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Treatment of delayed-union fractures of long bones with minimally invasive administration of allogeneic bone-forming cells differentiated from mesenchymal stem cells: a pilot clinical trial

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Abstract: *Although bone has remarkable intrinsic capacity to repair naturally after fracture, approximatively 5 to 15% of the fractures do not heal appropriately, resulting in delayed-unions (DU) or non-unions. These complications result in impaired quality of life for the patient and high socioeconomic burden. Current approaches for the treatment of an impaired fracture healing involve invasive surgery, which is painful, induces serious complications in up to 20-30% of patients and prolongs rehabilitation time. To address this unmet medical need, we developed ALLOB, an injectable allogeneic cell therapy product constituted of bone-forming cells derived from bone marrow mesenchymal stem cells (MSC). It displays potent bone formation and repair properties in relevant in vivo mouse models. ALLOB can be administered locally by a minimally invasive injection thereby preventing the need for additional surgery. The present first-in-man clinical trial was designed to evaluate the safety and efficacy of ALLOB, given as a single percutaneous administration, in patients diagnosed with non-infected DU fracture of long bones.*

Introduction

The present study was a 6-month prospective, multicentre, non-controlled, open-label trial conducted in patients diagnosed with a non-infected DU fracture of a long bone (femur, tibia, fibula, humerus, ulna or radius). DU was defined as an absence of healing at the time of screening, i.e. minimum 3 months and maximum 7 months (+/- 2 weeks) after fracture onset. ALLOB (2, 3 or 4 ml) was administered directly into the fracture site under general or loco-regional anaesthesia to 21 patients (Fig. 1). All patients were monitored for the occurrence of adverse events, especially hypersensitivity reactions. Patient clinical outcomes included health status using the Global Disease Evaluation (GDE) score and pain at palpation using a Visual Analogue Scale. Tomographic Union Score (TUS) assessed on Computed Tomography (CT) scan was used to quantify the progression of bone healing.

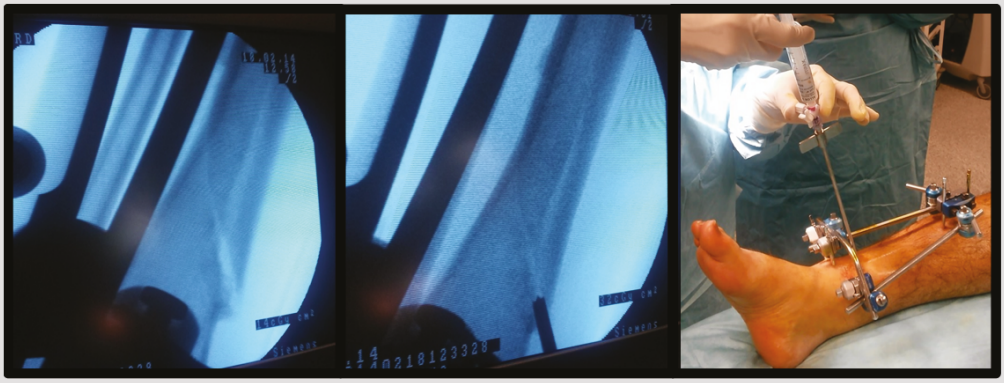

Figure 1: *Left: an immobilised leg with a delayed-union tibial fracture. Middle and Right: minimally invasive administration of ALLOB (allogenic osteoblastic cells) directly into the lesion site using a trephine.*

ALLOB was shown to be well tolerated in all patients without adverse reactions associated with its injection. No immediate hypersensitivity reactions occurred. In one patient, a hypersensitivity reaction without any established causal relationship was reported 4 weeks after administration, with full recovery. In line with published data from clinical trials investigating allogeneic mesenchymal stem cells or their derivatives, it was observed that blood samples of about half of the patients contained human leucocyte antigen (HLA) antibodies, either pre-existing or developed after administration.

At six months post-administration, 100% of the patients met the primary endpoint, defined as the absence of a rescue surgery together with an increase of at least 2 points on the TUS or an improvement of at least 25% of the GDE score. From a

radiological perspective, the patients improved by an average 3.84 points on the TUS score (statistically significant, $p<0.001$) compared to baseline. From a clinical perspective, the patients' health status measured by the GDE score, improved (statistically significant, $p=0.001$) by on average 48% compared to baseline. Pain at palpation at the fracture site was reduced by on average 61% compared to baseline (statistically significant, $p<0.001$).

Overall, these results (1) indicate that ALLOB was well tolerated and (2) provide preliminary efficacy evidence supporting further investigations on ALLOB in the DU indication.

Bone therapeutics and its progress in stem cell therapy

Bone Therapeutics is a leading cell therapy company addressing high unmet medical needs in the field of orthopaedics and bone diseases. The Company is developing a new and unique treatment approach using allogeneic osteoblastic cells (ALLOB) differentiated from MSC and administered via a minimally invasive percutaneous procedure or added through a simple addition/injection to the current standard-of-care, expected to offer significant benefits over or enhancing the current standard-of-care.

ALLOB is a proprietary advanced therapy medicinal product (ATMP) which has been developed in compliance with the European legislation and is classified as Tissue Engineered Product within the European regulatory framework governing advanced therapy in Europe (Regulation 1394/2007). ALLOB is manufactured in Bone Therapeutics according to strict GMP compliance.

Bone Therapeutics has an advanced clinical pipeline for ALLOB in orthopaedic conditions and bone diseases (currently, two Phase IIA clinical studies, in lumbar spinal fusion and in delayed-union fracture, see Fig. 2). These areas are characterised by high unmet medical needs due to the lack of efficacious and safe, non-invasive, treatments. Indeed, the existing current standard-of-care involves heavy surgery and long recovery periods.

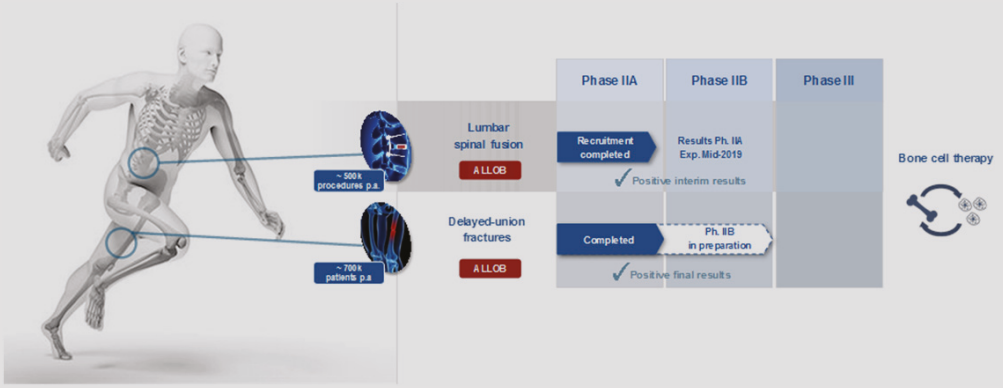

***Figure 2**: An advanced clinical pipeline for orthopaedic conditions with high unmet medical needs. Bone Therapeutics has successfully finished a Phase IIA clinical study in delayed-union and a second Phase IIA clinical study in lumbar spinal fusion is ongoing and showed positive interim results.*

Technology

The Company's technology platform is based on a unique approach in which MSC, derived from bone marrow of healthy volunteer donors, are stimulated in ex vivo culture to differentiate into ALLOB cells (Fig. 3).

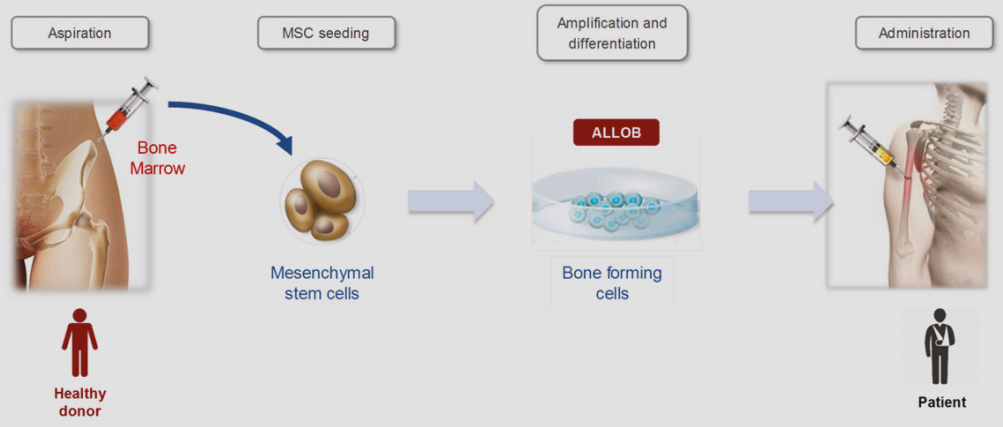

***Figure 3:** Key steps of ALLOB production and administration: bone marrow aspiration, mesenchymal stem cells seeding in the laboratory, differentiation into bone-forming cells in the laboratory and local administration to patients via a minimally invasive administration in the lesion site.*

The bone formation and repair capacities of undifferentiated MSC is limited and they act mainly via a paracrine mode of action stimulating host bone regeneration. To attempt to fulfil high unmet medical needs in the field of orthopaedics and bone diseases, MSC have been differentiated into human bone-forming cells (ALLOB) and tested for bone formation and repair capacities in animal models by a local and minimally invasive administration. These differentiated cells offer significant advantages regarding efficacy compared to undifferentiated stem cells.

Based on the results obtained in animal models, there are evidences that suggest that the mode-of-action of ALLOB is driven by: (Fig. 4),

- The presence of bone-forming cells which will create a healthy bone environment by recruiting haematopoietic and osteoprogenitor cells and stimulating osteoprogenitor cells to produce bone by secreting factors (paracrine effect), therefore amplifying the natural process of bone regeneration (osteo-inductive action).
- The bone-forming cells will replace the defective or missing bone cells at the lesion site and would repair the defective bone by forming new bone directly (osteogenic action).

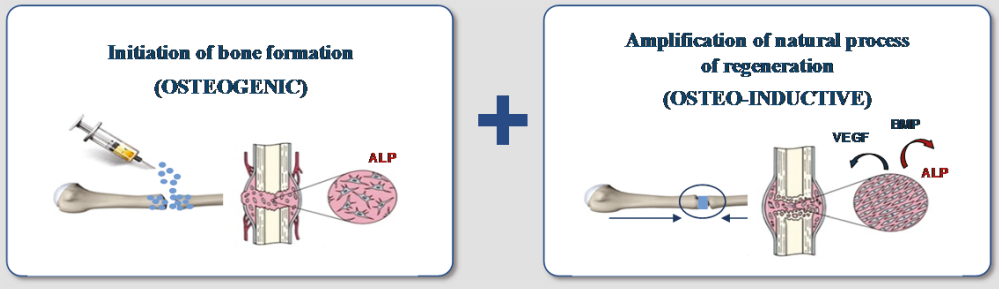

Figure 4: *Dual mode-of-action observed for ALLOB (bone-forming cells obtained after differentiation of MSC).*

The implanted ALLOB cells are expected to adhere onto the existing tissue and matrix, where they will produce new bone matrix that will be calcified. Finally, the cells will differentiate into osteocytes and become embedded into the calcified new bone matrix.

In summary, preclinical foundations and clinical results support Bone Therapeutics' research and development programs.