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Short Communication

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A Flowchart for the Translational Research of Cell-Based Therapy in the Treatment of Long Bone Defects

Johan Lammens*[1,2], Marina Marechal[2,5], Hendrik Delport[2], Liesbet Geris[2,3,4,5] and Frank Luyten[2,5]

Abstract

Autologous cell-based tissue engineering may offer new opportunities for the treatment of long bone defects. Experimental work in small animal models requires an important upscaling to meet the dimensions of geometrically large bone segments that need reconstruction, and this is of uttermost importance to make tissue-engineered cell-based therapies work in clinical conditions. Many fragmentary results in laboratory conditions using mice or rats are described, but a framework for a stepwise upscaling is lacking. Nevertheless, this is essential to guide the preclinical process to a reliable outcome and hence needs to be included in the translational research strategy to lead to predictive results in patients. A structured flowchart provides insight in the progress of the development of a robust tissue-engineered product, the process to reach the clinic, and the critical funding to support this translational step.

Keywords

Clinical translation; Skeletal tissue engineering; Combination product

Introduction

It remains a challenge for orthopaedic surgeons to reconstruct large defects of long bones originating from trauma, infection, prosthesis removal, tumour resection or native conditions such as congenital pseudarthrosis of the tibia. To avoid the morbidity associated with present techniques for the repair of bone loss, and to reduce the economic burden due to recurrent surgeries, prolonged hospitalization, functional impairment and working incapacity, the attention has become focused on tissue-engineered products. They are known as Advanced Therapy Medicinal Products (ATMPs) in Europe, and Human, Cells, Tissues and cellular and tissue based Products (HCTPs) in the United States [**1,2**]. The application of these products is based on strategies using the penta-concept, bringing cells, growth factors and an appropriate scaffold together in a well- prepared biological chamber in vivo, while the bone is mechanically stabilized [**3**].

However, upscaling a tissue-engineered construct towards clinically relevant dimensions is challenging, and a clear-cut scenario for translation from bench to bedside is lacking.

Current laboratory research is often restricted to the reconstruction of skeletal defects in small animal models (mice or rats), which is not always relevant for human applications [**4-7**]. The absolute geometrical dimensions of the specific defect under investigation must be taken into account, requiring suitable large animal models to ensure an effective translation of research findings towards use in patients.

Since different parts of the skeleton have different restorative capacities, animal models should target the anatomical part of the skeleton that is clinically relevant. Whereas the study of calvarial defects may be relevant for craniofacial reconstructions, a long bone model is most appropriate to study diaphyseal repair [**8**]. Researchers should also try to mimic the compromised environment characteristic of many patients undergoing skeletal defect reconstructions, where destruction of soft tissues and periosteum, and poor vascularization are rather the rule than the exception.

When comparing results of different studies, species, skeletal maturity of the animal, conditions of mechanical loading of the limb, follow-up period of the study, and most importantly a strict protocol of reproducible surgical procedures, are all key variables which must be managed properly as proposed by Sparks [**9**].

To address all aforementioned issues, we describe our three-stage approach involving animal models of increasingsize.

Animal Models

Nude mice model

Since a nude mouse model prevents immunological reactions, it is used extensively for the screening of ATMP combination products across species (e.g. human, rabbit, sheep) [**10,11**].

We use an ectopic model in which ATMPs of different composition with regard to progenitor cell type expanded bone marrow or periosteum-derived cells, growth factors and scaffolds, are evaluated for their capacity to form new bone. In this first screening, information is gathered about the osteogenic potential of the ATMP in conditions free from normal physiological loading. In addition, as alterations in the bone regulatory cascades will lead to a change in bone forming capacity, the mouse model allows studying specific signalling pathways through systemic or conditional knock-outs.

ATMP effectiveness is measured by examining the bone formation after implanting constructs subcutaneously. At removal 8 weeks postoperatively, the presence of bone, cartilage, blood vessels and bone marrow is studied with Nanofocus Computed Tomography (Nano CT) scanning and histologic analysis. When the implanted cells are from human origin, their contribution to the formed bone is assessed through in situ hybridization.

Once an ATMP has demonstrated good bone forming capacity, this construct is implanted in an orthotopic area. A tibial defect is used because the main final application involves long bones and most frequently the tibia, being one of the bones most prone to non- union [**12**]. As internal fixation devices may interfere with the biological processes of bone healing and create artefacts in imaging techniques, a custom-made external fixator is employed [**13**]. A bone

***Corresponding author:** Johan Lammens, Department of Orthopedic Surgery, University Hospitals KU Leuven, Leuven, Belgium, Tel: + 003216338827; E-mail: johan.lammens@uzleuven.be

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defect spanning 30% of the length of the tibia is created at a mid-diaphyseal level, in which the ATMPs are implanted (**Figure 1**). After 8 weeks, the tibias are collected and the bone forming capacity of the implanted ATMPs is evaluated using Nano CT, histology and in situ hybridization.

Although ATMPs can demonstrate an acceptable bone forming capacity in the nude mouse ectopic and orthotopic model this is not necessarily predictable for the effect in humans and therefore it is further evaluated at level 2 of the proposed flowchart.

Rabbit model

This model uses skeletally mature rabbits with a 3 cm tibial defect completely denuded of periosteum and stabilized by a circular custom-made frame (**Figure 1**) [**14**]**.** The defects are pre-treated for 6 weeks with a cement spacer to induce a foreign body reaction, resulting in a Masquelet membrane. Control groups free of treatment show these defects to be critical. These controls are essential to exclude any spontaneous regeneration of bone, which would interfere with the assessment of new bone formation induced by the ATMP.

Due to the relatively low cost compared to larger animals, large series of rabbits can be used to study the effect of varying doses of growth factor, the amount of cells per cubic millimetre, as well as modifications to the structure or composition of the scaffold.

Rabbit experiments also serve as a first pre-clinical phase in which the feasibility of upscaling the ATMP as well as its safety and efficacy can be monitored. Healing obtained in a 3 cm critical size defect in a rabbit can deliver a proof of concept, but taking into account the dimensions and geometry of the human tibia, these defects are still volumetrically too small to serve as an appropriate model for the defect repair in human patients.

Sheep model

To study anatomically equivalent defects with volumes comparable to those found in the human patient, we developed a non-union model in sheep as previously reported **(Figure 1**) [**15,16**]. In this model, we mimic the human non-union situation presenting with scars, fibrosis and compromised soft tissue/periosteum as close as possible. To this purpose, a critical size defect of 4,5 cm is created in the tibia. After installation of an external fixator, a 4, 5 cm fragment of bone and periosteum is resected and the defect left empty. After 6 weeks, the fibrotic tissue ingrowth at the defect area is removed and the bone gap is pre-treated with a cement spacer to induce a Masquelet membrane. Another six weeks later, the cement is removed and in the resulting cavity, which has become a 4.5 cm bio chamber, the ATMP is implanted. The bone forming capacity of the combination product is evaluated after a period of 16 weeks post implantation, most relevant being radiographical follow-up as to give a first indication of its efficacy. Long-term clinical observation with return to full unrestricted weight bearing, repeated radiographic and CT-imaging and final histologic and mechanical evaluation can complete the study [**16**].

Considerations

Most reconstructions in patients involve adults, making it a logical step to perform translational experiments in animals with closed growth-plates and thus comparable biological potential with regard to tissue regeneration. This is essential in order to assess the osteogenic effect of an implanted ATMP, since significant bone formation can occur in skeletally immature animals, even after creation of a critical size defect ≥ 4.5 cm[**17**].

Fresh defects, which are most commonly used in translational research, do not reflect the clinical situation of a 'biologically exhausted' defect. Our proposed model mimics this fibrotic, atrophic, defect/non-union, characteristic for many patients and research in this model has already proven that cell-based therapies are mandatory to regenerate bone in these conditions (**Figure 2**) [**16**].

For the treatment of critical size defects, the assumption that an equal defect in terms of percentage in a mouse versus a sheep tibia can be used to predict the clinical outcome in humans carries a significant risk. Although both defects have the same relative dimensions, there is a huge difference in absolute volume, which brings about significant biological, mechanical and manufacturing challenges (**Figure 3**). A mathematical mismatch arises between the amount of osteoprogenitor cells and the volumetric dimensions of the cavity to be filled due to the absent correlation between cell and body size. This necessitates much more cells for a large animal or human patient to fill a defect

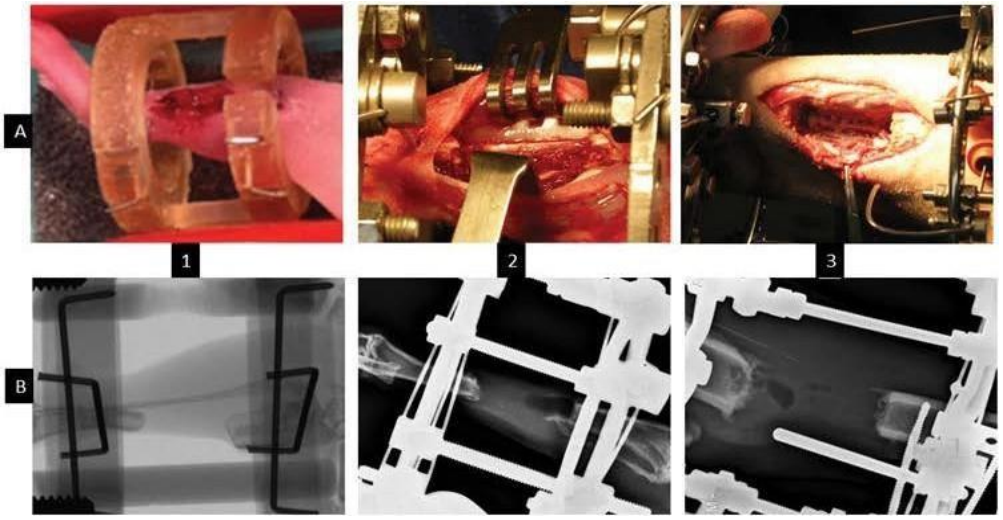

Figure 1: 1A (Clinical picture of mouse (1), rabbit (2) and sheep (3) model) , 1B = corresponding radiographies of the tibial defects.

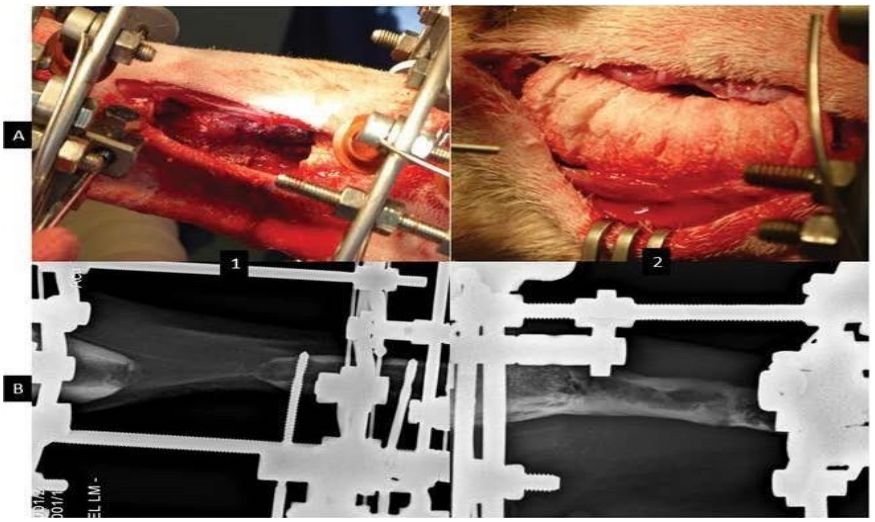

Figure 2: Clinical (A) and radiographical (B) illustration of sheep tibial defects: Empty (1) and treated (2) with tissue engineered cell-based implant.

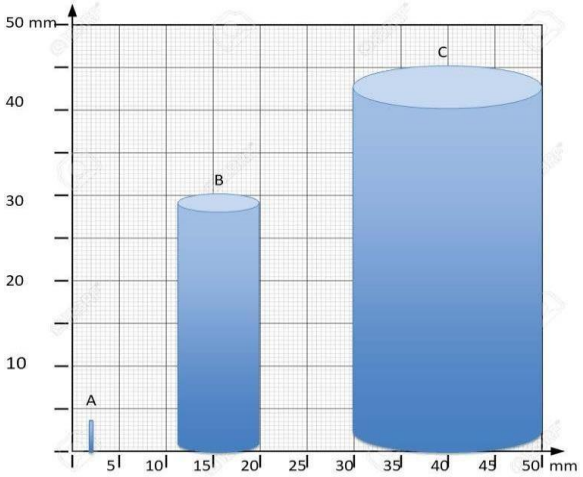

Figure 3: Schematic representation of the volumetric increase of defects using a 4 mm tibial defect in a mouse (diameter 1 mm), a 30 mm gap in a rabbit's tibia (diameter 8 mm) and a 450 mm sheep tibial defect (diameter 20 mm).

with the same relative (to body size) dimensions. This highlights the potential role of cell-based ATMPs, containing large populations of osteochondroprogenitor cells grown ex vivo, in bridging such critical size defects. It emphasizes the risk that extrapolating results obtained from small animal models directly to human patients, may not be clinically relevant and a systematic upscaling approach using sheep or goats is indispensable, tibial defects of ≥ 4 cm being most relevant for long bone critical size defects in human (**Figure 4**) [**18,19**].

Upscaling to a clinical relevant model allows testing feasibility by performing all steps in the procedure, including the surgical intervention, exactly as in human patients. Logistical requirements are also similar (e.g. harvesting, transportation and expansion of cells) and performed according to the same strict time-frames. As such, the model also reflects the overall financial implications of the procedure in patients since the amount of growth factors needed and the production costs associated with cell culture in a good manufacturing practice (GMP) environment are also comparable.

The use of the 3-stage approach advocated here has important financial implications. Upscaling translational research from the nude mouse model to rabbit and further to sheep requires a corresponding upscaling of funding. Items to be covered include the cost of animals, food and housing during the experimental period, surgical procedures and materials, postoperative care, cell culture and expansion, growth factors, scaffold, and ATMP assembly. Our experiments demonstrate an 11-fold increase in costs up-scaling from the nude mouse to the rabbit and a further 5-fold increase up-scaling from the rabbit to sheep.

Nevertheless, the investment brings important additional benefits. The consideration of geometric factors is key to ensure that potential products can be manufactured with the desired shape and the required structural integrity to fill the volume of defects equivalent to those found in humans. Moreover, information can be gathered about the amount of osteoprogenitor cells that can survive in such large constructs in vivo.

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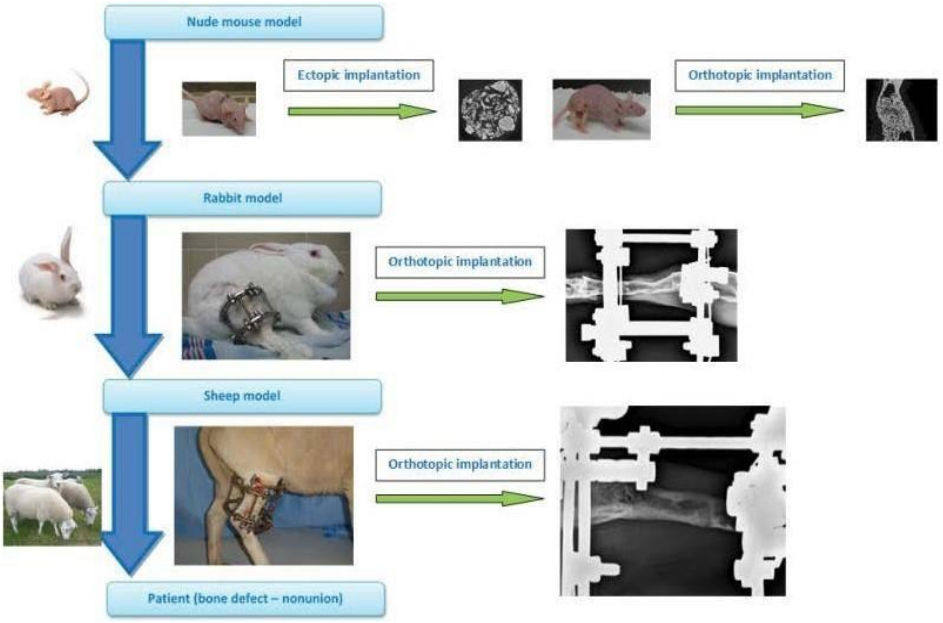

Figure 4: Flowchart for the translational research of cell-based therapy in the treatment of long bone defects.

The large animal model can also be used for training the rules of Good Manufacturing Practice (GMP) required by regulatory directives for manufacturing cell-based products [**20**]. These regulations were first introduced by the US Food and Drug Administration and also implemented in Europe (Commission Directive 2003/94/EC) to ensure the identity, quality, safety, purity and potency of tissue-engineered products. These guidelines are mandatory for use in clinical trials [21]. In contrast to biochemically derived biological products, the analysis and characterization of tissue-engineered products is complex. A quality control pipeline will help to guide the manufacturing process, in which manual operations are still dominant, but will progressively be replaced by automated cell-culture bioreactors.

Documentation standards as well as procedures to ensure reproducibility and traceability of critical components within this manufacturing process can also be developed, for eventual translation to a full GMP environment producing ATMPs for use in the clinical setting.

Whereas for conventional testing of drug development the classic three phasic trial model is used, this is not appropriate for tissue-engineered products. Seeking to repair damaged bone tissue the use of randomized controlled trials including healthy volunteers is not possible. Regulatory agencies agree on exploratory trials to establish safety and proof of concept. This should reflect the results in the large animal model to the best extent, illustrating once more the importance for the best 'human like' model in the preclinical phase.

Conclusions

In conclusion, skeletal defect research using small animal models still has its value, but rather as a screening method of potential ATMP candidates. Animal models, irrespective of their size, will never exactly predict the outcome of a therapeutic intervention in humans, as they are not exposed to systemic variables such as smoking, alcohol consumption, drug treatment or underlying morbidities.

The translational researcher must try to use models that mimic the human situation as close as possible. The use of relevant animal models in a progressive upscaling approach described here, achieves this aim. It provides information on not only safety and efficacy of a potential ATMP, but also on the biocompatibility of materials and the logistical feasibility and cost of translation to a clinical setting. It ensures that all critical elements are addressed at an early stage in the ATMP development process, allowing decisions on potential translation of findings to the clinical setting to be made on robust objective information.

Moreover, a well-organized experimental set-up such as that described here, will help administrators and decision takers, judging on the funding of translational research, and decide to which research activities they will commit their support.

Declarations

Ethics approval and consent to participate: All animal experiments had an ethical approval of the ethical committee of KU Leuven, Belgium and private facility for preclinical CRO Medanex Diest, Belgium.

Consent: Not applicable

Competing interests: The authors declare that they have no competing interests.

Conflict of interest statement: The authors have no conflicts of interest relevant to this article.

All authors have had an equal share in the intellectual contributions to this commentary.

References

1. European Medicines Agency (2019) Human regulatory:Advanced therapy medicinal products: Overview.
2. Food and Drug Administration (2021) Vaccines, Blood & Biologics: Cellular & Gene Therapy Pr006Fducts.

3. Lammens J, Laumen A, Delport H, Vanlauwe J (2012) The Pentaconcept in skeletal tissue engineering. A combined approach for the repair of bone defects. Acta Orthop Belg. 78: 569-73.
4. Young S, Patel ZS, Kretlow JD, Murphy MB, Mountziaris PM, et.al. (2009) Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. Tissue Eng Part A. 15: 2347-62.
5. Bernabe PE, Melo LN, Cintra LT, Gomes-Filho J, Dezan E , et. al. (2012) Bone healing in critical-size defects treated with either bone graft, membrane, or a combination of both materials: a histological and histometric study in rat tibiae. Clin Oral Impl Res. 23: 384-88.
6. Ben-David D, Kizhner TA, Kohler T, Müller R, Livne E, et. al. (2011) Cell-scaffold transplant of hydrogel seeded with rat bone marrow progenitors for bone regeneration. J Craniomaxillofac Surg. 39: 364-71.
7. Seebach C, Henrich D, Schaible A , Relja B, Jugold M, et. al. (2015) Cell-Based Therapy by Implanted Human Bone Marrow-Derived Mononuclear Cells Improved Bone Healing of Large Bone Defects in Rats. Tissue Eng Part A 21(9-10): 1565-78.
8. Spicer P, Kretlow J, Young S, Jansen J, Kasper K, et. al. (2012) Evaluation of Bone regeneration using the rat critical size calvarial defect. Nat Protoc 7:1918-29.
9. Sparks DS, Saifzadeh S, Medeiros Savi F, Dlaska CE, Berner A, et., al. (2020) A preclinical large-animal model for the assessment of critical-size load- bearing bone defect reconstruction. Nat Protoc 15(3):877-924.
10. Roberts SJ, Geris L, Kerckhofs G, Desmet E, Schrooten J, et. al. (2011) The combined bone forming capacity of human periosteal derived cells and calcium phosphates. Biomaterials 32: 4393-4405.
11. Peric M, Dumic-Cule I, Grcevic D, Matijasic M, Verbanac D, et. al. (2015) The rational use of animal models in the evaluation of novel bone regeneration therapies. Bone 70: 73-86.
12. Zura R, Xiong Z, Einhorn T, Watson JT, Ostrum RF, et. al. (2016) Epidemiology of Fracture Nonunion in 18 Human Bones. JAMA Surg151:162775.
13. van Gastel N, Stegen S, Stockmans I, Moermans K, Schrooten J, et. al. (2014). Expansion of murine periosteal derived progenitor cells with Fibroblast Growth Factor 2 reveals an intrinsic endochondral ossification program mediated by Bone Morphogenetic Protein 2. Stem cells 32:2407-18.
14. Bakker A, Schrooten J, Van Cleynenbreugel T, Vanlauwe J, Luyten J, et. al. (2008). Quantitative screening of engineered implants in a long bone defect model in rabbits. Tissue Eng Part C 14:251-60.
15. Lammens J, Maréchal M, Geris L, Van der Aa J, van Hauwermeiren H, et. al. (2017). Warning about the use of critical-size defects for the translational study of bone repair: analysis of a sheep tibial model. Tissue Eng part C 23(11):694- 99.
16. Lammens J, Marechal M, Delport H, Geris L, Oppermann H, et. al. (2020). A cell-based combination product for the repair of large bone defects. Bone 27;138: 115511.
17. Histing T, Garcia P, Holstein JH, Klein M, Matthys R, et. al. (2011) Small animal bone healing models: Standards, tips, and pitfalls results of a consensus meeting. Bone 49:591-99.
18. Reichert J, Epari D, Wullschleger M, Saifzadeh S, Steck R, et al (2010) Establishment of a preclinical ovine model for tibial segmental bone defect repair by applying bone tissue engineering strategies. Tissue Eng Part B 16:93- 104.
19. Pobloth AM, Schell H, Petersen A, Beierlein K, Kleber C, et. al. (2018) Tubular open-porous β-tricalcium Phosphate polycaprolactone scaffolds as guiding structure for segmental bone defect regeneration in a novel sheep model. J Tissue Eng Regen Med 12 : 897-911.
20. Arcidiacono JA, Blair JW, Benton KA (2012). US Food and Drug Administration international collaborations for cellular therapy product regulation. Stem Cell Res Ther 3(5): 38-42.
21. Erkki Iiikanen (2003). Laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use. The commission of the european communities.1-5.

Author Affiliations

Top

[1]*Department of Orthopedic Surgery, University Hospitals KU Leuven, Leuven, Belgium*

[2]*Department of Prometheus and Skeletal Tissue Engineering of the KU Leuven, Leuven, Belgium*

[3]*Department of Mechanical Engineering and Biomechanics Section, KU Leuven, Leuven, Belgium*

[4]*Department of Biomechanics Research Unit, GIGA In silico Medicine, University of Liege, Liege, Belgium*

[5]*Department of Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium*