Potential implications of cell therapy for osteogenesis imperfecta

Osteogenesis imperfecta (OI) is a brittle-bone disease whose hallmark is bone fragility. Since the disease is genetic, there is currently no available cure. Several pharmacological agents have been tried with not much success, except the recent use of bisphosphonates. Stem cells have been suggested as an alternative OI treatment, but many hurdles remain before this technology can be applied for treating patients with OI. This review summarizes what is known at present regarding the application of stem cells to treat OI using animal models, clinical trials using mesenchymal stem cells to treat patients with OI and the knowledge gained from the clinical trials. Application of gene therapy in combination with stem cells is also discussed. The hurdles to be overcome to bring stem cells close to the clinic and future perspectives are discussed.

KEYWORDS: bone fragility, gene therapy, osteogenesis imperfecta, stem cells

Christopher Niyibizi† & Feng Li

†Author for correspondence
Pennsylvania State University College of Medicine, Associate professor of Orthopaedics and Rehabilitation, Biochemistry and Molecular Biology and Anatomy, H089, 500 University Drive, Hershey, PA 17033, USA
Tel.: +1 717 531 5649
Fax: +1 717 531 7583
cniyibizi@hmc.psu.edu

Osteogenesis imperfecta (OI), or brittle-bone disease, is a heterogeneous disorder that affects skeletal tissues in which Type I collagen is the major protein component. The disease results from mutations that affect the genes that encode the polypeptide chains of Type I collagen [1,2]. Recently, some OI forms have been demonstrated to result from mutations in the genes that encode proteins that play a role in the post-translational modification of the Type I collagen polypeptide chains [3–5]. The hallmark of the disease is bone fragility, but other tissues rich in Type I collagen are also affected. Some patients with OI have teeth problems, a condition referred to as dentinogenesis imperfecta [6]. Owing to its clinical heterogeneity, Sillence classified the disease into four types, I, II, III and IV, based on clinical and radiographic findings (Table 1) [7]. Type I OI is the mildest form of the disease; this OI phenotype results from null mutations in *COL1A1* or *COL1A2*. Patients with Type I OI phenotype have approximately 50% of normal Type I collagen in their tissues. The patients may experience bone fractures but have fewer deformities. Type II OI is the most severe form; most of the infants with Type II OI do not survive, and they succumb to death immediately before or after birth. This OI type is often referred to as the lethal form of the disease [8], and results from structural mutations in either the α1 or α2 chains of Type I collagen. The mutations in Type II OI are dominant-negative, and most result from spontaneous mutations in *COL1A1* or *COL1A2*. In most cases the mutations involve substitutions of the conserved glycine that occurs in every third position of the polypeptide chains for amino acid residues with a bulky or charged side chain. The substitution leads to the destabilization of the triple collagen helices when the polypeptide chains assemble, thus leading to the intracellular degradation of the collagen molecules or deposition of the defective molecules in the extracellular matrix [2]. Type III and IV OI forms are progressively deforming, with severe skeletal deformities in some individuals [9,10]. Type III and IV OI are inherited in an autosomal dominant fashion and most result from the substitutions of the conserved glycine for amino acids with bulky or charged side chains, just as in the Type II OI phenotype. New additional OI phenotypes, Types V, VI, VII and VIII (Table 1) [9,11–14], have been added to the list; these phenotypes do not fall into the classical OI phenotypes originally described by Sillence [7]. Types V and VI OI are not caused by structural mutations in Type I collagen chains, but result from defects that have not yet been identified. These OI types exhibit characteristics of OI Type IV, but they were also shown to be distinct in their presentation [11,12]. Type V OI is inherited in an autosomal dominant pattern; the patients exhibit hyperplastic callus formation and mineralization of the interosseous membrane [12]. Type VI OI has an autosomal recessive form of inheritance; patients were described as sustaining more frequent fractures than patients with OI Type IV. The major characteristic of the patients with OI Type VI is a defect in mineralization [11]. Types VII and VIII OI are recessive forms that are also not caused by mutations in *COL1A1* or *COL1A2* [13,14]. Types VII and VIII OI result

future medicine part of fsg

Table 1. Classification of osteogenesis imperfecta phenotypes.

Type	Mode of inheritance	Clinical characteristics
I	Autosomal dominant	Osteoporosis, blue sclera, mild skeletal deformities, mild short stature and easy bruising
II	Autosomal dominant, mostly new mutations	Lethal form in perinatal period, severe skeletal deformities, beaded ribs and *in utero* fractures
III	Autosomal dominant	Progressive skeletal deformities, dentinogenesis imperfecta, severe bone fragility, hearing loss and short stature
IV	Autosomal dominant	Variable bone deformities, dentinogenesis imperfecta, normal sclera and short stature
V	Autosomal dominant	Moderately severe, hypertrophic callus and ossification of interosseus membrane
VI	Autosomal recessive	Defect in mineralization, osteopenia and bone fragility
VII	Autosomal recessive	Moderate-to-severe fractures at birth, early deformities, coxa vara, osteopenia and blue sclera
VIII	Autosomal recessive	Multiple fractures, decreased bone modeling and low bone mineral density

Adapted from [9].

from defects in the prolyl 3-hydroxylation complex [14]. The prolyl 3-hydroxylase complex consists of prolyl 3-hydroxylase, a cartilage-associated protein, and cyclophilin B. This complex is responsible for the hydroxylation of a single proline residue at position 986 in each of the α1 chains of Type I and II collagens [3–5,14,15], and several proline residues in Type IV and V collagen chains. Type VII OI results from defects in cartilage-associated protein, while Type VIII, identified in patients of West African origin, is due to a null mutation in *LEPRE1*, a gene that encodes prolyl 3-hydroxylase 1 [14].

Analyses of the cellular contribution to bone properties from two mice models of OI suggested that collagen mutations exert effects on the bone cellular function and survival [16,17]. The *Brtl* mouse, a knock-in model for moderately severe OI, displays osteoblasts and osteoclasts in balance, resulting in declining bone formation due to poorly functioning osteoblasts with a concomitant increase in bone-resorbing osteoclasts [16]. The *Aga2* mouse model of OI exhibits a mutation in *COL1A1*, which leads to the accumulation of mutant collagen chains intracellularly, resulting in the induction of an endoplasmic stress-specific unfolded protein response toward osteoblast apoptosis [17]. The cellular contribution to the bone properties in these mice models demonstrates further targets for cell therapies of OI. Clearly, cell therapy approaches for OI should be designed with consideration for the different types of mutations; for example, cell therapy approaches for dominant-negative mutations are more complex than the recessive forms of OI.

Pharmacological approaches for OI treatment

Since OI is a genetic disease, there is no cure available at present. Previous attempts to treat OI using different pharmacological agents were not successful. Calcitonin and growth hormone were attempted, but the results from these trials were mixed [18,19]. In addition, these treatments are not expected to change the course of the disease. Bisphosphonates have gained a lot of attention because they have shown efficacy in some patients; reduction in fracture rates and bone pain have been noted in patients receiving oral bisphophonates [20,21]. These drugs are currently being used for treating patients with OI, and clinical trials are being undertaken to evaluate the long-term effects of these drugs on growing children [22,23]. The rationale behind the use of bisphosphonates is to inhibit bone turnover, a common occurrence in patients with OI, thus allowing accumulation of matrix, although defective. Defective matrix in OI patients is susceptible to degradation by various proteases; by inhibiting matrix degradation, defective molecules accumulate in tissues and undergo mineralization, contributing to the structural integrity of bone. The long-term effects of these drugs on growing children with OI are not known, but studies in animal models suggest that there may be persistence of cartilage in the bones of the patients receiving bisphosphonate treatment [24]. Current efforts are directed toward understanding the long-term effects of these drugs on growing children with OI.

Stem cells

Stem cells have generated great interest and excitement because of the potential they possess in regenerative medicine. Application of stem cells to treat OI is attractive; the rationale for treating OI with stem cells is that osteoblasts in the bones of OI patients can be replaced with normal cells that will synthesize normal bone matrix and thus normalize tissue function. This is based on the premise that normal cells introduced into the bones of OI patients will have a growth advantage over the endogenous cells that synthesize defective matrix. However, in reality, is this possible? Essentially, the approach is to perform cell transplantation, and the transplanted cells will migrate into every bone of the body and synthesize and deposit normal bone matrix. Transplantation of bone marrow for the treatment of various diseases such as hematopoietic reconstitution after myeloablative procedure is routinely carried out. The transplantation of the stroma or cells that will home to bone and differentiate into osteoblasts and lay down bone remains to be established. For OI cell therapy, it is not clear which cells to transplant. Presently, two types of stem cells are recognized, adult-derived stem cells and embryonic stem cells (ESCs). ESCs will give rise to any cell type in the body; they can be expanded indefinitely without losing their stemness, and can be produced in high numbers. The ESCs are derived from the inner cell mass of the blastocysts after fertilization. Owing to the ethical concerns related to the mode of deriving the cells, progress in harnessing the power of these cells has been slow. Alternative approaches to generating ESCs include somatic nuclear transfer, a process by which a nucleus from a somatic cell is inserted into an enucleated oocyte to generate an ESC [25–27]. The nuclear transfer approach, also referred to as therapeutic cloning, is widely used for cloning various animals. Since most of these approaches still require the use of eggs to produce the ESCs, they still raise ethical concerns. Recent developments reporting generation of ESC-like cells, called induced pluripotent stem cells (iPS), by reprogramming fibroblasts using four factors (Oct3/4, Sox2, c-Myc and KLF4) have generated excitement and hope because ESCs could be produced without requiring eggs and, therefore, patient-specific stem cells could be generated [28–31]. As a proof of concept, Hanna *et al.* generated iPS-directed hematopoietic progenitors from a humanized mouse model of the sickle cell disease [32]. The defective human sickle cell hemoglobin allele was corrected by gene-specific targeting in the iPS. The corrected iPS-directed hematopoietic progenitors were transplanted into the same mouse model of sickle cell disease. The investigators showed that the iPS-directed hematopoietic progenitors were able to reverse the sickle cell defects in this mouse model. These data suggested that cell therapy for certain diseases using ESCs may be possible. Although iPS cells cannot be used in humans because of the viruses used to transfer the factors into the cells and because some of the factors themselves are tumorigenic, the cells hold promise once these concerns have been addressed. The inherent drawback in the application of ESCs for treating diseases is that the cells have to be directed for differentiation toward the desired cell lineage before their use in order to prevent teratoma formation. This is a challenge because methods to direct ESCs to the desired cell lineages are poorly understood at present. The application of ESCs for OI treatment therefore remains a future possibility.

Adult-derived stem/progenitors

In every adult tissue there are stem/progenitors whose role is to give rise to differentiated cells of the tissues in which they reside to aid in the tissue repair in cases of trauma or tissue damage. Mesenchymal stem cells (MSCs) or stromal cells, which were originally described by Fredestein, have gained a lot of attention because these cells are present in mesodermal tissues [33]; they can be easily harvested and expanded in culture. The major source is bone marrow, but they are also present in fat, muscle and other mesodermal tissues [34–36]. The cells are isolated by their adherence to tissue culture plates. Under appropriate conditions, the cells will give rise to osteoblasts, chondrocytes and adipocytes, and thus these cells are attractive targets for the repair and regeneration of musculoskeletal tissues. The most intriguing question is, however, are these cells transplantable and can they be used for treating OI? It is a well-known fact that hematopoietic stem cells are transplantable, as they have been used for many years for therapy of hematological malignancies and inherited or acquired disorders of the hematological/immune systems [37]. The cells are routinely used in the reconstitution of the recipients' hematopoietic system following myeloablative regimens. Early studies demonstrated that MSCs promote engraftment of cord blood-derived hematopoietic stem cells in severe combined immunodeficient mice and in fetal sheep [38,39]. The transplantability of MSCs has, however, generated much

controversy; though MSCs may enhance engraftment of hematopoietic stem cells, some reports suggested that MSCs exhibit poor engraftment capability following transplantation [40–43]. Clinical trials in animal models using MSCs to assess application of the cells for the repair and regeneration of various tissues and organs are being intensely investigated. The major focus has been on bone, cartilage and cardiac [44–46]. In some instances where MSCs were directly introduced into the tissues, reparative contribution of the cells to the recipient tissues has been noted [46,47]. Homing of the MSCs to bone and bone formation *in vivo* by the transplanted cells remains controversial. However, several studies have demonstrated MSC engraftment in bone, although at low levels [48–53].

Stem-cell therapies for OI

Initial studies to evaluate the potential to treat OI with stem cells began in mice. Pereira *et al.* transplanted primary adherent stromal cells harvested from a transgenic mouse harboring a collagen Type I mini gene into normal mouse recipients. Following the expression of the collagen mini gene to follow the fate of the donor cells, the authors reported that the donor cells accounted for 1.5–12% of the cells in bone, cartilage and lung, in addition to other tissues [48]. The authors concluded that cultured adherent cells from bone marrow can serve as long-lasting cells for mesenchymal tissues, as well as other tissues and organs. The authors used a similar approach and transplanted whole marrow or primary adherent normal cells into transgenic mice expressing the collagen Type I mini gene [49]. The rationale behind these experiments was to determine if stromal cells could rescue the mice with OI. The authors reported that the mice that received the cell transplant exhibited a small but significant increase in bone collagen content and mineral content after 1 month of cell infusion. These observations generated hope and excitement that marrow-derived stromal cell transplantation could potentially be of benefit to OI patients. These findings led to the clinical trials in which marrow-derived stromal cells were transplanted into patients with severe forms of OI.

Transplantation of MSCs into patients with OI

The initial clinical trial was conducted in babies with severe forms of OI. Three infants were transplanted with whole marrow from normal matched individuals. The results demonstrated that at 3 months after cell transplantation, two of the patients showed an increase in total body mineral content, increase in growth and reduction in fracture rates [54,55]. The number of donor cells that engrafted in the recipients ranged from 1.5 to 2% at 6 months after cell transplantation. How such low levels of engraftment produced such profound effects remains unclear. Subsequent studies involved transplantation of gene-marked MSCs into patients that had originally been transplanted with whole marrow [56]. The results from the clinical trial showed that the patients who received the cell transplant exhibited an increase in growth velocity, suggesting that MSCs contributed to the growth of the recipients. Although the studies showed promise, clear demonstration that the cells contributed to the benefits seen in the recipients is still lacking. These studies generated lots of controversies regarding the use of MSCs for OI treatment.

In utero transplantation of MSCs into patients with OI

In utero transplantation of MSCs into patients with a severe form of OI was attempted. In this clinical trial, MSCs were isolated from the fetal liver of a 10-week aborted, first-trimester fetus [57]. The cells were placed in culture and the adherent cells were processed for transplantation into a 32-week gestation fetus. The baby was delivered at 35 weeks of gestation following cell transplantation. The baby exhibited severe skeletal abnormalities and this necessitated treatment with bisphosphonates at 4 months postnatal. Analysis of the donor cells at 9 months demonstrated 0.3% donor engraftment. Fluorescence *in situ* hybridization demonstrated donor cells in the tissue sections made from the bone biopsies of the recipient, suggesting that MSCs differentiated into osteoblasts *in vivo*, although at very low levels. The authors reported that the patient exhibited an increase in growth velocity, presumably as a result of the cell transplantation. However, contribution of the cells to the bone structural integrity was not apparent.

The clinical trials using MSCs for treating OI generated more questions than answers; if there is such low engraftment of the donor cells, why is there improvement, although small, seen in the recipients? The emerging thought on MSC transplantation and function is that the cells are immunosuppressive and they also generate factors that may stimulate endogenous cells to initiate tissue repair [34]. Alternatively, initially there is a

high level of donor engraftment in the recipients' bones, but this level diminishes with time, and therefore, at the time of analysis only low levels of engraftment are detected [57]. These hypotheses await the use of animal models to test their validity. The notion that MSCs are immunologically privileged has generated interest, and clinical trials have been initiated to examine the application of the cells as novel immunosuppressive therapies for graft-versus-host diseases [37].

Studies in animal models

Since the human clinical trials have not provided conclusive data on the use of MSCs for OI treatment, investigators have gone back to animal models to assess the potential of MSCs to treat OI. Previous studies that led to the human clinical trials suggested that transplantation of MSCs via systemic circulation is possible, and that the cells will home to bone and will differentiate into osteoblasts and form bone *in vivo*. Several studies have confirmed these findings, but the level of donor engraftment in the skeletal tissues is very low [50–53].

Transplantation of MSCs in developing mice

Most of the studies assessing stem cell engraftment use adult mice as model systems. To emulate the clinical trials in which MSCs were transplanted in OI babies, the authors laboratory began to transplant the cells into a developing mouse model of the human OI. In these studies, a mouse model of OI that exhibits characteristics of OI Type III was used [51,53,58,59]. This mouse model of OI, called *oim*, has defective synthesis of proα2(I) chains due to a nucleotide deletion in the coding region of the carboxy-terminal propeptide of the proα2(I) chain [60]. The defective proα2(I) chains cannot associate with the proα1(I) chains, resulting in the accumulation of α1(I) homotrimers in tissues. We have used this mouse model in our studies to assess the efficacy of MSC transplantation for OI treatment [51,53,58]. MSCs harvested from normal mice and marked with green fluorescent protein gene were transplanted into the 2-day-old immunocompetent mice via the superficial temporal vein. The mice that received the transplant were examined at different time points following cell transplantation. The results showed that most of the transplanted cells were present in the lung [53]. The donor cells persisted in the lungs of the recipients and could still be detected 150 days after cell transplantation [53]. However, at 25 days after cell transplantation, very low levels of donor cells were detected in the bones of some of the recipient mice. Since only very low level engraftment was detected in bone, we asked whether the donor cells that had engrafted in bone would engraft at higher efficiency if they were reinjected into developing mice. Donor cells from the bones of the recipient mice that were retrieved at 25 days following transplantation and expanded in culture were subsequently reinjected in different neonatal mice. At 35 days following transplantation, examination of the recipient mice showed that few donor cells were present in the lungs of the recipients and the majority of the cells were in bone. Interestingly, the recycled cells also migrated to cartilages and differentiated into chondrocytes *in vivo* [53]. The results from these studies implied that cells exposed to bone microenvironments will return to bone upon reinjection via the systemic circulation. These data were confirmed by the transplantation of the adipose derived stem cells exposed to bone microenvironment by direct injection of MSCs into adult femur bone and retrieving them at 14 days following injection. Subsequent reinjection of the cells into the developing mice showed that the cells migrated to most of the bones of the recipients [61]. The level of engraftment in bone was, however, still low, as it ranged from 1–3% of the cells retrieved from the recipient animals at 30 days after cell transplantation.

To determine if higher levels of bone engraftment could be achieved by a defined population of MSCs, we generated single-cell subpopulations from the MSCs harvested from the marrow of normal mice. The single cells were expanded in culture, evaluated for efficiency in differentiation toward osteoblast *in vitro* and homing to bones following transplantation into the 2-day-old *oim* mice. By using this approach, subpopulations of MSCs were identified that exhibited very high efficiency for migration to bone. One of the identified clones migrated and engrafted in all the bones of the recipients at high efficiency following injection. The cells colonized all the femurs and tibia, differentiated into osteoblasts and deposited Type I collagen in the recipient bones (FIGURE 1) [58]. These results implied that there are cell subpopulations within bone marrow that are at different stages of differentiation, and that some of these cell subpopulations have a high affinity for migration to bone following transplantation. The level of engraftment, however, appeared to diminish

with time indicating loss of the cells, perhaps due to rejection by the host. Nevertheless, the results suggested that high levels of engraftment in bone can be achieved if correct subpopulations of cells for transplantation could be identified. Although a high level of engraftment was achieved using this cell population, these cells cannot be considered as stem cells because they expressed Runx2 an early marker of osteoblast differentiation. These observations suggest that the cells had progressed further along osteoblast differentiation and were therefore progenitors.

The above results have been confirmed by studies in which first-trimester human fetal blood MSCs were transplanted *in utero* into the fetuses of *oim* mice [62]. In this study, blood was collected from human fetuses at 10 weeks of gestation under ultrasound guidance. The adherent cells were expanded in culture and then transduced with a bicistronic lentiviral vector carrying renilla luciferase. The transduced cells, 10^6 fetal blood MSC/fetus, were then injected into *oim* fetuses at E13.5 to E15 gestation. Tissues harvested from the recipient mice were subjected to bioluminescence imaging for the detection of the luciferase activity. The authors reported that at 12 weeks following transplantation, the recipient mice had a reduction in the number of fractures and increased bone strength and length, as well as normalization of growth plate height. In addition, the donor cells were mostly found in the bones in the areas of active bone formation and remodeling. The authors concluded that *in utero* transplantation of MSCs into fetuses that are affected with OI may lead to the amelioration of OI phenotypes. *In utero* transplantation also led to the improvement of the glomerulopathy in this mouse model [63]. These findings are of interest because they suggest that MSCs may potentially be used for OI therapy if performed during the developmental stage. However, the long-term benefits of stem-cell transplantation for treating OI remain to be established. The mice that received the cell transplant were sacrificed at 12 weeks after birth; this time point is too short to assess the longer-term benefits of cell transplantation for OI patients. Nevertheless, *in utero* cell transplantation proved that MSCs may be beneficial for OI treatment, at least during the early stages of development.

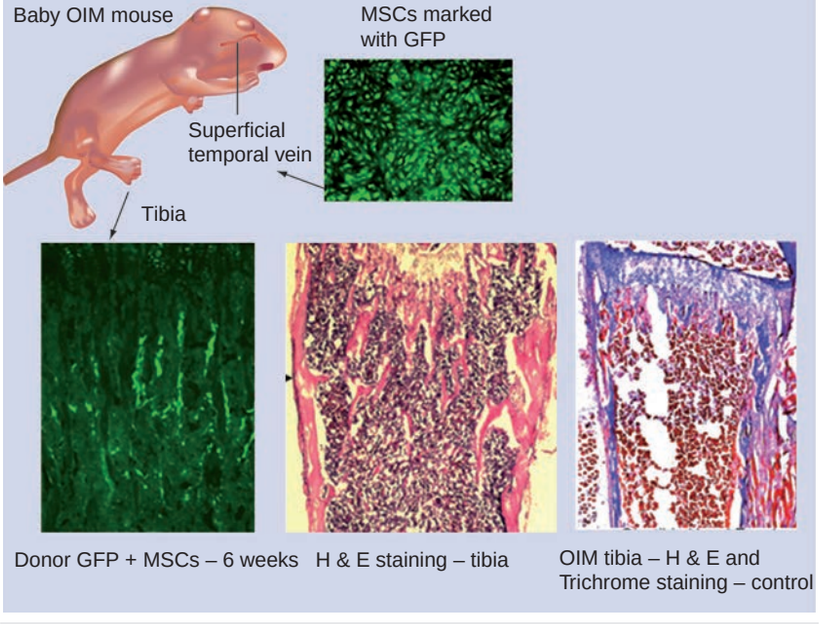

Figure 1. Schematic diagram showing transplantation of green fluorescent protein-marked mesenchymal stem cells into a mouse model of osteogenesis imperfecta. Bone marrow-derived mesenchymal stem cells expanded from a single cell clone were transduced with a retrovirus carrying green fluorescent protein and Zeocin-resistant genes. The cells were injected into a neonatal mouse with osteogenesis imperfecta via the superficial temporal vein [57]. Recipient mice were sacrificed at 4 weeks following transplantation and bones were harvested. The figure shows distribution of the donor cells in a tissue section of a tibia and the equivalent tissue section stained with hematoxylin and eosin. The femur from the osteogenesis imperfecta mouse that did not receive the cells has cortical thinning and near absence of trabecular bone [57]. The tissue section was stained with hematoxylin, eosin and trichrome to demonstrate absence of bone and collagen deposition.
E: Eosin; GFP: Green fluorescent protein; H: Hematoxylin; MSC: Mesenchymal stem cell; OIM: Osteogenesis imperfecta murine.

Stem and gene therapies for OI

As discussed previously, cell therapy for dominant-negative forms of OI will require a different approach to that of recessive forms of OI. Type II, III and IV OI forms result from mutations that affect the structure of α1(I) or α2(I) chains. Since abnormal collagen molecules are deposited in the matrix, a higher level of cell engraftment is necessary to generate sufficient matrix to counteract the presence of abnormal molecules. Studies of stem-cell transplantation in human and animal models showed that a low level of engraftment contributed to the improvement in the bone properties of the recipients [54,62]. Cabral *et al.* reported that a high proportion of mutant osteoblasts was compatible with normal skeletal function, suggesting that the presence of a low level of normal osteoblasts in OI patients is sufficient to normalize tissue function [64]. The inherent problem of cell replacement is that a normal donor is required to supply normal stem cells. In addition, the recipients need to be conditioned, for example, by myeloablative treatments to prevent rejection of the transplanted cells. The best approach would be to manipulate the

patient's own cells, for example, by eliminating the mutant allele or replacing the mutant gene in cases of recessive forms and then returning the cells to the patient.

Gene therapies in combination with stem cells are being evaluated as alternative approaches for treating OI [9,65–68]. Gene therapy approaches are complicated by the genetic heterogeneity of the disease and the fact that most forms of OI are autosomal dominant. Gene therapy approaches involving gene replacement in stem cells would not be possible in these patients with dominant-negative mutations without prior silencing of the mutant alleles. Several approaches for silencing mutant alleles have been suggested; use of oligonucleotides, RNAi suppression, use of ribozymes and gene targeting [9,65,67]. These approaches have inherent problems because treatment would have to be tailored to individual patients. Gene targeting proposed by Chamberlain *et al.* targets mutant alleles in stem cells, and the corrected stem cells can then be returned to the patients from whom the cells were harvested [65]. As a proof of concept, Chamberlain *et al.* used an adeno-associated virus vector carrying a neomycin-resistant gene and *COL1A1* to target the disruption of *COL1A1* in MSCs harvested from patients with OI. Using this approach, the investigators were able to show that the mutant allele could be disrupted, thus generating MSCs that synthesize normal Type I collagen [65]. The rationale behind this approach is that the stem cells corrected to synthesize normal Type I collagen would be returned to the patients from whom the MSCs were harvested. This attractive approach would entail harvesting stem cells from a patient, correcting the defect and then returning the cells to the same patient. Since the cells would be harvested from the patient, potential for cell rejection would be avoided. If this approach is possible, returning the cells to the respective patients poses a major challenge. In order to achieve this, methods to increase the level of engraftment of the donor cells in bones will have to be developed. In addition, it is not clear if the donor cells will persist in the patients' tissues following transplantation.

Gene replacement could also be possible in dominant-negative defects if a normal gene is overexpressed in the cells expressing abnormal protein. A report by Pochampally *et al.* showed that MSCs harvested from a patient with Type III OI due to a mutation in *COL1A1* made to express normal *COL1A1* cDNA by gene transfer, efficiently expressed the normal collagen *in vitro* and the cells differentiated efficiently into osteoblasts [69]. These data suggested that overexpression of the normal gene in stem cells expressing the abnormal gene could partially rescue the dominant-negative defect.

Conclusion

Stem-cell therapy for OI holds promise. Early treatment appears to be the best approach because the stem cells show higher engraftment in developing animals or at the fetal stage. For successful treatment of OI, cells to transplant and methods to increase cell engraftment in bone will need to be developed. Guillot *et al.* compared MSCs harvested from fetal and adult tissues, and concluded that stem-cell therapy for bone dysplasias such as OI may benefit from preferentially using first-trimester fetal blood or bone marrow MSCs rather than fetal liver or adult bone marrow MSCs [70]. These findings indicate that transplantation of the cells from fetal tissues may lead to high levels of stem cell engraftment in the bones of the recipients. The use of ESCs for transplantation is attractive, but derivation of the cells and methods to direct them to differentiate toward specific lineages remain challenges. The advantage of using ESCs is that gene correction is better suited to ESCs than to adult stem cells. Allogeneic transplantation is a major problem because of potential cell rejection; thus, the approach to combine stem cells and gene therapy offers the best approach for treating OI. Adult patients could be treated with stem cells by delivering cells locally in individual bones. This approach has not yet been attempted.

Future perspective

Over the next 5–10 years, extensive progress will be made in understanding the biology of stem cells, both the adult and the ESC. The ESC will gain extensive attention because of the potential they hold in regenerative medicine. Mechanisms of fibroblast reprogramming to generate ESC-like cells will be determined and this will pave the way for generating patient-specific ESC. Gene targeting to eliminate mutant alleles in stem cells will be improved, and this will open the way for gene replacements in stem cells. We anticipate improvements in designing novel methods to target stem cells to musculoskeletal tissues with high efficiency, and we will begin to understand the role of stem cells in treating various diseases including OI.

Financial & competing interests disclosure

This work was supported in part by a grant number R01 AR049688 from the National Institutes of Health to CN and Osteogenesis imperfecta fellowship to FL. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Osteogenesis imperfecta (OI) is a brittle-bone disease resulting from mutations in the genes that encode polypeptide chains of Type I collagen, the major component of bone.
- Some forms of OI result from mutations in the genes that encode noncollagenous proteins that play a role in post-translational modification of the collagen chains.
- Most mutations are dominant negative, thus creating complexity in developing treatments for the disease.
- Recessive forms of OI may be more amenable for cell therapy because defective molecules are not present in the matrix.

Pharmacological

- Drugs or hormones that have been tried for treating patients with OI are not expected to be effective because they will not alter the course of the disease.
- Bisphosphonates have proved effective, but long-term effects remain to be established.

Stem cells & OI treatment

- Stem cells hold promise for treating OI, especially recessive forms, but there are many hurdles to overcome before their application to OI treatment.
- Clinical trials with adult-derived stem cells showed potential of the adult stem cells for treating OI, but the treatment is not lasting.
- Animal studies showed that early treatment with stem cells may be more beneficial in treating patients with OI.
- The best approach will involve a combination of gene therapy and stem cells because of the complexity of the OI disease.

Conclusion

- Stem cells hold promise for treating OI if proper cells are identified.
- Methods of targeting stem cells to bone in order to achieve higher efficiencies in donor cell engraftment will need to be developed in order to apply stem cells to treat OI.
- Generating patient-specific stem cells will facilitate the use of embryonic stem cells for treating OI.
- A combination of gene and cell therapy may offer the best approach due to the complexity of the OI disease.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Pollitt R, McMahon R, Nunn J *et al.*: Mutation analysis of *COL1A1* and *COL1A2* in patients diagnosed with osteogenesis imperfecta Type I–IV. *Hum. Mutat.* 27, 716–722 (2006).

2 Prockop DJ: Mutations in collagen genes as a cause of connective tissue diseases. *N. Engl. J. Med.* 326, 540–546 (1992).

3 Morello R, Bertin TK, Chen Y *et al.*: *CRTAP* is required for prolyl 3- hydroxylation and mutations cause recessive osteogenesis imperfecta. *Cell* 127, 291–304 (2006).

4 Cabral WA, Chang W, Barnes AM *et al.*: Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nat. Genet.* 39, 359–365 (2007).

5 Barnes AM, Chang W, Morello R *et al.*: Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta. *N. Engl. J. Med.* 355, 2757–2764 (2006).

6 MacDougall M, Dong J, Acevedo AC: Molecular basis of human dentin diseases. *Am. J. Med. Genet. A* 140, 2536–2546 (2006).

7 Sillence DO, Senn A, Danks DM: Genetic heterogeneity in osteogenesis imperfecta. *J. Med. Genet.* 16, 101–116 (1979).

8 Martin E, Shapiro JR: Osteogenesis imperfecta:epidemiology and pathophysiology. *Curr. Osteoporos. Rep.* 5, 91–97 (2007).

9 Niyibizi C, Wang S, Mi Z, Robbins PD: Gene therapy approaches for osteogenesis imperfecta. *Gene Ther.* 11, 408–416 (2004).

10 Forlino A, Marini JC: Osteogenesis imperfecta and molecular therapeutics. *Mol. Genet. Metab.* 71, 225–232 (2000).

11 Glorieux FH, Ward LM, Rauch F, Lalic L, Roughley PJ, Travers R: Osteogenesis imperfecta Type VI: a form of brittle bone disease with a mineralization defect. *J. Bone Miner. Res.* 17, 30–38 (2002).

12 Glorieux FH, Rauch F, Plotkin H *et al.*: Type V osteogenesis imperfecta: a new form of brittle bone disease. *J. Bone Miner. Res.* 15, 1650–1658 (2000).

13 Ward LM, Rauch F, Travers R *et al.*: Osteogenesis imperfecta Type VII: an autosomal recessive form of brittle bone disease. *Bone* 31, 12–18 (2002).

14 Marini JC, Cabral WA, Barnes AM, Chang W: Components of the collagen prolyl 3-hydroxylation complex are crucial for normal bone development. *Cell Cycle* 6, 1675–1681 (2007).

15 Baldridge D, Schwarze U, Morello R *et al.*: *CRTAP* and *LEPRE1* mutations in recessive osteogenesis imperfecta. *Hum. Mutat.* 29, 1435–1442 (2008).

16 Uveges TE, Collin-Osdoby PC, Cabral WA *et al.*: Cellular mechanism of decreased bone in *Brtl* mouse model of OI: imbalance of decreased osteoblast function and increased osteoclasts and their precursors. *J. Bone Miner. Res.* 23, 1983–1994 (2008).

17 Lisse TS, Thiele F, Fuchs H *et al.*: ER stress-mediated apoptosis in a new mouse model of osteogenesis imperfecta. *PLoS Genet.* 4, E7 (2008).

18 Inzerillo AM, Zaidi M, Huang CL: Calcitonin: physiological actions and clinical

applications. *J. Pediatr. Endocrinol. Metab.* 17, 931–940 (2004).

19 Robinson RF, Long LD, Mahan JD: Treatment of poor linear growth in a patient with osteogenesis imperfecta Type III. *Clin. Ther.* 26, 1680–1683 (2004).

20 Glorieux FH: Osteogenesis imperfecta. *Best Pract. Res. Clin. Rheumatol.* 22, 85–100 (2008).

21 Land C, Rauch F, Glorieux FH: Cyclical intravenous pamidronate treatment affects metaphyseal modeling in growing patients with osteogenesis imperfecta. *J. Bone Miner. Res.* 21, 374–379 (2006).

22 Papapoulos SE, Cremers SC: Prolonged bisphosphonate release after treatment in children. *N. Engl. J. Med.* 356, 1075–1076 (2007).

23 Rauch F, Cornibert S, Cheung M, Glorieux FH: Long-bone changes after pamidronate discontinuation in children and adolescents with osteogenesis imperfecta. *Bone* 40, 821–827 (2007).

24 Camacho NP, Raggio CL, Doty SB *et al.*: A controlled study of the effects of alendronate in a growing mouse model of osteogenesis imperfecta. *Calcif. Tissue Int.* 69, 94–101 (2001).

25 Hochedlinger K, Rideout WM, Kyba M, Daley GO, Blelloch R, Jaenisch R: Nuclear transplantation, embryonic stem cells and potential for cell therapy. *Hematol. J.* 5(Suppl. 3), S114–S117 (2004).

26 Meissner A, Jaenisch R: Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. *Nature* 439, 212–215 (2006).

27 Beyhan Z, Iager AE, Cibelli JB: Interspecies nuclear transfer: implications for embryonic stem cell biology. *Cell Stem Cell* 1, 502–512 (2007).

28 Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676 (2006).

▪ **First report to demonstrate reprogramming of fibroblasts into an embryonic stem cell (ESC)-like state.**

29 Wernig M, Meissner A, Foreman R *et al.*: *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448, 318–324 (2007).

30 Takahashi K, Tanabe K, Ohnuki M *et al.*: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872 (2007).

▪▪ **Demonstrated that the human fibroblast can also be reprogrammed into an ESC-like state.**

31 Yu J, Thompson JA: Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917–1920 (2007).

32 Hanna J, Wernig M, Markoulaki S *et al.*: Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318, 1920–1923 (2007).

33 Friedenstein AJ, Chailakhyan RK, Gerasimov UV: Bone marrow osteogenic stem cells: *in vitro* cultivation and transplantation in diffusion chambers. *Cell Tissue Kinet.* 20, 263–272 (1987).

34 Caplan AI: Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J. Cell. Physiol.* 213, 341–347 (2007).

35 Gimble JM, Katz AJ, Bunnell BA: Adipose-derived stem cells for regenerative medicine. *Circ. Res.* 100, 1249–1260 (2007).

36 Cerletti M, Jurga S, Witczak CA *et al.*: Highly efficient, functional engraftment of skeletal muscle stem cells in dystrophic muscles. *Cell* 134, 37–47 (2008).

37 Jones BJ, McTaggart SJ: Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp. Hematol.* 36, 733–741 (2008).

38 in 't Anker PS, Noort WA, Kruisselbrink AB *et al.*: Nonexpanded primary lung and bone marrow-derived mesenchymal cells promote the engraftment of umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. *Exp. Hematol.* 31, 881–889 (2003).

39 Almeida-Porada G, Flake AW, Glimp HA, Zanjani ED: Cotransplantation of stroma results in enhancement of engraftment and early expression of donor hematopoietic stem cells *in utero*. *Exp. Hematol.* 27, 1569–1575 (1999).

40 Rieger K, Marinets O, Fietz T *et al.*: Mesenchymal stem cells remain of host origin even a long time after allogeneic peripheral blood stem cell or bone marrow transplantation. *Exp. Hematol.* 33, 605–611 (2005).

41 Anjos-Afonso F, Siapati EK, Bonnet D: *In vivo* contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. *J. Cell Sci.* 117, 655–5664 (2004).

42 Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B: Host origin of marrow stromal cells following allogeneic bone marrow transplantation. *Nature* 328, 429–432 (1997).

43 Wang L, Liu Y, Kalajzic Z *et al.*: Heterogeneity of engrafted bone-lining cells after systemic and local transplantation. *Blood* 106, 3650–3657 (2005).

44 Nesselmann C, Ma N, Bieback K *et al.*: Mesenchymal stem cells and cardiac repair. *J. Cell. Mol. Med.* 12, 1795–1810 (2008).

45 Tu Q, Valverde P, Li S, Zhang J, Yang P, Chen J: Osterix overexpression in mesenchymal stem cells stimulates healing of critical-sized defects in murine calvarial bone. *Tissue Eng.* 13, 2431–2440 (2007).

46 Tuan RS: Stemming cartilage degeneration: adult mesenchymal stem cells as a cell source for articular cartilage tissue engineering. *Arthritis Rheum.* 54, 3075–3078 (2006).

47 Amado LC, Saliaris AP, Schuleri KH *et al.*: Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc. Natl Acad. Sci. USA* 102, 11474–11479 (2005).

48 Pereira RF, Halford KW, O'Hara MD *et al.*: Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage and lung in irradiated mice. *Proc. Natl Acad. Sci. USA* 92, 4857–4861 (1995).

49 Pereira RF, O'Hara MD, Laptev AV *et al.*: Marrow stromal cells as a source of progenitor cells for non hematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc. Natl Acad. Sci. USA* 95, 1142–1147 (1988).

▪ **Demonstrated that mesenchymal stem cells (MSCs) may have potential for treating osteogenesis imperfecta (OI).**

50 Nilsson SK, Dooner MS, Weier HU *et al.*: Cells capable of bone production engraft from whole bone marrow transplants in non-ablated mice. *J. Exp. Med.* 189, 729–734 (1999).

51 Wang X, Li F, Niyibizi C: Progenitors systemically transplanted into neonatal mice localize to areas of active bone formation *in vivo*: implications of cell therapy for skeletal diseases. *Stem Cells* 24, 1869–1878 (2006).

52 Liechty KW, Mackenzie TC, Shaaban A *et al.*: Human mesenchymal stem cells engraft and demonstrate site specific differentiation after *in utero* transplantation in sheep. *Nat. Med.* 6, 1282–1286 (2000).

53 Niyibizi C, Wang S, Mi Z, Robbins PD: The fate of mesenchymal stem cells transplanted into immunocompetent neonatal mice: implications for skeletal gene therapy via stem cells. *Mol. Ther.* 9, 955–963 (2004).

54 Horwitz EM, Prockop DJ, Fitzpatrick LA *et al.*: Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat. Med.* 5, 309–313 (1999).

▪▪ **Reported the first clinical trial of MSCs in patients with OI.**

55 Horwitz EM, Prockop DJ, Gordon PL *et al.*: Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 97, 1227–1231 (2001).

56 Horwitz EM, Gordon PL, Koo WK *et al.*: Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc. Natl Acad. Sci. USA* 99, 8932–8937 (2002).

57 Le Blanc K, Götherström C, Ringdén O *et al.*: Fetal mesenchymal stem-cell engraftment in bone after *in utero* transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 79, 1607–1614 (2005).

58 Li F, Wang X and Niyibizi C: Distribution of single-cell expanded marrow derived progenitors in a developing mouse model of osteogenesis imperfecta following systemic transplantation. *Stem Cells* 25, 3183–3193 (2007).

▪▪ **Demonstrated that MSCs would engraft in the bone of developing mice and differentiate into osteoblasts and make bone.**

59 Phillips CL, Bradley DA, Schlotzhauer CL *et al.*: *Oim* mice exhibit altered femur and incisor mineral composition and decreased bone mineral density. *Bone* 27, 219–226 (1999).

60 Chipman SD, Sweet HO, McBride DJ Jr *et al.*: Defective proα1(I) collagen synthesis in a recessive mutation in mice: a mouse model of osteogenesis imperfecta. *Proc. Natl Acad. Sci. USA* 90, 1701–1705 (1993).

61 Liao X, Li F, Wang X, Yanoso J, Niyibizi C: Distribution of murine adipose-derived mesenchymal stem cells *in vivo* following transplantation in developing mice. *Stem Cells Dev.* 17, 303–314 (2008).

62 Guillot PV, Abass O, Bassett JH *et al.*: Intrauterine transplantation of human fetal mesenchymal stem cells from first-trimester blood repairs bone and reduces fractures in osteogenesis imperfecta mice. *Blood* 111, 1717–1725 (2008).

▪▪ **Demonstrated the rescue of the OI phenotype in a mouse model after *in utero* transplantation of MSCs.**

63 Guillot PV, Cook HT, Pusey CD *et al.*: Transplantation of human fetal mesenchymal stem cells improves glomerulopathy in a collagen Type I α2-deficient mouse. *J. Pathol.* 214, 627–636 (2008).

64 Cabral WA, Marini JC: High proportion of mutant osteoblasts is compatible with normal skeletal function in mosaic carriers of osteogenesis imperfecta. *Am. J. Hum. Genet.* 74, 752–760 (2004).

65 Chamberlain JR, Schwarze U, Wang PR *et al.*: Gene targeting in stem cells from individuals with osteogenesis imperfecta. *Science* 303, 1198–1201 (2004).

66 Chamberlain JR, Deyle DR, Schwarze U *et al.*: Gene targeting of mutant *COL1A2* alleles in mesenchymal stem cells from individuals with osteogenesis imperfecta. *Mol. Ther.* 16, 187–193 (2008).

67 Millington-Ward S, McMahon HP, Allen D *et al.*: RNAi of *COL1A1* in mesenchymal progenitor cells. *Eur. J. Hum. Genet.* 12, 864–866 (2004).

68 Millington-Ward S, McMahon HP, Farrar GJ: Emerging therapeutic approaches for osteogenesis imperfecta. *Trends Mol. Med.* 11, 299–305 (2005).

69 Pochampally RR, Horwitz EM, DiGirolamo CM, Stokes DS, Prockop DJ: Correction of a mineralization defect by overexpression of a wild-type cDNA for *COL1A1* in marrow stromal cells (MSCs) from a patient with osteogenesis imperfecta: a strategy for rescuing mutations that produce dominant-negative protein defects. *Gene Ther.* 12, 1119–1125 (2005).

70 Guillot PV, De Bari C, Dell'accio F, Kurata H, Polak J, Fisk NM: Comparative osteogenic transcription profiling of various fetal and adult mesenchymal stem cell sources. *Differentiation* 76, 946–957 (2008).