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Treatment of non-hypertrophic pseudoarthrosis of long bones with a Tissue Engineered Product loaded with autologous bone marrow-derived Mesenchymal Stromal Cells: Results from a phase IIa, prospective, randomized, parallel, pilot clinical trial comparing to iliac crest autograft

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ABSTRACT

Background: Atrophic pseudoarthrosis is a serious complication with an incidence of 5–10 % of bone fractures located in the diaphysis of long bones. Standard treatments involve aggressive surgical procedures and re-interventions requiring the use of autografts from the iliac crest as a source of bone-forming biological activity (Standard of Care, SoC). In this context, regenerative *ex vivo* expanded osteogenic cell-based medicines could be of interest. Particularly, Mesenchymal Stromal Cells (MSC) offer new prospects to promote bone tissue repair in pseudoarthrosis by providing biological activity in an osteoconductive and osteoinductive environment.

Methods: We conducted a phase IIa, prospective, randomised, parallel, two-arms, open-label with blinded assessor pilot clinical trial to compare SoC vs. a tissue-engineered product (TEP), composed of autologous bone marrow (BM)-derived MSCs loaded onto allogeneic decellularised, lyophilised spongy bone cubes, in a cohort of 20 patients with non-hypertrophic pseudoarthrosis of long bones. Patients were followed up for 12 months. Radiological bone healing was evaluated by standard X-ray and computed tomography (CT) scanning. Quality of life was measured using the EUROQOL-5D questionnaire.

Results: Ten patients were randomized to TEP and 10 to SoC with iliac crest autograft. Manufacturing of TEP was feasible and reproducibly achieved. TEP implantation in the bone defect was successful in all cases and none of the 36 adverse events (AE) reported were related to the treatment. Efficacy analyses were performed in the Full Analysis Set (FAS) population, which included 17 patients after 3 patients withdrew from the study. The degree of consolidation, estimated by measuring Hounsfield units (HU) on CT, showed no significant differences between the two treatment groups at 12 months post treatment (main efficacy variable) ($p = 0.4835$) or at 6 months.

Abbreviations: AE, Adverse Event; AEMPS, Agencia Española de Medicamentos y Productos Sanitarios (Spanish Agency for Medicines and Medical Devices); ATMP, Advance Therapy Medicinal Product; BM-MSC, Bone Marrow Mesenchymal Stromal Cells; BST, Banc de Sang i Teixits (Blood and Tissue Bank of Catalonia); GMP, Good Manufacturing Practices; HLA, Human Leukocyte Antigen; HU, Hounsfield Units; MedDRA, Medical Dictionary for Regulatory Activities; N-UF, Non-union fractures; PT, Preferred Term (MedDRA); QoL, Quality of Life; RUS, Radiographic Union Score; SAE, Serious Adverse Event; SF, Spinal Fusion; SOC, System Organ Class (MedDRA); SUSAR, Suspected Unexpected Serious Adverse Reaction; TEP, Tissue-Engineered Product; TUS, Tomographic Union Score; VAS, Visual Analogue Scale.

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Conclusions: Although only a small number of patients were included in our study, it is notable that no significant differences were observed between the experimental treatment and SoC, thus suggesting TEP as an alternative where autograft is not available or contraindicated.

Introduction

Bone healing after fracture may either resolve successfully or result in a delayed or altered process, also known as non-union (pseudoarthrosis), which is a serious complication of bone fractures that requires important surgical procedures [1,2]. These phenomena may be due to loss of vascularity in the healing site and destruction of periosteum and endosteum (atrophic pseudoarthrosis) or mechanical movement in the area while healing (hypertrophic pseudoarthrosis) [3]. In a recent analysis of more than 300 000 fractures in 18 bones, the non-union rate was 4.9 % [4]. Elevated non-union risk was associated with severe fracture (e.g., open fracture, multiple fractures), high body mass index, smoking and alcoholism. The study also found that women experienced more fractures but men were more prone to this condition. The non-union rate also varied with fracture location, with the scaphoid, tibia plus fibula and femur most likely to be non-union. In the UK alone, hospital costs attributed to the treatment of bone non-union have been estimated to be between £7000 and £79,000 pounds per patient, in addition to out-of-hospital and social health costs [2]. It is frequently located in the diaphysis of long bones, primarily the femur, tibia and humerus. The clinical presentation of pseudoarthrosis includes pain at the fracture site and functional impairment. Surgical treatment of pseudoarthrosis is based on providing: 1) stability at the fracture site in hypertrophic pseudoarthrosis; and 2) biological stimuli (the current standard of care, or SoC, being iliac crest) in non-hypertrophic pseudoarthrosis in addition to mechanical stabilisation where required.

Where iliac crest is not available for harvesting, or is contraindicated, the use of *ex vivo* expanded cells has emerged as an alternative for treatment. In particular, multipotent Mesenchymal Stromal Cells (MSC) are osteogenic progenitor cells that can be derived from virtually all vascularised tissues including the bone marrow and are capable of promoting bone tissue regeneration (either by direct osteogenic differentiation of by paracrine stimulating effect) while modulating inflammation in patients [5]. Although MSC-based medicinal products holding marketing authorisation are currently only available to treat Graft vs. Host Disease (GvHD) and complex perianal fistulas in Crohn's patients [6], there is intense research in the orthopaedics field to develop MSC-based treatments for pseudoarthrosis. We have previously completed a preclinical regulatory product development programme of a tissue-engineered product (TEP) composed of autologous bone marrow (BM)-derived MSC loaded onto allogeneic decellularised, lyophilised spongy bone cubes from tissue bank demonstrating its safety and signs of efficacy both in vitro and in vivo in translational animal models [7–10].

In the present study, we evaluated the feasibility of manufacturing clinical grade TEP and its experimental use in a pilot Phase IIa clinical trial to assess its feasibility, safety and efficacy in treating non-hypertrophic pseudoarthrosis of long bones compared to iliac crest autograft.

Materials, patients and methods

Aims

This study aimed to follow and monitor a cohort of patients diagnosed of non-hypertrophic pseudoarthrosis of long bones treated surgically and further evaluate the safety and efficacy of using either an experimental TEP or iliac crest autograft (SoC) according to the outcome (either "bone healing" or "failure").

Clinical trial design

We conducted an exploratory prospective, single-centre, open-label, two-arm, single-dose, randomised Phase IIa clinical trial with blind outcome assessment in which 20 patients aged 18 to 65 years affected with non-hypertrophic long-bone metaphyseal/diaphyseal pseudoarthrosis were selected. The study was carried out between 2014 and 2019 at ASEPEYO Sant Cugat Hospital (Sant Cugat, Spain), where the patients were recruited, treated and followed up. No previous sample size calculation was made because of the pilot nature of this study. Patients were randomised, following the PLAN procedure (SAS Institute Inc., Cary, North Carolina, USA) by Syntax for Science (Palma de Mallorca, Spain), to either one of the two study treatments described next: 1) Experimental Treatment (TEP) consisting of mechanical stabilisation (if required) combined with 10 cc to 20 cc TEP composed of *ex vivo* expanded autologous MSC loaded onto allogeneic cancellous bone graft as described previously [8,11] and prepared in accordance with current Good Manufacturing Practices (GMP) as reported elsewhere [9,12,13]; or 2) Control Treatment (SoC) consisting of mechanical stabilisation, if required, associated with autologous iliac crest graft (SoC) (Fig. 1). The Spanish Agency for Medicines and Medical Devices (*Agencia Española del Medicamento y Productos Sanitarios*, AEMPS) and the Clinical Research Ethics Committee of the IDCSalud Hospital General de Catalunya (Barcelona, Spain) approved the study protocol (EudraCT 2013-005025-23, NCT02230514). The study was carried out in accordance with the Declaration of Helsinki and the principles and standards of Good Clinical Practice. Signed informed consent form was obtained from all patients. Patients were subsequently screened for inclusion and exclusion criteria (Table 1) and randomised 1:1 using a previously programmed randomisation list, to either one of the two treatment arms. The most relevant patient demographic and clinical characteristics are described in **Supplementary Table 1**. We used the CONSORT checklist when writing our report [14].

After treatment, patients were followed for a period of 12 months with monthly follow-up radiographs (Rx) until month 6 and then at 9 and 12 months and a computed tomography (CT) was performed at month 12. Surgery was carried out by the same team of three orthopaedic surgeons (co-authors: DC, SG-V and FG-E) in all cases. All patients received the same post-operative analgesia and antithrombotic prophylaxis protocol. The primary objective was the efficacy assessment by quantification of Hounsfield units (HU) using CT at month 12 (primary endpoint). Secondary objectives included safety assessment by collecting adverse events (AE) reported throughout the experimental phase, characteristics of the callus by tomography and standard X-ray and quality of life (QoL) evaluation measured by EUROQOL-5D test (secondary endpoints).

Manufacture of tissue-engineered products

TEPs were prepared according to established protocols described comprehensively elsewhere [7,8,11,12,15]. Briefly, mononucleated cells (MNC) from BM aspirates were seeded on cell culture-treated plastic surfaces and MSCs readily proliferated using expansion medium consisting of Dulbecco's Modified Eagle's Medium (Gibco) containing 2 mM glutamine and supplemented with 10 % (v/v) pooled human inactivated serum B (hSerB; Banc de Sang i Teixits). All cultures were maintained at 37 °C, 5 % CO_2 and 95 % relative humidity. Medium was changed every 2−4 days. After 20 days of culture using a two-step expansion protocol, cells were harvested and automatic cell counting were performed by using Perfect-Count Microspheres (Cytognos SL) in a

FACSCalibur cytometer (Becton Dickinson). Cell viability was determined by cytometry using the 7-Amino-Actinomycin D (7-AAD; BD Biosciences) exclusion method and expressed as a percentage (%) of total cells and the phenotype was determined using a panel of 6 different markers as it was described previously [9]. Data from flow cytometry were analysed with the CellQuest Pro software (Becton Dickinson). Cells were loaded onto de bone grafts during 4 h (1×10^6 viable cells/cc bone) and colonisation were evaluated indirectly by counting the cells remaining in the supernatant once the process was finished. Cell viability in the TEP was assessed by using the ATP-based Cell Titer-Glo® Luminescent Cell Viability Assay (Promega, Madison WI, USA), as reported previously [7]. In short, cell-seeded bone cubes were placed in multiwell format plates and luminescent reagent was transferred to each well, after which they were incubated for 15 min in the dark. Then, 100 µL of supernatant from each well were transferred into opaque-walled 96-well plates and their luminescence was measured in triplicates on a Triad Multimode detector plate reader with Concert Triad Series software v2.1 (Dynex Technologies, Chantilly, VA, USA). Sterility tests were conducted both on bone marrow samples, intermedite and finished product according to EuPh 2.6.27. Endotoxin levels in the TEP were determined by LAL kinetic chromogenic methodology using the Endosafe-PTS system (Charles River) according to EuPh 2.6.14. and contamination by mycoplasma was ruled out by using EZ-PCR MycoplasmaTest Kit (Biological Industries, Beit Haemek Ltd.) according to Eur. Ph. Monograph 2.6.2.

Evaluation of efficacy in bone healing

To minimise inter-operator variability, bone healing was evaluated by the same researcher, a blinded radiologist (co-author JAP). Monthly follow-up radiographs (Rx) were performed until month 6 and then at 9 and 12 months. CT scan was used at month 12. We applied the Radiographic Union Score (RUS), Tomographic Union Score (TUS) and measurement of Hounsfield units to determine the degree of consolidation of the pseudoarthrosis area [16]. A TUS score greater than or equal to 11 was established to determine radiological healing [17–19]. Briefly, for each quadrant, the percentage of consolidation, maximum (Hounsfield Units, HU), minimum (HU), mean (HU), standard deviation (SD) and area (mm^2) were analysed at baseline and at 6 and 12 months, along with the difference between follow-up visits and baseline in each treatment arm.

Statistical analysis

Efficacy analysis was performed by intention-to-treat, using the Full Analysis Set (FAS). Demographic, safety and efficacy variables were summarised using descriptive statistics. For the quantitative variables we calculated: n (sample size without missing data), mean, standard deviation, 95 % confidence interval for the mean, median, 25th and 75th percentiles (p25, p75) and minimum and maximum. For the qualitative variables, we calculated the number of subjects at each level (absolute frequency) and their respective percentage (relative frequency). To compare groups of patients, we used parametric (Student's t or ANOVA) or non-parametric (Mann-Whitney or Kruskal-Wallis) tests for the quantitative variables, depending on the characteristics of the variables under study and the number of groups to be compared. For qualitative variables we used Chi-square tests and Fisher's exact test. All analyses were performed using the statistical package SAS v9.3 (SAS Institute, Cary, NC, USA).

Results

Cell expansion

Following a validated GMP-compliant process, MSC were successfully derived from BM aspirates of all patients and scaled up to generate clinical doses with a median of 7.9×10^5 viable MSC per cc of bone (range 1.8–9.9 $\times 10^5$ MSC/cc ($n = 9$, Table 2). First, MNC were plated and, after 7–10 days in culture, cells of fibroblastic morphology reached confluency and were replated for a total culture time of 21 days. The phenotypic characteristics of the human MSC were 99.7 % ± 0.2 % $CD45^-CD105^+$, 99.0 % ± 0.6 % $CD31^-CD73^+$ and 99.9 ± 0.3 % $CD90^+$. The combination of morphology assessment and cell surface marker expression confirmed the MSC nature of the cells used in this study. The MSC phenotype was compatible with mesenchymal identity with median values of markers 99.7 % $CD45^-/CD105^+$, 99.4 % $CD31^-/CD73^+$ and 99.8 % $CD90^+$. The final preparation involved a colonisation step onto 10 to 20 cc of cancellous bone cubes (deantigenised human bone particles) for 4 h, before delivery to the hospital. All MSC-based TEP tested negative for bacteria and *Mycoplasma* and endotoxin levels were always below 0.5 EU/mL except for two batches presenting values of <0.599 and ≤ 0.543 EU/mL, respectively.

Patients and treatment

The study enroled 20 patients (85 % men), mean age (SD) of 47.8

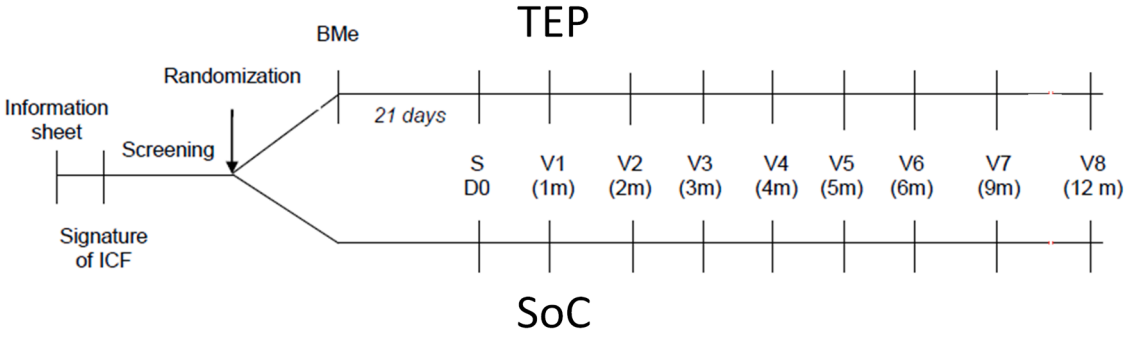

Fig. 1. Clinical study design. Patients randomised to experimental treatment with the tissue-engineered product (TEP) underwent bone marrow extraction 21 days before surgery. During this period, mesenchymal stromal cells (MSC) were expanded *ex vivo* from bone marrow aspirates and, the night before the surgery, cancellous bone cubes were loaded with cells to obtain the final TEP product. After the surgery, patients were followed up for 12 months. We established monthly follow-up visits for the first 6 months and at months 9 and 12. During the follow-up, we performed X-rays at each visit and computed tomography (CT) at 6 and 12 months. In addition, quality of life was measured with the questionnaire (EUROQOL-5D) at 1, 3, 6, 9 and 12 months. ICF: informed consent form; BMe: bone marrow extraction; S: surgery; D0: day 0; m: month; SoC: standard of care.

(8.9) years, with non-hypertrophic pseudoarthrosis of long bones and similar baseline clinical characteristics (men: age 24 to 60; women: age 44 to 59). Initial treatment of the fracture was either endomedullary (EM) nailing in 55 % of cases or using a plate (45 % of cases). To treat pseudoarthrosis, hardware was replaced in 10 cases (52.6 %) and the implant was retained in 9 cases (47.4 %). In 10 cases, an autologous iliac crest graft was implanted (52.6 %) and in 9 cases a TEP was used (47.4 %) according to the clinical trial protocol. Please note that one patient from the TEP group voluntarily self-excluded after randomization. Fig. 2 illustrates the procedure for the implantation of TEP in one of the patients enroled in the study.

Safety results

All randomised patients treated with one of the two treatments were included in the safety population. In total, 36 AEs were reported (21 in patients treated with TEP and 15 in patients treated with iliac crest graft), by 14 patients (6 in the experimental treatment group and 8 in the SoC group). None of the 36 AEs were related to the study treatment. In most cases, AEs required administration of concomitant medication or non-pharmacological treatment. There were no reported deaths. The most common frequent AE corresponded to musculoskeletal disorders, with a total of 13 events, the most common being pain in the extremity (see Table 3 for reported AEs). No clinically relevant changes in laboratory parameters, vital signs or physical examination were described during the follow-up visits. Two patients withdrew from the study for AEs (infection in both cases), while another patient from the TEP group withdrew voluntarily. These three patients were not included in the statistical analysis. Thus, 17 patients were finally studied.

Assessment of the efficacy of the experimental treatment

Analysis of the change in the consolidation percentage of treatments showed that from a baseline value (0.0 %), it reached 37.5 % (±24.8) at 6 months in patients treated with iliac crest auto-grafting and 28.4 % (±11.4) in the patients treated with TEP, respectively (Table 4, Fig. 3). At 12 months, the values were 48.3 % (±27.5) and 55.3 % (±24.1) in patients treated with TEP and with iliac crest autograft, respectively. There were no significant differences observed at either 6 months (p = 0.7209) or at 12 months (p = 0.5358).

The change in the consolidation percentage in the different quadrants followed the same trend as observed for the overall set of quadrants. In particular, the degree of consolidation using the RUS and TUS scales was determined at 6 and 12 months for the maximum (HU), minimum (HU), mean (HU), standard deviation (SD) and area (mm^2) values. No statistically significant differences were detected regarding the efficacy of treatments using radiographic techniques, except for the difference in SD in the anterior quadrant at 12 months between treatments (p-value=0.0140, the area in the posterior quadrant at 6 months of treatment with a (p-value=0.0076) and the maximum value at 6 months in the medial quadrant (p-value=0.0499) (see Table 5, RUS and TUS score).

The quality of life (QoL) of the patients was assessed by a linear regression model using the values obtained from the EUROQOL-5D questionnaire throughout the study. This analysis revealed that, although there was an evolution in the quality of life across visits, no significant difference was found in the patients' QoL based on the study arm to which they belonged (data not shown).

Discussion

Non-unions commonly need multiple procedures, strong chemotherapeutics and long hospitalisation periods, particularly in children [20]. Of the diverse and multifactorial aetiology of non-union, common tumours amongst the young population, such as osteosarcoma and lymphoblastic leukaemia, usually show a higher fracture risk in long-term survivors, since treatment factors such as chemotherapy, nutritional deficits and reduced physical activity levels can trigger premature osteoporosis [21]. Chronic rheumatic diseases in children also show a higher life-long risk for bone fragility [22]. In adulthood, the risk of mal-union, delayed union and non-union are reported to be higher, reaching rates of 55 per 1000 fractures for tibia and fibula non-union, meaning increased treatment costs, as well as a reduced QoL [2]. Moreover, diseases such as tuberculosis, diabetes, hypothyroidism and decalcifying osteopathy may increase the risk of developing pseudoarthrosis. These complications could be minimised by promoting bone regeneration and/or shortening the healing time, thus requiring fewer surgical procedures.

Cell therapy is considered the best option in case of atrophic non-union, with autografts regarded as the gold standard, since this intervention promotes the osteogenic niche through the contribution of biological activity of cells and extracellular matrix components harvested from the iliac crest of the patients themselves [23]. Treatment of bone pathologies often requires tissue deposition which is typically obtained from the patient's own iliac crest, although in some occasion previous history of surgical interventions may leave the patient with limited treatment options. In addition, iliac crest extraction is frequently associated with morbidity of the donor area which may last longer than

Table 1
Inclusion and exclusion criteria.

Inclusion criteria
■ 18 to 85 years of age (male and female).
■ Atrophic metaphyseal–diaphyseal pseudarthrosis of long bones, confirmed radiographically.
■ Signed Informed Consent Form.
■ The patient is able to understand the nature of the study.
Exclusion Criteria
■ Suspicion of pseudarthrosis focus infection diagnosed by clinical inspection and blood analysis.
■ Positive serology for human immunodeficiency virus (Anti-HIV I/II), Hepatitis B surface and core antigens (HBsAg and HBcAg, respectively), Hepatitis C (Anti-HCV) or Syphilis (Treponema pallidum, TP).
■ Significant abnormal laboratory tests contraindicating patient's participation in the study.
■ Pregnant woman or without proper anticonceptive measures according to the investigator, or breastfeeding.
■ Smoker of more than 15 cigarettes a day.
■ Congenital disorders of bones (hypophosphatemia), bone metabolic disorders associated to primary or secondary hypoparathyroidism.
■ Badly managed diabetes mellitus.
■ Patients diagnosed with peripheral arterial disorders.
■ Previous therapeutic radiation (5 previous years) of the affected bone.
■ Neoplasia within the previous 5 years, or without remission.
■ The patient is legally dependant.
■ Participation in another clinical trial or treated with an investigational medicinal product within the previous 30 days.
■ Other pathologic conditions or circumstances that difficult participation in the study according to medical criteria.
■ The patient does not accept to be followed-up for a period that could exceed the clinical trial length.

Table 2
Critical Quality Attributes of the Tissue Engineered Product used in the clinical study.

Patient No.		2	3	5	8	9	15	16	18	19
MSC-loaded bone cubes (cc)	**[≥5]**	15	15	15	20	15	20	10	20	20
Cell count (x10^5 viable cells/cc of bone)	**[≥ 3]**	6.83	9.88	9.39	8.30	8.62	8.04	7.45	9.95	9.09
Viability (by metabolic activity)	**[+]**	+	+	+	+	+	+	+	+	+
Endotoxin (EU/mL)	**[≤ 0.5]**	<0.500	<0.500	<0.500	<0.599	<0.500	≤ 0.543	<0.500	<0.500	<0.500
Sterility	**[Sterile]**	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile
Mycoplasma	**[-]**	–	–	–	–	–	–	–	–	–

Acceptable results (conformity) in square brackets;+: positive; -:negative.

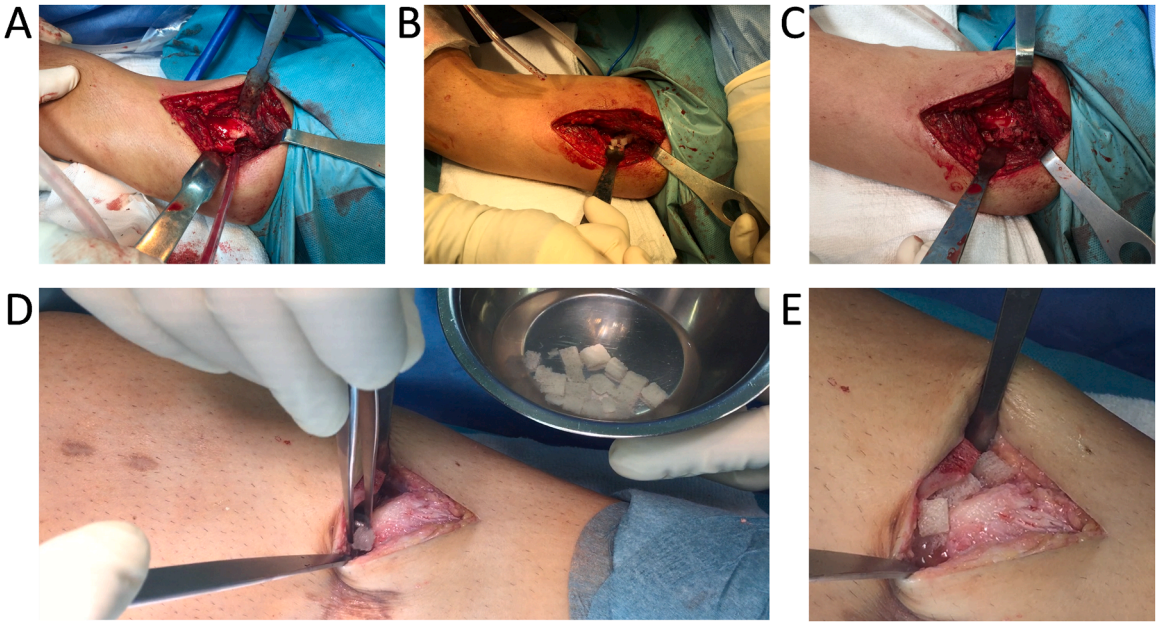

Fig. 2. Experimental treatment. Administration of the experimental product in patients 5 (A, B and C) and 18 (D and E). Patient 5 was a 36-year-old male with a history of humerus open fracture (Gustilo I) surgically treated with endomedullary nailing who developed non-hypertrophic pseudoarthrosis. After checking the stability of the osteosynthesis, the treatment was fibrosis debridement and implantation of a tissue-engineered product (TEP) in the defect. Image showing non-union focus after curettage (A), details of TEP implantation (B) and appearance of the bone defect filled with the experimental TEP treatment. Patient 18 was a 42-year-old male with a history of open fracture of the tibia (Gustilo IIIA) surgically treated first with external fixation and them with a tibial endomedullary nail. Nine months later, he developed non-hypertrophic diaphyseal pseudoarthrosis of the tibia. (D) Details of application of the TEP in the pseudoarthrosis site after fibrosis debridement. (E) Pseudoarthrosis focus fill-up with 10 cc of TEP consisting of bone matrix loaded with autologous bone marrow-derived mesenchymal stromal cells.

the original bone pathology [24]. Furthermore, it is limited in quantity and sourcing entails aggressive surgical procedures. The devitalised allogeneic option is also commonly used, since the supply is easier. However, 60 % of allografts fail to integrate, resulting in non-unions and delayed healing [25]. Cellular grafts for bone regeneration can induce strong osteoinduction, by recruiting MSCs throughout their secretome. These molecules can develop vascularisation and osteoblastic maturation, contributing to the formation of the early callus [23,25].

Bone tissue engineering approaches are concerned with creating implantable bone substitutes for critical skeletal defects that cannot heal on their own. This is achieved by providing a structural osteoconductive scaffold for osteogenic cell attachment, proliferation and differentiation into bone cells. Several TEPs have been tested in clinical trials [26]. It is important to highlight that clinical trials like the one presented herein are complex due to the medicinal nature of TEP and the strict European legislation for Advanced Therapy Medicinal Product (ATMP) development, where cells are considered 'engineered' if they have been subjected to substantial manipulation and their intended function in the transplanted location differs from that of the site where they were harvested [27].

Strengths and weaknesses

Herein we report the feasibility, safety and efficacy results in the clinical use of an ATMP based on autologous BM-expanded MSCs loaded onto allogeneic cancellous bone compared to SoC in bone regeneration. We acknowledge that a weak point of our study is its small sample size (20 recruited patients, 17 of them completing the study). However, similar studies found in the literature share the same weakness. It should be noted that pseudoarthrosis is not a prevalent pathology (especially in our case, focusing on non-hypertrophic pseudoarthrosis with restrictive inclusion criteria) and large samples are extremely difficult to recruit (in our case, patient recruitment took 4 years in a single centre). Alternatively, multi-centre and even multinational studies, as described by Gómez-Barrena and collaborators, may be one possible solution for future research [28]. We also observed a gender bias in the treated population (17 male vs. 3 female), although this was probably due to the casuistry, given that no evidence of sex dependence has been described in the literature for bone healing. Regarding endotoxin levels in the TEPs, although they were higher than our initial internal criteria in two cases, these results were always below the maximum limits established in chapter 5.1.10 of the European Pharmacopoeia for medicines administered intravenously, therefore their clinical use did not represent any risk to the patient's health, as also confirmed in the post-treatment

Table 3
Reported adverse events. Terminology according to the Medical Dictionary for Regulatory Activities (MedDRA) 21.1 [29].

System Organ Class *Preferred Term*	Iliac crest (n = 10) Patients (%); Events (n)	TEP (n = 9) Patients (%); Events (n)
Total	8 (80.0 %) (15)	6 (66.7 %) (21)
Musculoskeletal and connective tissue disorders (Total)	4 (40.0 %) (6)	5 (55.6 %) (7)
Musculoskeletal discomfort	1 (10.0 %) (1)	3 (33.3 %) (3)
Pain in extremity	1 (10.0 %) (1)	3 (33.3 %) (3)
Back pain	1 (10.0 %) (1)	0 (0.0 %) (0)
Muscle atrophy	0 (0.0 %) (0)	1 (11.1 %) (1)
Muscle contracture	1 (10.0 %) (1)	0 (0.0 %) (0)
Pseudoarthrosis	1 (10.0 %) (1)	0 (0.0 %) (0)
Tendonitis	1 (10.0 %) (1)	0 (0.0 %) (0)
General disorders and administration site conditions (Total)	2 (20.0 %) (3)	3 (33.3 %) (6)
Medical device site discomfort	2 (20.0 %) (2)	1 (11.1 %) (1)
Discomfort	0 (0.0 %) (0)	1 (11.1 %) (1)
Gait disturbance	0 (0.0 %) (0)	1 (11.1 %) (1)
Medical device site joint discomfort	1 (10.0 %) (1)	0 (0.0 %) (0)
Medical device site joint pain	0 (0.0 %) (0)	1 (11.1 %) (1)
Medical device site pain	0 (0.0 %) (0)	1 (11.1 %) (1)
Pain	0 (0.0 %) (0)	1 (11.1 %) (1)
Product issues (Total)	2 (20.0 %) (2)	1 (11.1 %) (1)
Device breakage	2 (20.0 %) (2)	1 (11.1 %) (1)
Infections and infestations (Total)	0 (0.0 %) (0)	2 (22.2 %) (3)
Cystitis	0 (0.0 %) (0)	1 (11.1 %) (1)
Post-procedural infection	0 (0.0 %) (0)	1 (11.1 %) (1)
Viral infection	0 (0.0 %) (0)	1 (11.1 %) (1)
Nervous system disorders (Total)	1 (10.0 %) (1)	1 (11.1 %) (1)
Dysaesthesia	1 (10.0 %) (1)	0 (0.0 %) (0)
Hypoaesthesia	0 (0.0 %) (0)	1 (11.1 %) (1)
Psychiatric disorders (Total)	1 (10.0 %) (1)	1 (11.1 %) (1)
Anxiety	0 (0.0 %) (0)	1 (11.1 %) (1)
Encopresis	1 (10.0 %) (1)	0 (0.0 %) (0)
Gastrointestinal disorders (Total)	0 (0.0 %) (0)	1 (11.1 %) (1)
Haemorrhoids	0 (0.0 %) (0)	1 (11.1 %) (1)
Injury, poisoning and procedural complications (Total)	1 (10.0 %) (1)	0 (0.0 %) (0)
Post- procedural discomfort	1 (10.0 %) (1)	0 (0.0 %) (0)
Metabolism and nutrition disorders (Total)	1 (10.0 %) (1)	0 (0.0 %) (0)
Vitamin D deficiency	1 (10.0 %) (1)	0 (0.0 %) (0)
Surgical and medical procedures (Total)	0 (0.0 %) (0)	1 (11.1 %) (1)
Limb operation	0 (0.0 %) (0)	1 (11.1 %) (1)

follow-up.

Finally, we would like to highlight that we traditionally measure bone healing times based on the model or pattern of the acute fracture. However, according to the observations made in our study patients, if we start from a pathological situation (non-hypertrophic pseudoarthrosis), bone regeneration/consolidation times are slower. Of the eight patients who did not achieve radiological union at month 12, four did so at month 18. The other four required additional surgical procedures to achieve union, i.e. of the overall sample analysed (17 patients), 13 (76.4 %) achieved both clinical and radiological consolidation at month 18 (76.4 %) with a single surgery. This is consistent with the figures described in the bibliography for this pathology.

Conclusions

Our results indicate that the combination of a precise fixation technique with application of osteoinductive and osteogenic TEP supplies an optimum environment to support the healing microenvironment at the fracture site, with a positive impact on clinical outcomes. Despite the small number of patients studied, the fact that no differences were observed between treatments suggests that TEP could be a feasible, safe and effective alternative to autografts for those patients ineligible for iliac crest harvesting.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Clinical Research Ethics Committee (CEIC IDCsalud, Catalonia) (protocol XCEL-PSART-01, January 31st 2014).

Table 5
Radiographic and Tomographic assessment. The radiographic union score (RUS) and tomographic union score (TUS) were used.

			Illiac crest (n = 10)	TEP (n = 10)	p-value
6 months	**RUS**	**n**	8	8	1.000
		Consolidated	5 (62.5 %)	4 (50.0 %)	F
		Not consolidated	3 (37.5 %)	4 (50.0 %)	
	TUS	**n**	8	8	0.119
		Consolidated	5 (62.5 %)	1 (12.5 %)	F
		Not consolidated	3 (37.5 %)	7 (87.5 %)	
12 months	**RUS**	**n**	8	7	0.569
		Consolidated	7 (87.5 %)	5 (71.4 %)	F
		Not consolidated	1 (12.5 %)	2 (28.6 %)	
	TUS	**n**	8	7	0.315
		Consolidated	6 (75.0 %)	3 (42.9 %)	F
		Not consolidated	2 (25.0 %)	4 (57.1 %)	

Missing values correspond to patients who withdrew from the study after randomisation, patients excluded due to adverse events and patients with treatment failure. F: Fisher's exact test.

Table 4
Evolution of consolidation of bone fracture by computerized tomography. Change in consolidation at each visit and overall in the FAS population.

			SoC (n = 10)	TEP (n = 10)
6 months	**% consolidation**	n	8	8
		Mean (STDEV)	37.45 (24.79)	28.41 (11.38)
		Median (p25, p75)	32.35 (17.10, 56.15)	28.80 (19.20, 39.05)
		(Min, Max)	(9.70, 78.70)	(11.40, 41.80)
12 months	**% consolidation**	N	8	7
		Mean (STDEV)	55.31 (24.12)	48.30 (27.48)
		Median (p25, p75)	55.70 (45.65, 70.05)	38.70 (27.30, 64.20)
		(Min, Max)	(9.70, 90.00)	(14.00, 96.60)

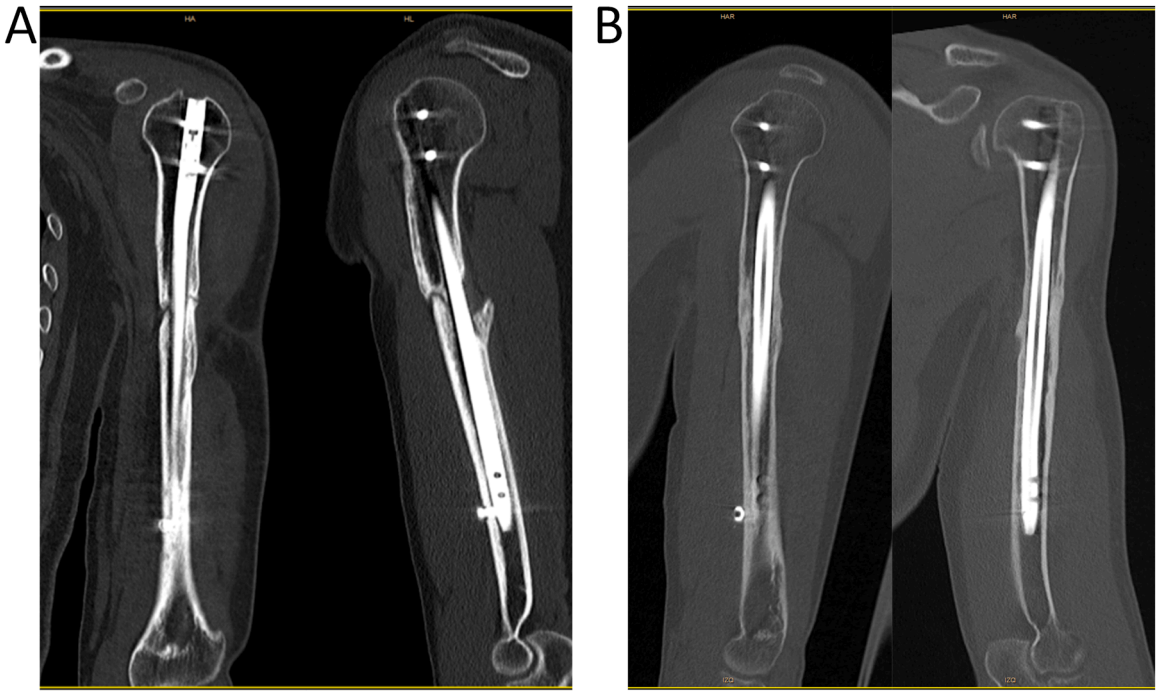

Fig. 3. Patient follow-up. Patient no. 5 from the experimental treatment group. This was a 36-year-old male with no relevant clinical history who presented an open diaphyseal fracture of the left humerus. Evolution to pseudoarthrosis was diagnosed by CT scan 9 months after fracture. Note the absence of consolidation in all cortices with a large defect in the posterior cortex (A). In B, complete consolidation was observed after 12 months, at the last CT follow-up visit.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Daniel Chaverri: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Santiago Gallardo-Villares:** Writing – review & editing, Validation, Investigation. **Javier A. Pinto:** Writing – review & editing, Investigation, Formal analysis. **Luciano Rodríguez:** Writing – review & editing, Investigation, Formal analysis. **Margarita Codinach:** Writing – review & editing, Investigation, Formal analysis. **Joan García-López:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Sergi Querol:** Writing – review & editing, Resources, Methodology, Funding acquisition, Formal analysis. **Ruth Coll:** Writing – review & editing, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Joaquim Vives:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Fernando Granell-Escobar:** Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare there is no conflict of interests.

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Supplementary materials

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