highly osteoinductive, have been associated with safety concerns following off-label applications of greatly supraphysiological morphogen doses, leading to severe complications in the cervical spine.[42] Similarly, allogeneic bone protein extracts, whilst offering an acceptable efficacy and safety profile,[35] may be limited in specific countries by the paucity of human donor bone. Commercial allogeneic DBM batches have also been found to suffer from product intra- and inter-variability.[23,42] (see Fig. 2)

OBM may represent a reliable graft substitute candidate that offers certain advantages of safety, biocompatibility, abundant supply and efficacy. OBM is a humanized DBM derived from single source bone of adolescent closed swine herds (*Sus scrofa*) which are endowed with abundant levels of bone morphogens in their bone matrix.[27] Xenogeneic transplantation of porcine BMP is osteoinductive in cross-species experiments in rodents[26,26] pointing to the evolutionary conservation of these morphogens amongst mammals.[34] Injectable OBM also offers the advantage that it can be injected into poorly accessible defect locations to achieve improved biomaterial delivery. Clinical safety and efficacy may be improved by employing humanized OBM containing an immobilized bone morphogenetic protein complex, as opposed to a large dose of diffusible recombinant BMP. The histological analysis of the OBM implant taken from a treated patient shows new bone grafting directly onto the matrix surface (Fig. 3) and demonstrates the humanized and biocompatible status of the OBM implant.

For a homologous matrix ECM graft material to be safe, it must not cause an allergic response, transmit diseases, be cytotoxic or cause excessive inflammation. Mammalian-derived ECM-based biomaterials are composed mainly of Type I collagen and have been approved by the Food and Drug Administration (FDA) for numerous orthopedic applications, examples include porcine ECM bone void filler (Pioneer Surgical Technology, MI, USA),[42] bovine ECM combined with recombinant BMP-7/OP-1 (Stryker Biotech, MA, USA), and bovine collagen sponge combined with BMP-2 for spine fusion and long bone non-unions (InductOs, Wyeth Biotech/Genetics Institute, MA, USA). Porcine ECM-based biomaterials such as small intestinal submucosa, heart valves and skin have been used for many years in more than a million patients and it has been generally concluded that collagenous biomaterials pose no threat or risk, and no related clinical manifestations have been evidenced.[24,43]

In a clinical study investigating the safety of a bovine collagen carrier with rhBMP-2 for open tibial fractures, no patient had a clinical manifestation of an allergic response to bovine collagen and no relationship

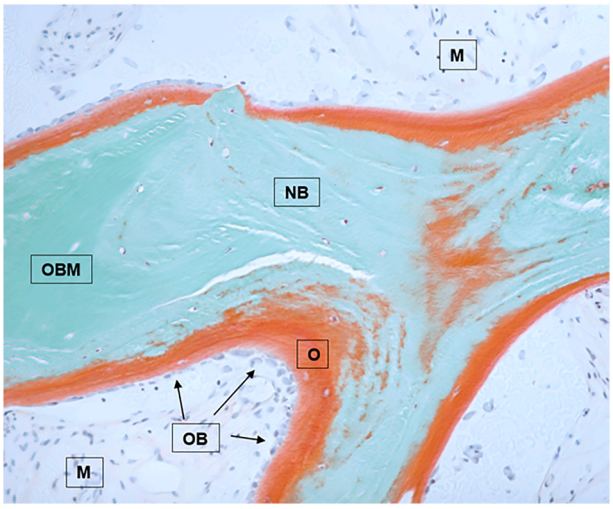

Fig. 3. Photomicrograph of undecalcified section of an OBM biopsy specimen retrieved 4 months post-surgery from the distal tibia of a trial participant. The specimen was embedded in methylmethacrylate resin, cut to 10 μm thickness, and stained with Villanueva stain (A is 40× magnification; B is 200× magnification). Abundant new bone (NB) formation replete with bone marrow (M) and thick osteoid seams lined with osteoblasts (OB, arrows) is evidenced throughout the section. The implanted OBM (OBM) is evidenced in dark green color, with newly forming human bone (NB) grafting directly onto the decellularized ECM (OBM) evidenced as a lighter staining area.

between the immune response to bovine collagen and treatment failure was evident.[15] In contrast, intradermal cosmetic bovine collagen injections may cause localized hypersensitivity at treatment sites in 1 %–2 % of the treated patients, but no systemic effects have been reported to date.[39,44,43] In the present study, no hypersensitivity reactions to the OBM implant were evidenced at the implant site in participants that developed humoral anti-collagen antibodies, and there were no related clinical manifestations in these participants that were followed for a total of 30 months. Additionally, no patient developed anti-human type I collagen antibodies in whom antibodies to porcine Type I collagen had been detected. At the clinical study endpoint of 6 months in the OBM group, 7 (70 %) of 10 participants had bridging of the bone defect, 8 (80 %) had weight-bearing, and 6 (60 %) of subjects had mobility (Table 6; Table 7). OBM was well tolerated and the pain scores of investigators and subjects showed improved scores in all the subjects (Table 7). There was no evidence that OBM had any adverse effect on vital signs or clinical laboratory results. The laboratory results show that biochemical and hematological parameters of the OBM group were generally within the normal reference values. The incidence of a small number of laboratory findings could not be linked to the OBM implant and no adverse events were related to these findings.

5. Limitation of the study

The efficacy study showed a trend of better bridging in the OBM versus SOC group, however, the small population sizes in this study pose limitations in the strength of the statistical analyses to discriminate between the two groups. Findings can be considered clinically but not statistically significant due to the effect size and small cohort number. Most of our comparisons are characterized by a lack of statistical power, due to the small sample sizes. Larger sample size in a subsequent randomized pivotal device study is being planned. Further limitations include the potential religious, cultural, and personal objections to the use of porcine implants. The strict exclusion criteria might affect the broader applicability of the findings as many patients would have been excluded from the study. in the study.

6. Conclusions

This safety and efficacy study showed that treatment with OBM, an enhanced homologous demineralized bone matrix, was well tolerated and there was no evidence of immunological, biochemical, hematological or clinically relevant toxicity or adverse reaction due to the use of OBM. The highest fusion was experienced in the Gustilo-Anderson type II and IIIA subgroup achieving an 86 % fusion rate at 6 months. Pharmacoeconomic analysis showed OBM to be cost-effective versus SOC. The quality of life study revealed that there was an increased level of satisfaction as compared with the baseline data.

Funding

The study was funded by the Technology Innovation Agency (formerly the Innovation Fund) of the Department of Science and Technology of South Africa.

Conflict of interest statement

Dr Nicolaas Duneas is a director of and owns shares in Altis Biologics (Pty) Ltd, the manufacturer of Osteogenic Bone Matrix (OBM).

Ethical review committee

This work was reviewed and approved by the Human Research and Ethics Committee of the University of the Witwatersrand, trial number 050503 and the Medicines Control Council of South Africa trial (N2/19/8/2).

Statement of location of study

This trial was performed in the Republic of South Africa.

Ethical approval

The study was approved by the Medicines Control Council of South Africa (N2/19/8/2). The study was conducted per the ethical principles that have their origins in the Declaration of Helsinki (1964). The protocols were reviewed and approved by the ethics committee (institutional ethics approval number 050503) of the University of the Witwatersrand, Johannesburg, before their implementation.

Informed consent

All patients gave their informed consent before participating in this study.

Availability of data and material

The data sets are available upon request from the corresponding author.

Endnotes

MedDRA® the Medical Dictionary for Regulatory Activities terminology is the international medical terminology developed under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). MedDRA® trademark is owned by IFPMA on behalf of ICH.

CRediT authorship contribution statement

Marshall Murdoch: The study was designed, Surgeries were conducted. **Craig Wittstock:** Concetualiztion. **George Psaras:** The study was designed. **Alan Widgerow:** The study was designed, Surgeries were conducted. **Mkhululi Lukhele:** The study was designed. **Mmampapatla Thomas Ramokgopa:** The study was designed, principal investigators, Surgeries were conducted. **Jacques Snyman:** Concetualiztion. **Jane Hutchings:** was the trial monitor, LM: clinical were trial site managers. **Elizabeth Marcos:** Concetualiztion. **Anna Grisillo Biscardi:** clinical were trial site managers, BR: contributed technical assistance. **Duncan Cromarty:** performed, analyzed and interpreted the antibody study. **Xu Zheng:** performed data analyses, interpretations and reviews, The manuscript was written through the contributions of all authors, All authors read and approved the final manuscript. **Nicolaas Duneas:** The study was designed, RR: principal investigators. **Shunmugam Govender:** The study was designed, national principal investigator.

Acknowledgments

We thank the WITS Health Consortium for their role as a clinical research organization, Microtherapeutic Research Pvt (India) for data management, Nuno Pires for project management, Markus Labuschagne for manuscript review and editing, Department of Microscopy and Microanalysis of the University of Pretoria for histological analyses, Kureha Laboratories (Japan) for methylmethacrylate resin embedding of histological samples and Dr. DJM Frantzen and his team for radiologic analyses. The authors would also like to acknowledge the late Brian Rothman for his co-authorship in this research.

List of Abbreviations

aCCPs	anti-cyclic citrullinated peptide antibodies
AE	Adverse Event
ALP	Alkaline phosphatase

BMP	bone morphogenetic protein
DBM	demineralized bone matrix
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
ICER	incremental cost-effectiveness ratio
ICH	International Conference on Harmonization
IgG	immunoglobulin gamma
LEAP	Lower Extremity Assessment Project
MCC	Medicines Control Council of South Africa
MeDRA	Medical Dictionary for Regulatory Activities
OBM	Osteogenic Bone Matrix
ORIF	Open Reduction Internal Fixation
RF	rheumatoid factor
rhBMP	recombinant human Bone Morphogenetic Protein
SAE	Serious Adverse Event
SOC	standard of care
TGF-β1	Transforming growth factor-β1
WHO	World Health Organisation

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