EFORT open reviews

Frontiers in non-union research

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- Multifactorial aetiology defines non-unions, with a biological and a mechanical distortion of the timeline of bone healing.

- Research on new advances to increase osteogenesis and promote non-union healing is strongly directed towards new forms of cell products.

- Basic science and research on non-union treatments is needed to compile preclinical data on new treatments.

- Bone marrow concentration and expanded mesenchymal stromal cells still require extensive clinical research to confirm efficacy in non-union treatment.

- Solid preclinical studies, precise cell product definition and preparation, and appropriate ethical and regulatory approvals are needed to assess new advanced therapy medicinal products.

Introduction

Non-union or pseudarthrosis represents the inability of bone to heal after a fracture. Although this is a general definition that is well accepted by most orthopaedic trauma surgeons, it is not a simple concept. The absence of timely bone healing after a fracture, during the expected consolidation process, is associated with the presence of specific signs that confirm that the fracture will not spontaneously heal and therefore may require specific actions to obtain the desired bone healing. As surveys among specialists clearly show, both the consolidated bone fracture and the absence of consolidation producing a non-union or pseudarthrosis both deserve more precise definitions.[1,2]

Fracture non-union is diagnosed not only based on the absence of consolidation (or the absence of bone bridging and the presence of a fracture line), but rather by the radiological presence of specific signs that indicate the incapability of completing bone healing, with pain and eventually abnormal motion at the fracture site. These specific signs include the cessation of biological reaction at the bone ends, the obliteration of the medullary canal, and the interposition of a fibrous or cartilaginous tissue between the bone ends (Figs 1 and 2).

Interest in non-union treatment research is not only concerned with the insufficient and sometimes ineffective current treatments. It is worth noting that non-unions are also a serious fracture complication, with associated morbidity, repeated hospitalization, significant secondary functional limitations for 40% to 70% of patients,[3] and significant resource consumption due to repeated surgeries.[4]

The rate of aseptic non-union of fractures after acute treatment is consistent among observational and interventional studies and varies from 5% to 10%, after two years of follow-up. Diaphyseal fractures of the humerus, femur and tibia evolve to non-union at three to five-times higher risk than other fracture locations in the same bones.[5–8] High-energy trauma, open fractures, bone loss, fracture location, consumption of opioids, analgesics or anticoagulant drugs, are well known to predispose a fracture to delayed union or non-union.[9–13] In the case of open fractures, the delayed or non-union may be associated with infection and loss of vascularity.[14] Other authors have not found any influence of age, sex, body mass index, general health status (American Society of Anaesthesiology ASA score) or tobacco use on fracture consolidation.[9,11,15] Therefore, multifactorial aetiology is involved in non-union, and risk factors associated with the patient, the fracture, and its treatment, may well be the origin of the problem.

In Europe, about one million patients require a surgical bone reconstruction annually. The 'gold standard' treatment of non-unions in the orthopaedic trauma field has long been based on the autograft, frequently obtained from the iliac crest. Although little scientific evidence is available, empirical rationale based on the pathophysiology of bone healing over the decades strongly supports its
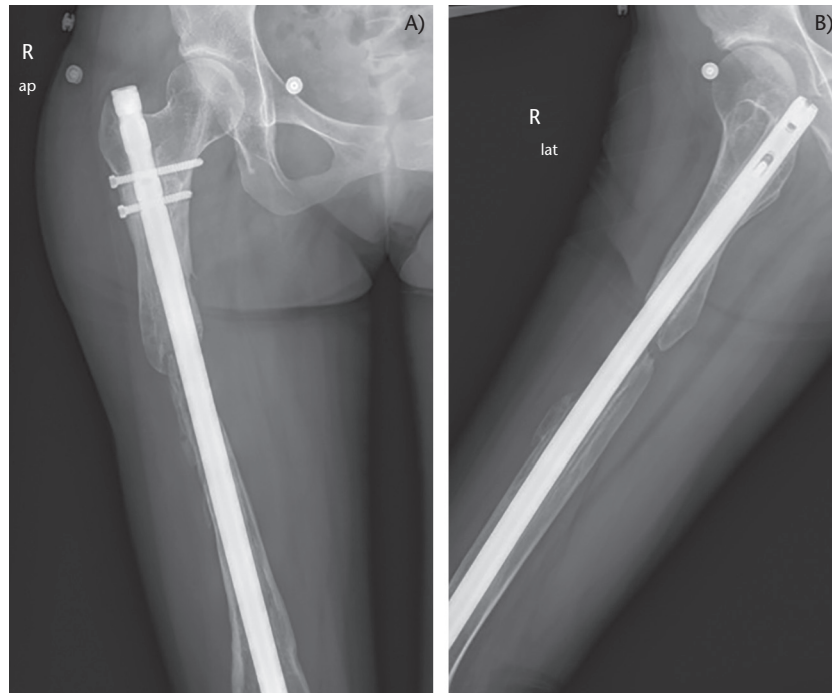
Fig. 1 Radiographic assessment of a proximal femoral diaphyseal atrophic non-union in (A) anteroposterior and (B) lateral views.
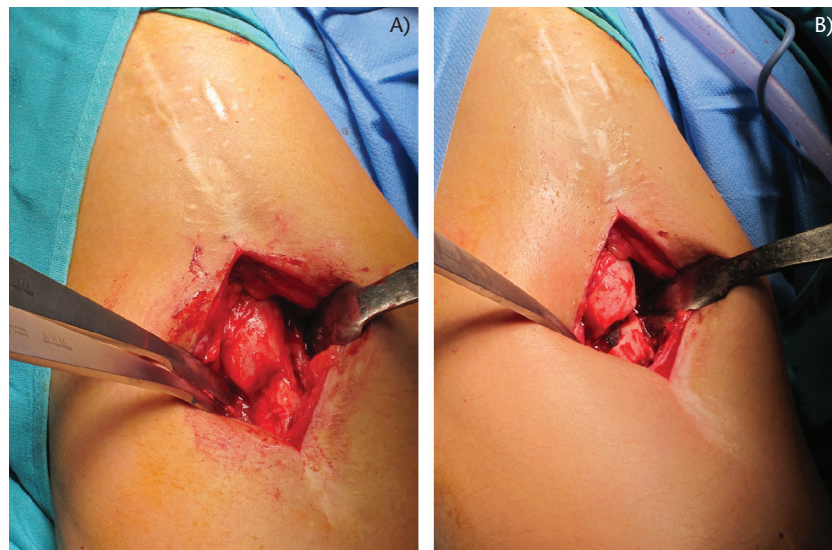

Fig. 2 Intra-operative view of the previously shown femoral atrophic non-union. Note in (A) the exposed non-union, and (B) the non-union after debridement of the fibrous tissue, showing the intramedullary nail.

wide use in non-union treatment. Bone autograft is the safest and most effective grafting procedure, since it contains the patient's mesenchymal stromal cells (MSCs) to enhance osteogenesis and growth factors to enhance osteo-induction,[16] while providing a calcified osteoconductive framework for the new bone to grow into. Although not without complications,[17] these are usually minor (about 20%), though sometimes significantly affecting the donor site and thus the patient (approximately 5%).

Alternatives have also focussed on osteoconduction and osteo-induction methods to stimulate bone healing. These include augmentation with biomaterials or devitalized grafts such as allografts, surgical techniques to optimize the mechanical environment, physical methods to

mechanically induce osteoblastic differentiation (e.g. extracorporeal shock wave therapy or low intensity pulsed ultrasound), and growth factors to stimulate cell growth and differentiation (most studies focussing on bone morphogenetic proteins (BMPs).

A most frequently debated alternative is currently the surgical administration of biological products involving cells. This review will put in perspective non-union research, including preclinical and basic science research, but will extensively focus on cell product therapies such as bone marrow concentration and expanded MSC technologies.

Definition of non-union and bone healing

Adequate research on the topic requires clear definitions of non-union and bone healing to confirm the non-union under treatment and the end-point of bone healing. A clear non-union definition is needed to set inclusion criteria in any research study, and different definitions have been proposed. Although timing of the fracture to heal is no longer accepted alone, being highly variable among fractures and patients, a long time without healing suggests a non-union if no biological progression is observed in the fracture site over several months. In this sense, several European trials (EudraCT 2009-017039-16, 2011-005584-24) have considered six months after the fracture, with more than three months without biological progression. Similarly, a recent survey confirms that the majority of surveyed surgeons considered six months as the timeframe to classify a painful delayed union as a non-union.[2] However, and despite the general belief, the most commonly used definition of a non-union in clinical trials today was proposed by the Food and Drug Administration (FDA): 'A non-union occurs when fracture healing is not completed within 9 months following injury, with absence of progressive signs of healing on serial radiographs over the course of 3 consecutive months'.[18]

Bone healing or fracture consolidation, particularly in long bones where thick cortices require more time and more biological 'effort' to heal, can be radiologically confirmed when bone bridging is observed across the fracture gap, and the fracture line disappears when the continuity of the fractured bone can be ascertained. In long bones, three out of four cortices (meaning two cortices in the anteroposterior (AP) view and two cortices as evaluated in the lateral view) need to be bridged to accept the fracture as healed as per radiological evaluation. These features have been usefully employed by bone healing scores in recent fractures, such as the RUST (Radiographic Union Scale for Tibial fractures)[19] or the RUSH (RUS for Hip fractures).[20]

Yet some differences are encountered in the non-union healing evaluation. Re-modelling may be slow and therefore the resolution of the fracture line may occur much later than bone bridging. Furthermore, the present 'gold standard' to assess bone healing or persistence of the non-union is computed tomography (CT) bone evaluation. In this context, a score to assess non-union bone healing after treatment with biomaterials and MSCs has been recently defined and validated by our group,[21] considering an intermediate category of bone bridged non-union with still observable fracture line due to slow remodelling, and a good correlation between radiographs and CT in evaluating non-union.

From basic science to preclinical models of non-union

Fracture evolution to delayed union and non-union is related to timely bone healing failure. However, the consolidation cascade[22] is highly variable among individuals and fractures. The general frame of the consolidation process is well known, yet the specific sequence, with its drawbacks and regulation, is modulating the prognosis for each bone fracture and each patient and is possibly modified at different moments of the bone healing process.

The cellular events occurring during bone healing are like normal bone embryogenesis, except for the associated inflammation, the lower number of osteoprogenitors in the adult, and the potent mechanical influence that occurs in the adult bone. The bone cells directing bone healing after a fracture are osteoprogenitors from the periosteum or pluripotent cells from the bone marrow, modulated by signalling and transcription factors towards osteoblastic differentiation and osteogenesis.[23]

New insights of how a disbalance may translate into clinical disorders are attracting research towards non-union biology. Downregulation of effector memory regulatory T cells, effective at suppressing Receptor Activator for Nuclear Factor κ B Ligand (RANKL), was recently observed in patients with tibial fracture delayed unions.[24] The biology in the vicinity of the non-union has been also studied, confirming a less favourable environment than other bone locations. In five patients with atrophic non-union, the MSCs at the non-union site and the iliac crest were similar, although the differentiation capability was not evaluated.[25] However, Hernigou and Beaujean[26] showed in 35 patients that the number of colony-forming units-fibroblastic (CFU-F) both at the non-union site and the iliac crest bone marrow was decreased in patients sustaining the bone healing problem, compared with control donors of bone marrow. The current conclusion is that a biological impairment of the non-union site may relate to different reasons, including vascularity, cell activity and, basically, an unfavourable biological scenario. This may also include decreased local osteogenesis, as concluded by Bajada et al,[27] who demonstrated increased levels of the Wnt signalling inhibitor, DKK1, secreted by cells in the

vicinity of the non-union site. This Wnt pathway inhibition could limit BMP-mediated osteoblastic differentiation at the non-union site.

Besides molecular and cellular events, preclinical studies based in animal models are required to evaluate new treatments for the non-union. To create a non-union model, a critical size defect on a long bone is needed. This relates to a defect that does not heal spontaneously and remains unchanged in control animals when properly immobilized by an external fixator, a plate or a nail. The size of the critical defect depends of the animal and the bone.

Reviews of animal models for bone therapies[28,29] detail the process to design an animal study. The protocols must respect the rules to ensure the well-being of laboratory animals, including the 3Rs (Replacement, Reduction and Refinement) to reduce the number of animals without compromising the quality of the study. This is described in EU Directive 2010/63/EU 'On the protection of animals used for scientific purposes', which entered into force in all EU Member States on 1 January 2013. The access to in vivo imaging methods, such as X-rays, CT or magnetic resonance imaging (MRI), should be possible to adequately follow in vivo the regeneration process, and therefore reduce the number of animals.

The non-union experimental model must be as close as possible to human long bone injuries, in term of size and weight-bearing. Large animals such as the sheep, the minipig or the dog may be preferred for this reason. But research in large animals presents drawbacks such as high cost, long time for bone healing, and requirements of appropriate facilities.[30] Currently, there is no technical alternative to simulate bone regeneration than the long bone critical defect. Before testing an orthotopic therapy in large animals, a preliminary in vitro screening is necessary, followed by in vivo studies on small animals at ectopic sites (subcutaneous tissue or calvaria). The osteoinduction and osteoconduction potential of cells, biomaterials, scaffolds or growth factors under study are then confirmed.

No animal model fully mimics the human injury, and studies are usually performed in healthy animals with a normal tissue environment.[29] On the contrary, bone defects are often associated in clinical practice with fibrous soft tissues, scars and vascularity impairment. Sheep tibia or metatarsal models have been validated to evaluate bone regeneration in critical size defects of 2.5 cm.[31–33] The commercial pig grows too fast to be used, but this is not a problem with the minipig, whose bone characteristics approach those of human bone[34] and even load-bearing is similar,[28,30] even if more expensive than the sheep. Dog bones are quite similar to human bones, but the feelings of today's society restrict their use. This concern is even more of a factor for non-human primates.

The design of the study should assess efficacy not only against a placebo, but, rather, should compare new treatments against already validated standard therapies. The number of animals in a placebo group must be as low as possible, even more so if the model has been already validated in the literature. For valid statistical analysis, a minimum number of seven animals per group is usually necessary, with repeated in vivo imaging over a period which goes beyond the expected time of physiological union.[28]

The operative technique must be very precisely described, particularly regarding the periosteum. Indeed, the simple resection of a bone cylinder leaving the periosteum in place can regenerate even in a critical size defect, which can distort the results. The osteosynthesis depends on the study design. The external fixator is theoretically ideal due to the absence of material interference with bone regeneration that starts from bone ends or soft tissues. But the stability is imperfect, particularly in cases allowing weight-bearing, and it requires protection to avoid secondary injuries. The locked intramedullary nail may provide stability as in human patients, but it may suppress bone regeneration induced from the medullar cavity and impede the placement of grafts or biomaterials. The plate is probably the best compromise, and produces no nursing problems, although the weight-bearing may require cast protection.[31] At the end of the study, animals are euthanized and bone explants are collected for processing (imaging, mechanical testing, histology).

Clinical research in non-unions: bone marrow concentration

The percutaneous autologous bone marrow grafting principle is based on osteo-inducing cell activity in the fracture site. It was demonstrated by Paley et al in rabbits in 1986.[35] These cells correspond to MSCs and are also called colony-forming units-fibroblastic (CFU-F). Connolly et al proposed bone marrow centrifugation to increase the CFU-F rate and tested it in rabbits.[36] Hernigou and Beaujean further applied this technique to patients.[26]

Bone matrix is synthesized by osteoblasts which originate in CFU-Fs.[37] Under physiological conditions, there are very few CFU-Fs at the fracture site, and even fewer in patients sustaining a non-union, both at the pseudarthrosis site and at the iliac crest.[38] This is part of the rationale behind proposing and using procedures to engraft autologous bone marrow, whether concentrated[39–44] or not.[22,45–48]

Hernigou et al were the first team to use autologous bone marrow concentration (BMC) in a large cohort of pseudarthrosis after open or closed tibial fractures. They obtained 88% success in 60 tibiae with pseudarthrosis.[42] With this concentration technique, an increase in the

number of CFU-F was obtained, the starting material with 600 CFU-F/mL showed 2,500 CFU-F/mL after concentration.[43] A significant correlation was found between the CFU-F rate in the BMC graft and bone consolidation. Bone union was obtained when the injected bone marrow contained more than 1500 CFU-F/mL, at an average in total of 55,000 ± 17,000 CFU-F.[42] Sugaya et al also used BMC in 17 non-union cases (10 femurs, 5 tibia, 1 humerus and 1 ulna), with 76% success.[49] Le Nail et al, in a retrospective study of 43 open tibial fracture cases with initial surgical treatment and non-union, or delayed union treated with BMC, also observed a threshold of 360,000 CFU-F to obtain 100% success.[50] The timing of the graft was also important, and if the BMC was carried out earlier than 110 days after fracture, the success was 47%. But if the BMC was carried out later than 110 days after the fracture, success increased to 73%. BMC success rate decreased with increasing open fracture severity, and no success was obtained in cases in which the gap was wider than 4 mm. A post-operative fracture gap greater than 4 mm is associated with a high rate of procedures to obtain consolidation.[9,51,52] Therefore, a large fracture gap is a contraindication for bone marrow grafting.

Reviewing the literature on autologous bone marrow grafting, one can see a relative homogeneity with nucleated cells numbers, whereas CFU-F numbers show very large variation, confirmed by the variations reported by different teams. Possible explanations may include the automatic process for nucleated cell numeration (cell counter) instead of cell culture for CFU-F. Variations exist even in the same centre, confirming the difficulties of quantifying CFU-F. However, a precise definition of the injected product, both in research and patient treatment, is of the utmost importance to clarify efficacy.

BMC risks are low. Bone marrow extraction offers very mild complications, if any, but the injected product could carry a risk for the patient. Concerning the infection risk, even if it is possible to have positive bacteriological systematic examinations in a non-union, there is no report of secondary infection in the series. Concerning the oncological risk, a recent study by Hernigou and al[53] confirmed the absence of increased incidence of oncological pathologies after autologous bone marrow injection.

Percutaneous autologous concentrated bone marrow graft is therefore a safe technique that has shown good results for the treatment of delayed union and non-union. Advantages include the intra-operative extraction and injection after concentration, a procedure that can be redone after a few weeks if needed, preserving bone stock and avoiding iliac bone harvesting or surgical exposure complications. Although it is not useful in cases with a large fracture gap or infection history, its results are interesting and need further study, especially CFU-F

osteoblastic differentiation capacities, and randomized studies are needed to obtain comparative clinical results.

Clinical research in non-unions: expanded MSCs

The rationale behind using expanded MSCs to treat non-unions depends on the cell dose that is required to obtain efficacy. Currently, this cell dose is unknown. The previous approach to cell dose threshold to heal non-unions, as described in the preceding section, was established as 360,000 CFU-F, associating a higher success rate with the higher dose.[43,50] Those BMC studies inspired MSC expansion to deliver enough cells to the non-union site.

The fate of these cells is also uncertain, but we recently published the results of a Phase I/IIa clinical trial implanting 100–200 x 10^6 MSCs (CD90+, CD63+, CD105+, CD45–) with a cell viability of 97% at release.[54] With these cells, we obtained radiological consolidation at 12 months in 92% of cases, thus confirming the possible efficacy of this cell dose after expansion with this protocol (Figs 3 and 4).

Cell expansion may be efficacious, but this research is much more complex. Regulation 1394/2007 of the European Parliament states that cell and tissue engineering should be considered as 'advanced therapy' and requires marketing authorization (pre-market approval), demonstration of quality, safety and efficacy, and post-authorization vigilance.[55] In this context, cells are considered engineered if they have been subjected to substantial manipulation (which occurs when expanded in the Good Manufacturing Practice – GMP – facility to obtain the demonstration of quality and thus the authorization) or if the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor (which can be considered if intended to regenerate bone).

To clinically launch this research, advanced therapy medicinal products, or ATMPs (such as expanded cells) require regulatory and ethical approval (from national regulators or competent authorities and ethical committees). For this, strong preclinical support is needed with adequate research methodology that includes in vitro and in vivo cell assessment, plus safety and efficacy evaluation of the cells in small and large animal models. When the cell product under study (given the cell source, the process and manufacturing technique, and the dose among other standardized parameters) is approved, an adequately designed clinical trial may be launched.

Of course, this complex procedure is very conservative and carries significant difficulties for the performance the clinical research that is required to eventually obtain marketing authorization in Europe. Nevertheless, an hospital exemption clause may facilitate in-hospital research on
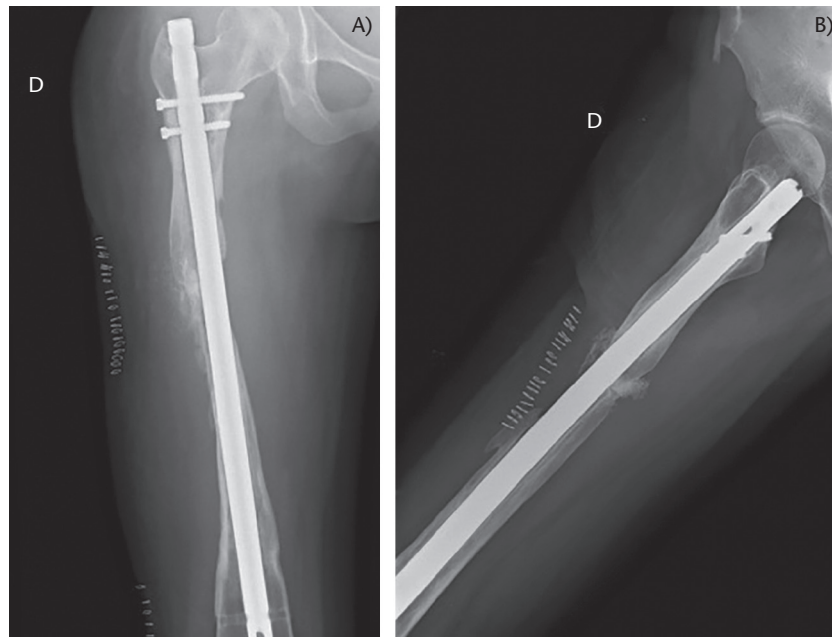
Fig. 3 Previously shown femoral atrophic non-union immediately after surgical treatment with expanded mesenchymal stromal cells (MSCs) combined with a Ca-P biomaterial, in (A) anteroposterior and (B) lateral radiographs.
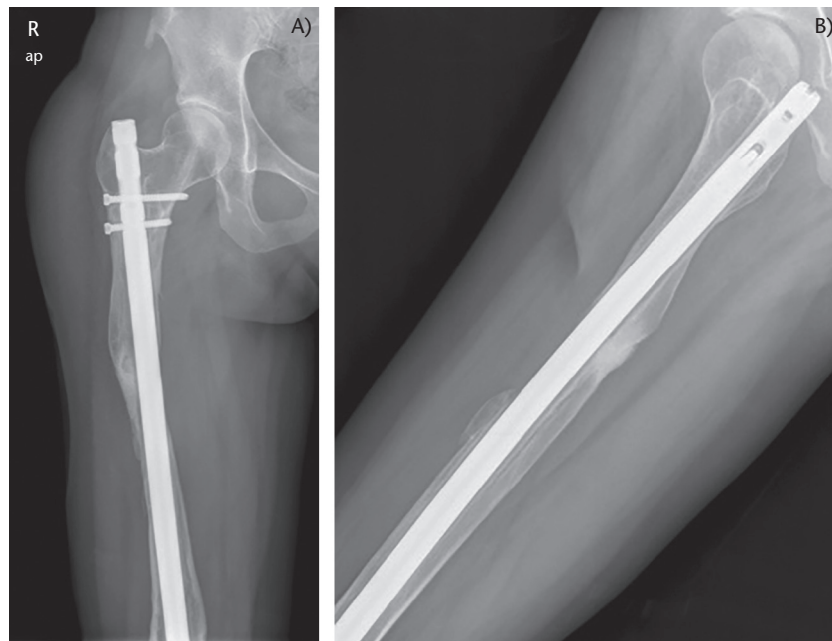

Fig. 4 Femoral atrophic non-union shown in Figs 1 to 3, surgically treated with expanded mesenchymal stromal cells (MSCs) combined with a Ca-P biomaterial, in (A) anteroposterior and (B) lateral radiographs after 12 months follow-up, confirming bone healing.

the topic and thus autologous therapies may undergo hospital research permission to eventually obtain treatment authorization.

In a 2015 to 2019 brief revision of the literature regarding the use of ATMPs in orthopaedics, we identified a total of 18/59 studies related to bone healing/bone formation after an intervention with an ATMP. Of these, eight were reviews, two consensuses on manufacturing process, one consensus on clinical evidence, five were results from a European survey on the use of ATMP, and only two were

clinical trial results. We also revised the clinical trial registries (EudraCT and ClinicalTrial.gov) to identify studies using ATMP for bone healing, specifically for long bone non-unions.

The results of European surveys show an increasing trend in the use of ATMPs for bone regeneration (4% of the total use of ATMPs, as reported in 2015).[56] In the musculoskeletal area, the most frequent research focusses on cartilage and maxillary bone. From clinical trial registries, we identified 17/92 (18%) studies using ATMP to heal non-union fractures, from 2007 to 2019 (see Table 1). Twenty four per cent of these studies (4/17) proposed the use of allogenic cells while the other 76% proposed the use of autologous cells. Treatments included percutaneously aspirated autologous bone marrow cells ($n = 1$), pre-osteoblast cells from bone marrow ($n = 2$), and mesenchymal stem cells from bone marrow ($n = 9$). Two trials were withdrawn, four showed an unknown status, and four were completed. Even though more than 15 registered clinical trials were found (after 2010) about bone healing/formation, results were not found in the same proportion.

Authors agree regarding the need to better understand cell behaviour (mechanism of action) and the impact of production processing on the therapeutic response. Another common observation is the heterogeneity of the doses used and the method to count the delivered cells

Table 1. List of clinical trial using ATMP from 2007–2019

Identification: Title	Start / Last Update	Status	ATMP	Application
NCT00424567: TRC autologous bone marrow cells for the treatment of appendicular skeletal fracture non-union	2007 / 2017	Terminated (closed early for business reasons)	Aastrom tissue repair cells (TRC): percutaneously aspirated autologous bone marrow cells	Appendicular skeletal fracture non-union
NCT00916981: Treatment of refractory non-union fractures by pre-osteoblast cells grafting: a pilot study	2009 / 2012	Completed	Cultured pre-osteoblast cells from bone marrow	Pseudarthrosis (non-union fractures) of long bones
NCT01206179: Effect of bone marrow-derived mesenchymal stem cell transplantation in reconstructing human bone defects	2010 / 2011	Completed	Autologous mesenchymal stem cell	Pseudarthrosis (non-union fractures) of long bones
2010-019380-11: Autologous cell therapy of fracture non-union – cell phenotype as a predictor of outcome (09/0278)	2010 /	Prematurely ended	Autologous mesenchymal stem cell	Pseudarthrosis (non-union fractures)
NCT01429012: Treatment of atrophic non-union fractures by autologous mesenchymal stem cell percutaneous grafting. A randomized, double-blind, controlled study (placebo)	2011 / 2013	Not yet recruiting	Aut. BM-hMSC: autologous bone marrow mesenchymal stem cells	Pseudarthrosis (non-union fractures)
NCT01435434: Mononucleotide autologous stem cells and demineralized bone matrix in the treatment of non-union/delayed fractures	2011 / 2014	Unknown	Mononucleotide autologous stem cells	Pseudarthrosis (non-union fractures) of long bones
NCT01813188: Phase II clinical trial of tissue engineering based on the use of mononuclear cells from autologous bone marrow seeded on porous tricalcium phosphate biomaterial in patients with pseudarthrosis	2011 / 2017	Completed	Mononuclear cells from autologous bone marrow	Pseudarthrosis (non-union fractures) of long bones
NCT01756326: A pivotal phase 2b/3, multicentre, randomized, open, controlled study on the efficacy and safety of autologous osteoblastic cells (PREOB®) implantation in non-infected hypotrophic non-union fractures	2012 / 2018	Active, not recruiting	Autologous osteoblastic cells	Pseudarthrosis (non-union fractures) of long bones: hypotrophic
NCT01788059: The efficacy of mesenchymal stem cells for stimulate the union in treatment of non-united tibial and femoral fractures in Shahid Kamyab Hospital	2013	Unknown	Aut. BM-hMSC: stem cells derived from iliac bone marrow	Pseudarthrosis (non-union fractures) in tibia and femur
NCT01842477: Evaluation of efficacy and safety of autologous MSCs combined to biomaterials to enhance bone healing (C11-12: OrthoCT1)	2013 / 2017	Completed	Aut. BM-hMSC: autologous bone marrow mesenchymal stem cells	Pseudarthrosis (non-union fractures) of long bones
NCT02020590: A pilot phase 1/2a, multicentre, open proof-of-concept study on the efficacy and safety of allogeneic osteoblastic cells (ALLOB®) implantation in non-infected delayed-union fractures	2013 / 2017	Active, not recruiting	ALLOB®: cultured allogeneic osteoblastic cells	Pseudarthrosis (non-union fractures) of long bones

(continued)

Table 1 (continued)

Identification: Title	Start / Last Update	Status	ATMP	Application
NCT02177565: Autologous stem cell therapy for fracture non-union healing	2014	Completed	Carrier plus in vitro expanded autologous BMSCs	Pseudarthrosis (non-union fractures) of long bones
NCT02307435: Potency of allogenic bone marrow, umbilical cord, adipose mesenchymal stem cell for non-union fracture and long bone defect, directly and cryopreserved	2014	Unknown	Allogenic mesenchymal stem cell (AMSC)	Pseudarthrosis (non-union fractures) of long bones or bone defect
NCT02230514: A phase IIa, single centre, prospective, randomized, parallel, two-arms, single-dose, open-label with blinded assessor pilot clinical trial to assess XCEL-MT-OSTEO-ALPHA in non-hypertrophic pseudarthrosis of long bones	2014 / 2018	Active, not recruiting	XCEL-MT-OSTEO-ALPHA: ex vivo expanded adult autologous mesenchymal stromal cells fixed in allogeneic bone tissue	Pseudarthrosis (non-union fractures) of long bones
NCT02448849: Use of autologous bone marrow derived mesenchymal stromal cells in combination with platelet lysate product for human long bone non-union treatment, a phase 2-3 clinical trial	2015	Unknown	Aut. BM-hMSC: Bone marrow –derived mesenchymal stromal cell) in combination with PL (platelet lysate product)	Pseudarthrosis (non-union fractures) of long bones
NCT02483364: A phase II clinical trial to assess the effect of HC-SVT-1001 (autologous fat stem adult mesenchymal cells expanded and combined with a tricalcium phosphate biomaterial) and HC-SVT-1002 (allogeneic fat stem adult mesenchymal cells expanded and combined with a tricalcium phosphate biomaterial) in the surgical treatment of atrophic pseudarthrosis of long bones	2015/2019	Recruiting	HC-SVT-1001 (autologous fat stem adult mesenchymal cells expanded) HC-SVT-1002 (allogeneic fat stem adult mesenchymal cells expanded	Pseudarthrosis (non-union fractures) of long bones: atrophic
NCT03325504: A comparative study of 2 doses of BM autologous H-MSC+ biomaterial vs iliac crest autograft for bone healing in non-union (ORTHOUNION)	2017 / 2019	Recruiting	Aut. BM-hMSC: autologous bone marrow mesenchymal stem cells	Pseudarthrosis (non-union fractures) of long bones

Note. ATMPs, advanced therapy medicinal products; BMSCs, bone mesenchymal stem cells.

which support Phinney et al's request[57] to establish a guideline to better harmonize the manufacturing process and yield more homogeneous products globally.

Conclusions and future directions

Current research on non-union treatment has been fostered by the uncertain efficacy of standard methods based on autograft augmentation. But obviously, treatment requires first an adequate surgical performance that certainly includes consistent pseudarthrosis debridement, including canal permeation, and appropriate bone fixation.

Non-unions are better defined and assessed today due to more specific diagnostic criteria, and different scores have helped to objectively define bone healing after a fracture, or its evolution to non-union, particularly in long bones with thick diaphyseal cortices. Risk factors and non-union epidemiology will help understanding of the patient and fracture characteristics to select treatments. Basic science and experimental models to sustain preclinical research in non-unions are also crucial to launch new treatment options. Major interest is today given to bone marrow concentration procedures and expanded MSC technology, addressing both clinical and regulatory aspects.

When doubts arise about osteo-induction fostered by administered growth factors, due to unclear dose, short-term effect and potential risks, surgically managed strategies such as bone marrow concentration have attracted significant attention. However, the amount of concentration, the exact composition of the re-infused material (cells of different types, growth factors, and other factors), or the association with grafts and/or biomaterials, are not yet clarified and compared. So, it is expected that substantial research may still be performed in the near future on this strategy, with advantages for the surgical management and administration in the operating room.

New options such as cell expansion are complex and expensive, and evidence-based research is definitely required, although the cell dose and product composition may be better defined than in bone marrow concentration. Bioreactors are also under scrutiny to ensure higher cell doses that may be associated with higher efficacy, although still undefined. Adequate cell product characterization (cell surface markers, phenotype, cell doubling), osteo-inductive properties of the product and osteoconductive matrix to deliver it, are all aspects that may modify the efficacy of the final product. Risk factors to the fracture and the patient may orient to autologous or allogeneic treatment research depending on cell reactivity, and

potency assays need to be defined to better understand each patient's potential to heal with autologous cells.

Although this research is not so close to wide clinical application, most research efforts are concentrated today on the biological treatment of non-unions. This biological side needs to be further explored to heal a non-union when a well-performed surgery is not sufficient.

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