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OSTEOGROW – NOVEL BONE DEVICE FOR BONE REGENERATION

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Summary

Molecular processes required for bone repair are a prerequisite for the development of new biological procedures for stimulation of bone healing. Currently, there is no adequate therapy available that can accelerate long bone fractures healing. Specifically there is a need for the development of a new osteogenic device that will offer safe healing in particular of the trabecular bone. The Osteogrow project has developed a new therapy that promises to be safe and cost-effective and might decrease the need for secondary interventions. The Osteogrow device contains an autologous blood coagulum (ABC) made from the peripheral blood and recombinant bone morphogenetic protein 6 (BMP6). BMP6 has been selected as compared to BMP2 or BMP7/OP1 as it does not bind avidly to the BMP antagonist Noggin. ABC was chosen as a substrate for the delivery since BMP6 binds tightly to the number of plasma proteins resulting in the sustained and linear release over seven to ten days without provoking inflammation and immune responses. With support of the EU FP7 grant we have completed the preclinical development of Osteogrow and started Osteogrow first in humans (FIH) clinical studies. Osteogrow is tested clinically in two indications: the distal radial fracture and high tibial osteotomy to establish the safety and potential efficacy for Osteogrow for regeneration of the metaphyseal bone. Beyond currently tested clinical indications, this therapy would also be employed for posterolateral spinal fusion to treat degenerative spine disorders.

Keywords: Osteogrow; BMP6; blood carrier; bone regeneration.

BONE REGENERATION

Bone fracture healing is a tightly regulated process which entails a complex series of biological events, with the interplay of different cell types and

the orchestration of several intracellular and extracellular signaling pathways. The first hours after trauma are characterized by hematoma formation and an acute inflammatory response. When injured, bones have a rare property of endogenous self-repair by regenerating new bone without forming a fibrotic scar that would modify their mechanical characteristics. The healing of adult bones follows the steps mimicking of the bone formation during embryogenesis and organogenesis, where the regenerated bone is finally not distinguishable from the initial tissue (1).

While the human population is aging, the incidence of bone trauma will increase. Although skeletal tissue has a robust regenerative capacity, the healing process may fail and result in delayed healing, malunions and non-unions. Susceptibility to bone fracture is also majored by an increasing number of women and men with osteoporosis. In the US and in the European Union (EU), about 5 and 10% of bone fractures exhibit disunion or late healing and therefore remain a key management in orthopedic surgery (2,3). Spinal fusions, fracture non-unions and repairs of critical-size bone defects caused by trauma, infections, tumors, and abnormal skeletal development are some examples of surgeries frequently performed with important clinical need for the development of new healing possibilities to locally induce and stimulate the bone healing, but also to fill the bone defect and stimulate the physiological bone development, without donor site morbidity (4-7).

A major limitation preventing the development of efficient therapies so far is the lack of an appropriate carrier for the delivery of osteoinductive molecules (8-11) to cells during bone repair (12). The effectiveness of most morphogens critically depends on their concentration in the microenvironment, the kinetics of release and the stability of the protein. Release kinetics is defined by non-specific interactions between the protein and the material where boost release may lead to non-physiological, excessive micro environmental doses which result in edema, strong inflammatory reaction and ectopical ossification (13). In this paper we will present a novel bone device OSTEOGROW containing all of the elements important for proper bone healing.

BMP THERAPY IN FRACTURE HEALING

The bone morphogenetic proteins (BMPs) are growth and differentiation factors and form a large family of proteins structurally related to the TGF-β super family. BMPs signal through a set of specific Ser-Thr kinase receptors and act

under the influence of a concentration gradient, which is governed by extracellular matrix proteins and BMP antagonists (14,15). BMPs serve as inductive signals for cell migration, growth, and subsequently differentiation. Osteogenic BMPs applied locally support formation of new bone, cartilage, and ligaments. Recombinant BMPs (BMP-2,-7 and-6) when implanted with an appropriate collagenous matrix are capable of inducing new bone and this effect is dose dependent (16-18). Currently, two rhBMP-based treatments have been approved for marketing in EU (BMP2 InductOS and BMP7 Osigraft /BMP7 Opgenra) and in both the BMP active ingredient is combined with a bovine-derived collagen matrix (bovine collagen type 1). The FDA granted approval for the use of rhBMP2 for the treatment of open tibial shaft fractures and fusion of the lumbar spine. BMP7 has been also approved as an alternative to autograft in compromised patients requiring revision posterolateral lumbar spinal fusion (3). Owing to its bovine source, the collagen must be highly purified to eliminate immunogenicity and viral contamination and the risk cannot be completely eliminated. Therefore, the need still remains for a safe, effective and affordable means of delivering osteogenic proteins to the sites of bone defects.

OSTEOGROW – NOVEL THERAPY FOR BONE REPAIR

A novel bone device is composed of rhBMP6 that was previously found in the plasma of healthy humans confirmed by proteomic analysis (19). rhBMP6 has an important role in the body including involvement in a large number of physiological and pathophysiological processes. BMP6 is a paralog of BMP7 and rhBMP7 has been approved for treating tibial non-unions. BMP6 and BMP7 have high sequence similarity and act through the same receptor mechanisms and the profile of their biological effects is almost identical. The only major difference is the higher potency of rhBMP6 regarding the bone formation because of its resistance to noggin, the BMP antagonist due to the amino acid lysin in the position 60 of the mature BMP6 domain. This lysine allows for reversible binding of BMP6 to Noggin, and unlike BMP7 or BMP2, BMP6 can dissociate from Noggin and escape from Noggin inhibition when binding to BMP receptors (20,21). This explains why BMP6 is more potent in promoting osteoblast differentiation *in vitro* and inducing bone regeneration *in vivo* when compared with its closely related BMP7 paralog (20). The first hours after trauma are characterized by hematoma formation and inflammatory response where blood and bone marrow derived leukocytes initiate the healing process. During characterizati-

on of rhBMP6 pharmacokinetic properties we discovered that it binds to blood components and disappears from the serum after blood coagulation (18,22-24). In experiments using rhBMP6 labeled with radioactive technetium (99mTc), it was proven that ABC serves as an ideal carrier for BMP6 since it tightly binds to the serum proteins.

The new rhBMP6 containing medicinal product OSTEOGROW is composed of a biologically compatible autologous carrier ABC, to which small amounts of rhBMP6 are added to accelerate and enhance bone formation. Osteogenic activity may be detected using osteogenic assays that include ectopic bone formation in which a carrier and rhBMP6 are implanted at an ectopic site in a rodent and then monitored for bone formation (25).

MECHANISM OF ACTION OF RHBMP6 IN OSTEOGROW

Ectopic bone formation mediated by BMP6 and endogenous factors in the ABC containing extracellular matrix and soluble molecules serve as an incubator for the ectopic formation of new bone via ingrowth of surrounding blood vessels, and cells to initiate osteogenesis (*Figure 1*).

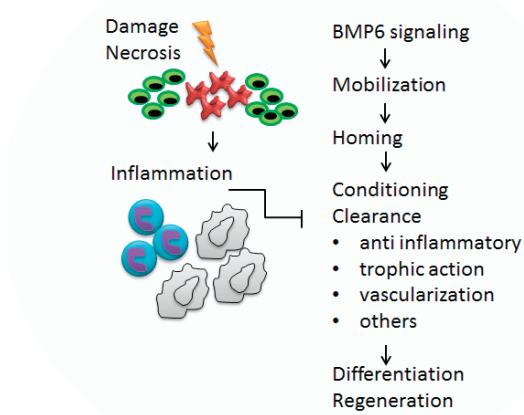

Figure 1. The rhBMP6 role in the new ectopic bone formation.

In *in vivo* testing of rhBMP6 activity in a rat subcutaneous assay condensations of extracellular matrix (ECM) and cells are denser at day 3 of implantation

from which the cortical bone will form (*Figure 2A*). On day 7 bone is already formed in the area of peripheral condensations which is significantly earlier then with other carriers (*Figure 2B*). At day 35 there is a cortical bone formed outside the ossicle with trabecular bone and bone marrow inside the implant (*Figure 2C*). Implanted ABC with rhBMP6 did not exhibit inflammation and swelling which was observed with other commercial bone devices which use bovine collagen as carrier.

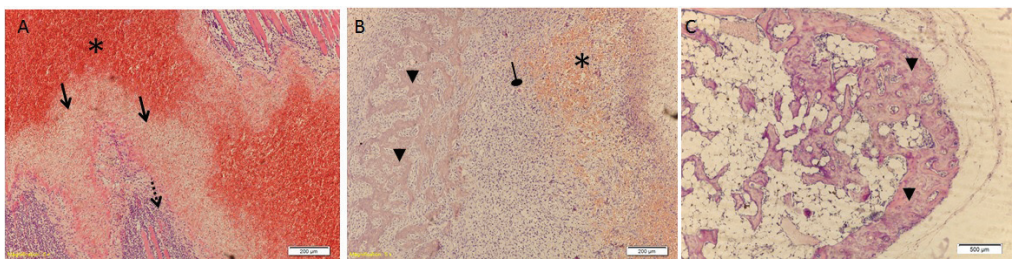

Figure 2. Histology evaluation of ABC plus rhBMP6 in rat subcutaneous implants harvested at day 3, 7 and 35 at an amount of 25 μg rhBMP6 per implant. A) Dashed black arrows denote condensation of cells of extracellular matrix; black arrows indicate the "yellow osteoprogenitor zone" within ABC gradually penetrated by cells from outside the ABC (asterix) (day 3); B) black arrowheads denote newly formed bone while oval arrow denotes hypertrophic chondrocytes in endochondral bone formation area (day 7); C) At day 35 there is a cortical bone formed outside the ossicle (black arrowheads) and trabecular bone with bone marrow inside the ossice. Size marker: left 200 μm (magnification 10×); middle image 200 μm (magnification 10×), right image 500 μm (magnification 4×).

Safety and efficacy studies have been conducted in the rat and rabbit and in the rabbit ulna critical size defect model (UCSD) (20,23,26-28). The UCSD implants consisted of ABC as a carrier to which different amounts of rhBMP6 were added. A 1.5-centimeter segmental osteoperiostal defect was created in the middle of the ulna with an oscillating saw. The radius was left intact for mechanical stability, and no internal or external fixation devices were used. The Osteogrow implant was packed carefully into place to fill the defect. The soft tissues were closed meticulously in layers to contain the implant. Osteogrow preparation included several steps: reconstitution of rhBMP6 with water for injection and mixing with freshly sampled blood and calcium chloride, incubation on room temperature for 60-90 minutes and maintaining red to deep red appearance in color and a cylindrical shape (*Figure 3*).

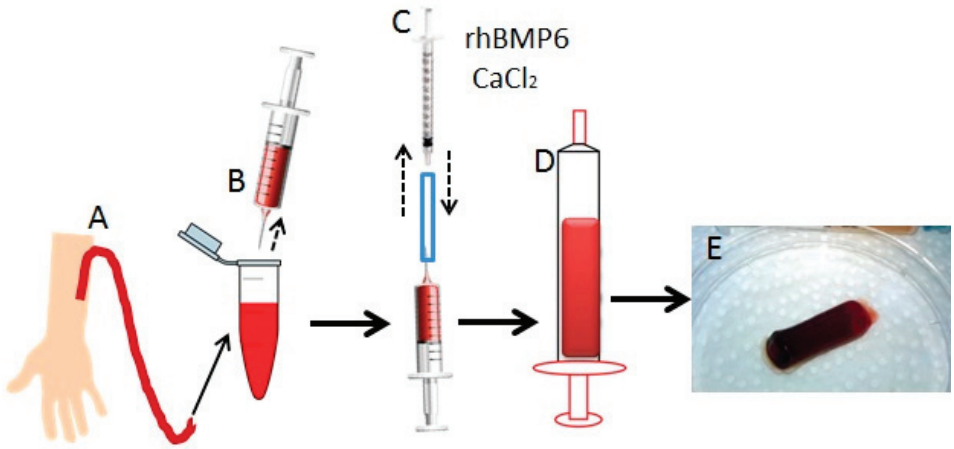

Figure 3. Osteogrow preparation. A) Blood withdrawal; B) Aspiration of the well-defined volume blood in a syringe; C) Mixing of freshly sampled blood with rhBMP6 and calcium chloride; D) Incubation of Osteogrow on room temperature for 60-90 minutes; E) Quality control of the Osteogrow implant before implantation.

The result showed that 100 μg of rhBMP6 in ABC enhanced the radiographic bone union across the defect and induced a complete radiographic osseous union. None of the control animals treated with ABC only (i.e., no BMP) achieved the full defect rebridgement (*Figure 4*).

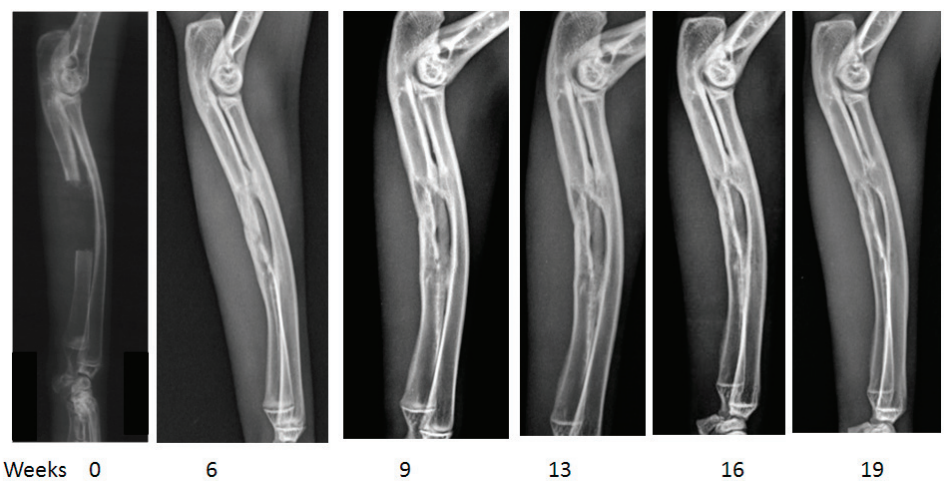

Figure 4. Evaluation of Osteogrow efficacy on the new bone formation in the rabbit ulna segmental defect during the 19 week period. Rabbits (n=8 per group) treated with 100 μg rhBMP6 showed a complete restoration with cortical and trabecular bone in the diaphysis._

General toxicology studies were conducted in rats and rabbits while local tolerance of the implant was tested in rabbits. Single doses (30-450ug/kg) were safe, and similar amounts injected for 14 days did not cause any systemic toxicology signs. Also no signs of local intolerance after transcutaneous paraosseous injection or intraosseous implantation were observed (23). OSTEOGROW is currently clinically tested for bone repair. The initial Phase I/II study of fracture healing is conducted in patients with closed distal radius fracture (DRF) and high tibial osteotomy (HTO). It is the first trial of OSTEOGROW in humans. DRF is a two-stage; no-treatment standard of care (SOC) controlled randomized trial, placebo (PBO) and one dose-level (SoC+ABC-rhBMP6 250mg, 1.0 mL). The total planned sample size is 75 and the trial consists of two stages: Stage 1A to address the "first-in-human" (FiH) safety evaluation and Stage 1B safety continuation testing. The difference between stages is in the rate of patient enrollment. Stage 1 is planned to generate sufficient data (a total of 36 patients, enrollment 1:1:1) for a reasonable safety assessment to justify expansion of the trial to a 2nd site in Stage 2. Progression between stages is determined by safety and tolerability. HTO is the first trial of Osteogrow with local intraosseal administration in varus deformity of the forelegs. It is a randomized, double-blind, placebo controlled trial conducted in 2 stages to address Phase I-II clinical development.

All enrolled patients (N=20) will receive the standard of care (SoC) and will be followed-up for 24 weeks with a post-trial follow-up for 18 months after the surgery (SoC time of removal of osteosynthetic material). They are being randomized (2:1 in Stage 1 (N=6), 3:4 in Stage 2 (N=14), to obtain the final 1:1 assignment) in respect to locally administered treatment. Primary objectives are safety and tolerability and evaluation of systemic pharmacokinetics (PK) of rhBMP6 of a single dose of OSTEOGROW delivered locally and secondary objective is related to efficacy. Phase I DRF and Phase I HTO clinical study reports by Independent Drug Safety Monitoring Boards did not observe any serious side effect in patients following local implantation. A new indication for Osteogrow testing is the posterolateral interbody spinal fusion (PLIF), a technique used to treat a medical condition with inadequate pain management and poor efficacy in restoring load bearing capacity. Based on our preclinical data and preliminary evidence of safety in man, we believe that our novel bone device may represent a long term solution for the treatment of degenerative disc diseases.

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Declaration of interest statement

The authors declare that they have no conflict of interest.

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Sažetak

OSTEOGROW – novi lijek za koštanu regeneraciju

Molekularni procesi potrebni za regeneraciju kosti su preduvjet za razvoj novih bioloških postupaka neophodnih za stimulaciju koštanog cijeljenja. U ovome trenutku na tržištu ne postoji adekvatna terapija koja može ubrzati cijeljenje prijeloma dugih kostiju. Postoji potreba za razvojem nove koštane naprave koji će ponuditi sigurno i ekonomično liječenje. Projekt Osteogrow razvio je potpuno novu terapiju koja obećava da će biti sigurna i isplativa te će smanjiti potrebu za sekundarnim intervencijama. Osteogrow sadrži autologni krvni ugrušak (ABC) kao nosač koji se sastoji od periferne krvi u koji se dodaje rekombinantni protein BMP6 (engl. Bone Morphogenetic Protein). BMP6 je odabran kao poželjni koštani morfogentski protein u usporedbi s BMP2 ili BMP7 / OP1 jer se ne veže na BMP antagonist, Noggin. Autologni krvni ugrušak odabran je kao nosač BMP6 molekule, jer se veliki broj proteina plazme čvrsto veže na BMP6. Uz potporu EU FP7 programa završena su pretklinička testiranja Osteogrow-a i započela je prva primjena Osteogrow lijeka u ljudi (FIH) unutar odobrene kliničke studije. Osteogrow se testira unutar dvije indikacije: distalna radijalna fraktura i visoka osteotomija goljenične kosti koje su odabrane kako bi se utvrdila sigurnost i potencijalna učinkovitost Osteogrow lijeka u regeneraciji metafizalne kosti. Osim trenutno testiranih kliničkih indikacija, ova terapija će se koristiti za liječenje degenerativnih bolesti kralježnice.

Ključne riječi: Osteogrow; BMP6; krvni nosač ; koštana regeneracija.

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