A systematic review and meta-analysis on the efficacy of stem cell therapy on bone brittleness in mouse models of osteogenesis imperfecta

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ABSTRACT

There is no cure for osteogenesis imperfecta (OI), and current treatments can only partially correct the bone phenotype. Stem cell therapy holds potential to improve bone quality and quantity in OI. Here, we conduct a systematic review and meta-analysis of published studies to investigate the efficacy of stem cell therapy to rescue bone brittleness in mouse models of OI. Identified studies included bone marrow, mesenchymal stem cells, and human fetal stem cells. Effect size of fracture incidence, maximum load, stiffness, cortical thickness, bone volume fraction, and raw engraftment rates were pooled in a random-effects meta-analysis. Cell type, cell number, injection route, mouse age, irradiation, anatomical bone, and follow up time were considered as moderators. It was not possible to investigate further parameters due to the lack of standards of investigation between the studies. Despite the use of *oim* mice in the majority of the investigations considered and the lack of sham mice as control, this study demonstrates the promising potential of stem cell therapy to reduce fractures in OI. Although their low engraftment, cell therapy in mouse models of OI had a beneficial effect on maximum load, but not on stiffness, cortical thickness and bone volume. These parameters all depend on bone geometry and do not inform on its material properties. Being bone fractures the primary symptom of OI, there is a critical need to measure the fracture toughness of OI bone treated with stem cells to assess the actual efficacy of the treatment to rescue OI bone brittleness.

1. Introduction

Osteogenesis imperfecta (OI) is a genetic disorder of collagen and collagen-associated genes characterized by bone fragility and skeletal deformities. OI affects 1 in 10,000 births, and both genders equally (Van Dijk and Sillence, 2014). It is an inherited dysplasia with prenatal onset that has been categorized into 21 genetically distinct types (Rossi et al., 2019). Types of OI differ in modes of inheritance, ranging from dominant, recessive, and X-linked and result in a range of phenotypic severity and symptoms (Kang et al., 2017). OI also ranges in its clinical manifestations, from mild symptoms with normal lifespans to perinatal lethality (Huber, 2007). Most OI cases result from mutations affecting the genes COL1A1 and COL1A2 encoding for the α1 and α2 chains that constitute type I collagen. Collagen type I is the most abundant form of collagen in the body and is the major protein found in bone, tendon, ligament, skin, sclera, cornea, and blood vessels. It is composed of two identical α1 polypeptide chains and one distinct α2 polypeptide.

Mutations in the COL1A1 and COL1A2 genes can either affect the amino acid sequence, thus resulting in abnormal collagen production, or cause haploinsufficiency, which prevents polypeptide chains from forming. Mutant procollagen chains unable to be incorporated into heterotrimers are either degraded via proteasome, eliminated through autophagy, degraded through alternate pathways, or secreted into the extracellular matrix. These pathways result in bone fragility, the hallmark of OI (Forlino et al., 2011; El-Gazzar and Högler, 2021).

There is no cure for OI. Current available treatment options include pharmaceuticals, physiotherapy, rehabilitation, and surgery (Millington-Ward et al., 2005). Bisphosphonates have been the predominant therapeutic for the OI population and act by reducing osteoclastic activity. While bisphosphonates have demonstrated beneficial effects in increasing bone mineral density and improving mobility (Roldán et al., 1999; Biggin and Munns, 2017; Shi et al., 2016), the effects of bisphosphonates on fracture rate remain unclear. Clinical meta-analysis studies have shown that bisphosphonates did not significantly reduce

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the proportion of people with OI experiencing fracture compared to controls (OI with no treatment, OI with placebo and OI with other comparator interventions) (Hald et al., 2015; Dwan et al., 2016). Furthermore, bisphosphonates have been shown to cause other potential adverse skeletal consequences, including delayed tooth development and delayed healing of osteotomy sites (Roldán et al., 1999; Biggin and Munns, 2017; Shi et al., 2016). Thus, as bisphosphonates do not address the issue of poor bone quality in OI, questions remain regarding the ability of this treatment to improve bone toughness as well as its efficacy with long-term treatment in growing children with OI (Biggin and Munns, 2017). Therefore, a need remains for treatment strategies that tackle the underlying genetic defect to improve OI bone quality.

Stem cell therapy may represent a viable solution for OI as it holds the potential to correct the bone phenotype by utilizing genetically healthy cells early in their development to remodel OI bone. Mesenchymal stem cells (MSCs) are mononuclear progenitor stem cells with the ability to self-renew. They have the capacity to differentiate into osteoblasts (OB), chondrocytes, myocytes, adipocytes, and neurons (Rohban and Pieber, 2017). These cells can be found in the bone marrow, umbilical cord, umbilical cord blood, placenta, amniotic fluid, and adipose tissue (Rohban and Pieber, 2017). Notably, MSCs have a homing ability, as they can migrate to injured sites, differentiate into local components, and secrete chemokines, cytokines, and growth factors to aid in tissue regeneration (Zhang et al., 2016). Additionally, MSCs have immunosuppressive, anti-apoptotic, and anti-inflammatory properties (Ranzoni et al., 2016). These qualities make MSCs good candidates for clinical transplant therapies. Commonly investigated sources of MSCs include bone marrow-derived mesenchymal stem cells (BMSCs or bone marrow stromal cells) and human fetal mesenchymal stem cells. Cell therapy for the treatment of OI aims to overcome the consequences of dominant-negative OI mutations by introducing non-differentiated cell progenitors with the possibility that they will successfully engraft to the bone, undergo osteogenic differentiation, and participate in bone modeling and remodeling, replacing mutant endogenous cells. Recent findings suggest that transplanted cell progenitors are able to contribute to bone remodeling through the release of paracrine factors of bioactive molecules, proteins and RNAs that can deliver signals for intercellular communication (Phinney et al., 2015).

In 1998, Pereira et al. (1998) were the first to investigate the potential ability of wild type (WT) MSCs to rescue the fragile bone phenotype of OI mice. Primary adherent MSCs were infused via intraperitoneal injection to OI-transgenic mice expressing the collagen type I mini gene. Mice displayed a small but significant increase in mineral content and bone collagen content one month following treatment. Donor cell DNA was detected in the bone, bone marrow, cartilage, and lungs 2.5 months after infusions. An experiment, in which male MSCs were infused into female OI-transgenic mice, utilized fluorescence in-situ hybridization assays for the Y chromosome and showed that at 2.5 months following infusions donor cells accounted for 4–19% of fibroblast-like cells in the primary cultures of lung, calvaria, cartilage, long bone, tail, and skin tissues (Pereira et al., 1998). This initial study demonstrated the possibility of using cell progenitors for the treatment of OI.

Following the results of this and other pre-clinical models showing the potential of MSCs to correct collagen-related disorders, Horwitz et al. (1999) investigated allogeneic bone marrow transplantation (BMT) in OI children. Five patients were intravenously infused with unmanipulated bone marrow from human leukocyte antigen matched or single-antigen mismatched siblings after ablative conditioning therapy (Horwitz et al., 1999). Engraftment was assessed in two children about 12–14 weeks following BMT, and showed only 1.5–2.0% donor-derived OB (Horwitz et al., 1999). Increased growth acceleration and total body bone mineral content (TB-BMC) was reported for three children in the six months following transplantation compared to two non-transplanted OI controls. Growth rates slowed or plateaued with extended follow up time up to six months following transplantation, however bone mineral

content continued to increase (Horwitz et al., 2001). A decrease in fracture rate was observed up to 12 months after BMT (Horwitz et al., 1999). Due to unsustained improvements, patients then underwent two rounds of infusions with cultured, retroviral vector gene-marked BMSCs from the prior donors 18–34 months after BMT. Between four and six weeks after infusions, five out of the six patients showed engraftment in bone among other tissues. Over the six months following infusions, these five patients displayed increased growth velocities, ranging from 60 to 90% of the predicted median compared to negligible growth in the six months preceding therapy. However, only one patient showed increased TB-BMC three months post therapy (Horwitz et al., 2002). These case studies show that injected MSCs are able to engraft in the bone, marrow stroma, and skin without preparative chemotherapy and not elicit severe immune responses. Initially the cells were able to produce clinically measurable benefits; though it is unclear why these outcomes were short-lived. Additionally, confirmation of engraftment in these studies was not clear as OB were not identified so donor cells may have been hematopoietic in origin (Horwitz et al., 1999, 2001, 2002). Finally, despite increased amount of normal collagen fibers deposited by these cells in the bones (Horwitz et al., 1999), there was no evidence that cells were capable to produce a healthy matrix.

These studies set the go for further research to continue investigating the effects of stem cell therapies on OI bone in the following decades. Different stem cell types, cell dosages, routes of delivery, age at injection and cell culture conditions are just some of the parameters under investigation in an attempt to rescue OI bone fragility. This meta-analysis aimed to define the effects of stem cell therapy on the mechanical, structural and compositional properties of the OI bone. This analysis reports the current knowledge on the effects of stem cell therapy on bone brittleness in OI and emphasizes gaps that need further investigation for a comprehensive understanding of the effect of stem cell treatment on OI bone quality and fragility, and for ensuring the effective translation of stem cell therapy for OI from preclinical to clinical setting.

2. Meta-analysis methods

The Preferred Reporting Items for Systematic Reviews and Meta-analyses statement was used to guide our methods. A literature search was conducted using databases including PubMed, Embase, ScienceDirect, SciFinder, and Science.gov, from their inception dates to April 2021 (Fig. 1). Keywords used for search included (Osteogenesis Imperfecta) AND (Stem cell OR Stromal cell).

2.1. Eligibility criteria

2.1.1. Study types
Inclusion criteria included controlled trials utilizing stem cell therapy for the treatment of mouse models of OI. Clinical trials, case reports, and in vitro studies were excluded. Reviews, editorials, comments, and conference articles were excluded.

2.1.2. Mouse types
Studies using any mouse model of osteogenesis imperfecta were included. Studies were excluded if the untreated OI mouse model did not demonstrate phenotypic bone fragility in their control OI group.

2.1.3. Intervention types
Studies using stem cell therapy, including human- or mice-derived bone marrow or stem cells compared with a control group were considered, as well as those using retrovirally transduced reporter genes for cell tracking purposes. Studies using genetically engineered stem cells were instead excluded.

2.1.4. Outcome types
Bone compositional, structural, mechanical properties were reviewed. This analysis includes the outcomes common between three
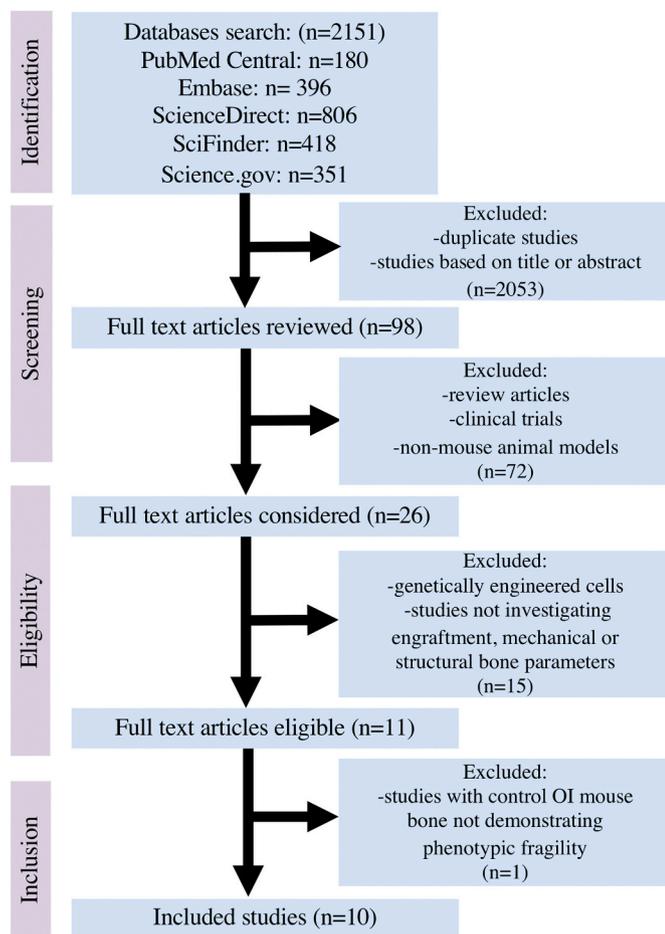
Fig. 1. Study flow diagram of the selection process. A total of 2151 studies were retrieved from the literature review. Abstracts and titles were reviewed and excluded when not matching eligibility criteria. Duplicates were also excluded. 98 full texts were reviewed with clinical trials, review articles, and studies using non-mouse animal models then being excluded. Subsequently 26 full text articles were considered with 10 studies meeting all inclusion criteria.

or more studies (Green et al., 2008; Hooijmans et al., 2014): cortical thickness, bone volume fraction (BV/TV), stiffness, and maximum load. The effects of other compositional, structural, and mechanical parameters were not analyzed due to low number of studies (two or less) that

investigated these properties. Biological properties common to the selected studies, such as cell engraftment (expressed as the raw percentage of engrafted transplanted cells to total cells) and fracture incidence, calculated as the total number of fractured long bones over the total number of long bones considered, were also considered for this meta-analysis.

2.2. Data extraction

Titles and abstracts and then the full-text articles were screened according to the eligibility criteria. Studies satisfying the inclusion and exclusion criteria were enrolled in the meta-analysis. The mean results, standard deviations pertaining to the results, and number of animals of experimental and control groups were extracted to calculate effect size, which represented the magnitude of the observation. Standardized mean difference (SMD), mean difference (MD), and risk ratio (RR) served as effect size measurements for the variables as appropriate. Other information regarding mouse model, cell type, route of delivery, age of treatment, cell dosage, and follow up times were incorporated in the database (Table 1). In studies using different follow up time points for data measurement, the last time point was considered for analysis. Corresponding authors were contacted in the case of missing data. When original study data was not available, GraphClick software was used to approximate data from bar graphs.

2.3. Study quality assessment

Due to the nature of these basic science research studies, that were principally to evaluate effects of transplantation using different readouts, a formal quality assessment was not applicable. However, study characteristics including choice of controls, blinding, biases, and baseline characteristics are reviewed in the discussion.

2.4. Statistical analysis

Data review and analyses were performed using Review Manager 5.3 software by the Cochrane Collaboration (Review Manager Web (Rev-Man Web), 2019) and SPSS Statistics (IBM Corp., Armonk, N.Y., USA) (Field and Raphael, 2005). SMD, MD, and RR were used as a measure of effect size in a random effects model. Each effect was expressed as a 95% confidence interval (CI). The effect size was considered significant if the relative 95% CI did not include 0. Risk ratios lower than one signified a decrease in risk. Heterogeneity was considered significant at $p < 0.10$ (Green et al., 2008). Inconsistency was estimated using the I^2 statistic, describing the percentage of total variation across studies that is due to

Table 1

Study characteristics of stem cells injected in mouse models of OI. iSCP = induced single cell progenitors; MSC = mesenchymal stem cells; BM = bone marrow; fSC = fetal stem cells; BMSC = bone marrow stromal cells; fMSC = fetal mesenchymal stem cells; NA-BMC = non-adherent bone marrow cells, e-CSC = early chorionic stem cells, AFSC = amniotic fluid stem cells; IUT = intrauterine transplantation; IP = intraperitoneal.

Study	Cell type	Number of cells (×e6)	Route of delivery	Irradiation (cGv)	Mouse type	Age (wk) at injection	Follow up time (wk)
Li et al. (2007)	B6C3Fe iSCP	0.05	Superficial temporal vein	350	oim/oim	Neonates	4
Guillot et al. (2008)	Human fetal MSC	1	IUT embryonic	None	oim/oim	Fetus	4, 8, 12
Panaroni et al. (2009)	CMV/eGFP CD-1 BM	5	IUT embryonic	None	BrtlIV	Fetus	8
Vanleene et al. (2011)	Human fSC	1	IUT embryonic	None	oim/oim	Fetus	8
Li et al. (2010)	WT BMSC	1	Femur medullary space	350	oim/oim	4	6
Jones et al. (2012)	Human fMSC	1	IP	None	oim/oim	Neonates	8
Otsuru et al. (2012)	H2K-GFP NA-BMC, MSC	2	Intradermal tail vein	1125	oim/oim	4–6	3
Jones et al. (2014)	Human fetal e-CSC	1	IP	None	oim/oim	Neonates	8
Ranzoni et al. (2016)	Human AFSC	1	IP	None	oim/oim	Neonates	8
Sinder et al. (2020)	SMA9/Col2.3GFP BMSC	1	Femur medullary space	900	oim/oim	8–10	12

heterogeneity rather than chance. Values of 25, 50, and 75% were considered low, moderate, and high inconsistency (Higgins et al., 2003).

Categorical and continuous moderator analyses were conducted in order to examine the impact of study factors on the results. Factors including mouse model, cell type, route of delivery, age at time of treatment, cell dosage, time at follow up, and experimental anatomical bone type were analyzed when appropriate and with sufficient study numbers.

2.5. Publication bias within the meta-analysis

Funnel plots were created to visualize the potential of publication bias with Meta-Essentials for Microsoft Excel (Suurmond et al., 2017). Tests for funnel plot asymmetry were not included, as recommended by Stern et al. (Sterne et al., 2011) for the analyses of less than 10 studies.

3. Results

3.1. Study selection

The literature search identified 2151 articles, 10 of which were eligible for this review (Fig. 1) (Ranzoni et al., 2016; Li et al., 2007; Guillot et al., 2008; Panaroni et al., 2009; Li et al., 2010; Vanleene et al., 2011; Jones et al., 2012; Otsuru et al., 2012; Jones et al., 2014; Sinder et al., 2020). All selected studies were in English. Characteristics of included studies are depicted in Table 1. The studies meeting all criteria and included in the meta-analysis used *oim/oim* (B6C3Fe-a/aCol1a2$^{oim/oim}$) and/or BrtlIV/+ (Col1a1$^{tm1.1Jcm}$/Col1a1$^+$) mouse models of OI. *Oim* mice have a mutation in the gene encoding the α2 chain of type I procollagen, preventing the proper assembly of collagen propeptides. This mouse model is representative of moderate to severe forms of OI in its heterozygotes and homozygotes forms, respectively (Saban et al., 1996; Enderli et al., 2016). BrtlIV/+ is a knock-in mouse with a classical glycine substitution in type 1 collagen exhibiting a phenotype resembling moderately severe and lethal OI (Enderli et al., 2016; Forlino et al., 1999). The *oim/oim* and BrtlIV/+ mice treated with cell progenitors received intrauterine transplantation, intraperitoneal, tail vein, temporal vein, or local femoral injections. At the time of injection, mice ranged from neonates to 10-week-old mice with follow up durations ranging from four weeks to three months. Within the ten included studies, two investigated BMSCs (Li et al., 2010; Sinder et al., 2020), one bone marrow (Panaroni et al., 2009), two used derivatives of BMSCs (Li et al., 2007; Otsuru et al., 2012), four studied types of human fetal stem cells (Guillot et al., 2007, 2008; Vanleene et al., 2011; Jones et al., 2012; Jones et al., 2014), and one amniotic mesenchymal stem cells (Ranzoni et al., 2016). Though these cell treatments exhibit varying levels of stemness, we will refer to them as stem cells in our discussion. Number of cells delivered and amount of irradiation also varied across studies. Only one study (Millard et al., 2015) was excluded from this meta-analysis because control *oim* mice treated with phosphate buffered saline intrauterine transplantation (PBS-IUT) did not exhibit the phenotypic fragility of the OI bone. Most of the selected studies used original OI mice, and not sham treated mice, as controls (Ranzoni et al., 2016; Li et al., 2007; Guillot et al., 2008; Panaroni et al., 2009; Vanleene, 2011; Jones et al., 2012; Jones et al., 2014). Multiple parameters were investigated within this meta-analysis to understand the efficacy of stem cell therapy on OI bone mechanical and structural properties (Fig. 2).

3.2. Mechanical properties

The meta-analysis showed that stem cell therapy had a significant beneficial effect on increasing maximum load (SMD = 2.36, 95% confidence interval = 0.41–4.31, $p = 0.02$, I^2 = 94%). Moderator analyses showed anatomical bone (categorical, $\chi^2 = 5.014$, $p = 0.025$) and follow up time (continuous, $\chi^2 = 9.855$, $p = 0.043$) to significantly moderate these results. Cell therapy had no significant effect on stiffness (SMD =

2.09, 95% confidence interval = −0.45-4.64, $p = 0.11$, I^2 = 96).

3.3. Structural properties

No significant effect of stem cell therapy was shown on the structural properties here analyzed. Both cortical thickness and BV/TV showed trends of a beneficial effect but not statistical significance as their CIs intersected 0 (SMD = 0.64, 95% confidence interval = −0.85-2.12, $p = 0.4$, I^2 = 90%; SMD = 1.38, 95% confidence interval = −1.26-4.02, $p = 0.31$, I^2 = 92%). Follow up time was shown to significantly, categorically moderate the effect of cell therapy on cortical thickness (categorical $\chi^2 = 6.354$, $p = 0.042$; continuous $\chi^2 = 4.996$, $p = 0.172$).

3.4. Cell engraftment

Five of the 10 included studies reported engraftment rate in terms of percentage of engrafted donor cells to total cell number, measured through either histological section (Panaroni et al., 2009; Otsuru et al., 2012) or quantitative PCR (Li et al., 2007; Vanleene et al., 2011; Jones et al., 2012) cell counting of green fluorescent protein (GFP) positive cells/human cells on bone. A forest plot in Fig. 2 shows the low and variable raw engraftment percentages (the mean difference between engraftment rates of transplanted and non-transplanted mice).

3.5. Fracture incidence

The fracture incidence was shown to be significantly decreased with stem cell therapy (RR = 0.27, 95% confidence interval = 0.19–0.38, $p < 0.00001$, I^2 = 0%).

3.6. Heterogeneity

High heterogeneity and inconsistency were found in all outcomes investigated except in fracture incidence. Random effects analysis was utilized to partially address this heterogeneity as well as decrease risk of erroneous estimates.

4. Discussion

This meta-analysis shows that stem cell therapy in mouse models of OI has a significant beneficial effect in decreasing fracture incidence and increasing maximum load, despite low rates of cell engraftment. Maximum load was shown to be influenced by anatomical bone (tibia vs. femur). This is not surprising as maximum load depends on the geometry of the bone, and femur and tibia greatly differ in their shape despite being both long bones. Cell therapy was shown to not have an effect on bone stiffness, cortical thickness, or BV/TV. These findings predominantly coincide with the results from clinical trials (Horwitz et al., 2001; Amin and Shazly, 2014; Le Blanc et al., 2005; Götherström et al., 2014).

The low cell engraftment in OI-stem cell treated bone has been demonstrated in numerous studies using animal models and is in agreement with the few existing human clinical trials (Pereira et al., 1998; Horwitz et al., 1999, 2001, 2002; Li et al., 2007; Guillot et al., 2008; Panaroni et al., 2009; Vanleene et al., 2011; Otsuru et al., 2012). The use of different cell types, dosages, delivery routes, and follow up times resulted in low but variable engraftment rates both within and between studies (from 0.3% to 28% in a given bone chip) (Li et al., 2007). Regardless the variety of parameters considered in these studies, vast and consistent improvements in the engraftment (i.e. the percentage of engrafted transplanted cells to total cells) have not been shown. Among the considered studies, only Guillot et al. (Guillot et al., 2008) studied the engrafted cells longitudinally using qRT-PCR at 1, 2, 4, 8, and 12 weeks. They reported engraftment of 5% at week one, with levels slightly decreasing over time and then stabilizing around 4% from weeks four to 12. Despite the low engraftment rate, stem cell therapy in mouse models of OI has been shown to decrease their bone fracture incidence,

A) Maximum Load

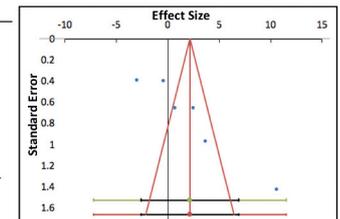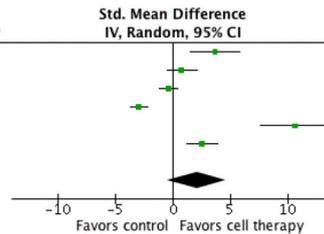| Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Guillot 2008	4.3	0.2	8	2.5	0.2	4	10.2%	8.31 [4.08, 12.54]	2008
Panaroni 2009	28.56	4.97	5	20.41	3.68	8	17.9%	1.81 [0.41, 3.20]	2009
Li 2010	9.126	0.693	5	7.925	1.941	5	18.1%	0.74 [-0.56, 2.05]	2010
Vanleene 2010	6	2.1	11	6.1	2.8	15	19.1%	-0.04 [-0.82, 0.74]	2010
Jones 2014	4.5	0.3	22	4.8	0.3	34	19.4%	-0.99 [-1.55, -0.42]	2014
Ranzoni 2016	7.5	0.4	13	4.6	0.3	17	15.4%	8.15 [5.82, 10.47]	2016
Total (95% CI)			64			83	100.0%	2.36 [0.41, 4.31]	

Heterogeneity: Tau² = 5.01; Chi² = 81.15, df = 5 (P < 0.00001); I² = 94%
Test for overall effect: Z = 2.38 (P = 0.02)
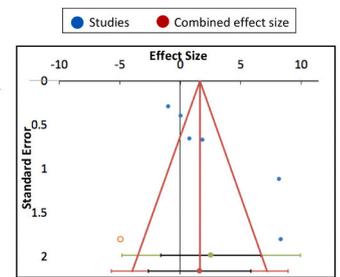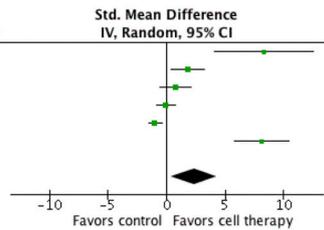
B) Stiffness

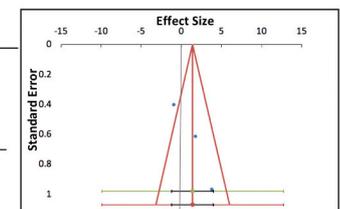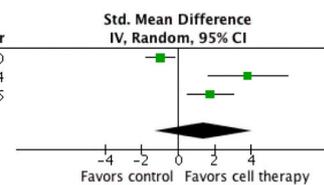| Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Guillot 2008	10.9	1	8	7.4	0.5	4	15.9%	3.67 [1.51, 5.83]	2008
Li 2010	63.8	6.4	5	50.6	21.2	5	17.2%	0.76 [-0.55, 2.07]	2010
Vanleene 2010	35.5	10.4	11	41.3	16.4	15	17.7%	-0.40 [-1.18, 0.39]	2010
Jones 2014	29.7	2.1	22	35.3	1.7	34	17.7%	-2.96 [-3.74, -2.18]	2014
Ranzoni 2016	42.9	1.7	13	26.1	1.4	17	14.4%	10.64 [7.67, 13.62]	2016
Sinder 2019	3.3	0.3	10	2.5	0.3	7	17.1%	2.53 [1.16, 3.90]	2019
Total (95% CI)			69			82	100.0%	2.09 [-0.45, 4.64]	

Heterogeneity: Tau² = 9.38; Chi² = 128.29, df = 5 (P < 0.00001); I² = 96%
Test for overall effect: Z = 1.61 (P = 0.11)

C) BV/TV

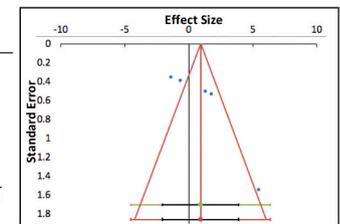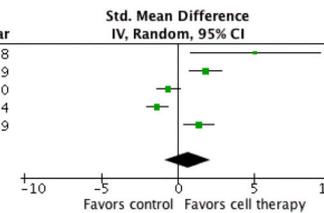| Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Vanleene 2010	0.1	0.03	12	0.13	0.03	16	36.0%	-0.97 [-1.77, -0.17]	2010
Jones 2014	4.1	0.6	6	2	0.4	6	29.7%	3.80 [1.63, 5.98]	2014
Ranzoni 2016	5.2	0.7	9	3.9	0.7	6	34.3%	1.75 [0.48, 3.01]	2016
Total (95% CI)			27			28	100.0%	1.38 [-1.26, 4.02]	

Heterogeneity: Tau² = 4.88; Chi² = 24.58, df = 2 (P < 0.00001); I² = 92%
Test for overall effect: Z = 1.02 (P = 0.31)

D) Cortical Thickness

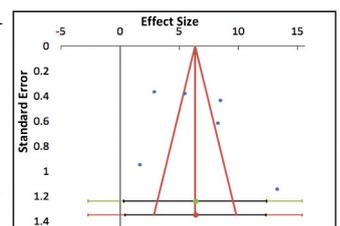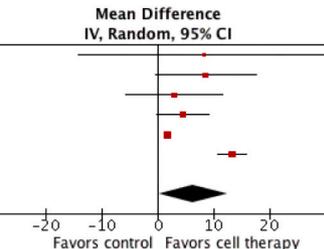| Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Guillot 2008	0.27	0.02	4	0.17	0.01	3	8.2%	5.03 [0.77, 9.29]	2008
Panaroni 2009	0.203	0.022	8	0.164	0.02	12	22.1%	1.80 [0.71, 2.88]	2009
Vanleene 2010	0.164	0.02	12	0.183	0.034	16	23.5%	-0.64 [-1.41, 0.13]	2010
Jones 2014	17.2	0.5	19	17.9	0.5	20	23.8%	-1.37 [-2.08, -0.67]	2014
Sinder 2019	0.16	0.017	10	0.14	0.01	9	22.4%	1.35 [0.33, 2.37]	2019
Total (95% CI)			53			60	100.0%	0.64 [-0.85, 2.12]	

Heterogeneity: Tau² = 2.29; Chi² = 39.30, df = 4 (P < 0.00001); I² = 90%
Test for overall effect: Z = 0.84 (P = 0.40)

E) Engraftment

Study or Subgroup	Mean Difference	SE	Weight	Mean Difference IV, Random, 95% CI	Year
Li 2007	8.3	11.55	5.6%	8.30 [-14.34, 30.94]	2007
Panaroni 2009	8.51	4.61	15.2%	8.51 [-0.53, 17.55]	2009
Vanleene 2010	2.93	4.4	15.6%	2.93 [-5.69, 11.55]	2010
Jones 2011	4.52	2.4	19.8%	4.52 [-0.18, 9.22]	2011
Otsuru 2012 MSC	1.7	0.2	22.3%	1.70 [1.31, 2.09]	2012
Otsuru 2012 NA-BMC	13.3	1.3	21.5%	13.30 [10.75, 15.85]	2012
Total (95% CI)			100.0%	6.35 [0.14, 12.55]	

Heterogeneity: Tau² = 44.88; Chi² = 81.25, df = 5 (P < 0.00001); I² = 94%
Test for overall effect: Z = 2.01 (P = 0.04)

F) Fracture Incidence

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Fixed, 95% CI	Year
Guillot 2008	6	41	61	127	21.4%	0.30 [0.14, 0.65]	2008
Vanleene 2010	1	24	11	40	5.9%	0.15 [0.02, 1.10]	2010
Jones 2011	9	108	26	96	19.8%	0.31 [0.15, 0.62]	2011
Jones 2014	12	120	23	78	20.0%	0.34 [0.18, 0.64]	2014
Ranzoni 2016	10	168	44	156	32.8%	0.21 [0.11, 0.40]	2016
Total (95% CI)		461		497	100.0%	0.27 [0.19, 0.38]	
Total events	38		165				

Heterogeneity: Chi² = 1.58, df = 4 (P = 0.81); I² = 0%
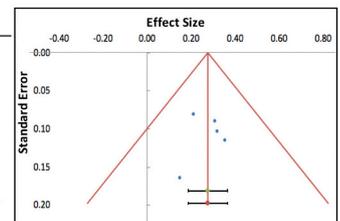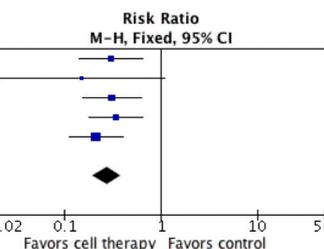Test for overall effect: Z = 7.51 (P < 0.00001)

Fig. 2. Forest plots showing the effect size of stem cell therapy on mouse models of OI. Compared with a control group, **A)** standardized mean difference (SMD) of maximum load, **B)** SMD of stiffness, **C)** SMD of BV/TV, **D)** SMD of cortical thickness, **E)** mean difference (MD) of raw engraftment percentages, **F)** risk ratio (RR) of fracture incidence. Studies considered for each analysis are presented on the left side and in the forest plot their weight is represented with a square and with a line representing their 95% confidence interval (CI). Black diamonds in the forest plot represent the total effect size, with the width representing its 95% CI. Diamonds falling completely to the right side of the graph, favoring cell therapy, show a significant effect. The diamond representing RR of fracture incidence is shown to be significant as the risk is less than 1, thus representing a decrease in fracture incidence. Each forest plot is shown with its funnel plot, a scatter plot of the study effect size against its standard error. These plots are used for the assessment of potential publication bias, with asymmetry within the plots resulting from possible non-reporting biases, poor methodological quality, or true heterogeneity. Fracture incidence does not include error bars as standard risk ratio does not include standard deviations. Chi² measures heterogeneity and I² inconsistency. MSC = mesenchymal stem cells; NA-BMC = non-adherent bone marrow cells.

as demonstrated in this meta-analysis. Substantial improvements are not shown in most other investigated parameters, such as structural and mechanical measurements of the cortical bone, which surprisingly are actually neglecting to analyze the bone resistance to fracture (or toughness) of the stem cell treated bone to quantify changes in OI bone brittleness with therapy. Indeed, only a few studies examined improvement in OI mouse bone quality after stem cell treatment. These studies mostly considered *oim* mice, and all but three, which used the contralateral leg of the same mouse (Li et al., 2010; Sinder et al., 2020) or PBS-injected tail vein (Otsuru et al., 2012) as control, compared results from stem cell treated OI mice to naïve OI mice as controls, and not to sham operated OI mice, thus highlighting the need of further investigation in the field. These collective results led us to three conclusions: (1) the current laboratory strategies are not completely adequate in identifying all possible beneficial effects of stem cell therapy on OI mouse bone brittleness; (2) the use of different mouse models of OI, as well as the use of original mice and shams as control, are needed to fully comprehend the effect of stem cell treatment on OI bone quality and its brittleness; and (3) the mechanisms of action of stem cells to enhance properties of OI bone is still unclear and may be independent from cell engraftment.

Regarding the adoption of adequate laboratory strategies to identify the beneficial effects of stem cell therapy on OI bone fragility, this meta-analysis suggests the methodologies and outcomes investigated thus far are not completely sufficient for analyzing the total effect of the treatment. An extremely important aspect of bone not yet been studied widely is the proper assessment of the mechanical, structural, and compositional properties of bone after stem cell therapy in order to fully determine improvements of this treatment for OI bone. Bone fragility is the primary symptom of OI, thus bone from OI mice treated with stem cells must be tested for their brittleness to determine the efficacy of treatment. To date, the most accurate measure of bone fragility is determined by assessing its fracture toughness (Ritchie, 2011; Inzana et al., 2013). Methodologies have been developed for testing fracture toughness in mouse bone (Ritchie et al., 2008; Carriero et al., 2014a, 2014b; Rodriguez Florez et al., 2014) and they have successfully been applied to investigate bone toughness in *oim* mice (Carriero et al., 2014c; Docaj et al., 2020; Docaj and Carriero, 2020) as well as in other mouse strains (Carriero et al., 2014d; Miller et al., 2017). Fracture toughness informs on the material properties of the bone to resist crack propagation – thus there is a critical need to make sure OI treated bone has increased fracture toughness in order to reduce its fragility. Stiffness and maximum load determined from the load-displacement curve highly depend on both material properties and geometry of the bone considered. Thus, these parameters do not appropriately inform on the bone resistance to crack propagation. Bone strength from the stress-strain curve should also be tested to inform on the properties of the bone material to resist deformations. Because microcracks form in regions of high strains, full field strain distributions on the OI mouse bone treated with stem cells, as those acquired on other mouse models (Carriero et al., 2021; Javaheri et al., 2018; Carriero et al., 2018; Poulet et al., 2016; Javaheri et al., 2015; Carriero et al., 2014e), can inform on the ability of the treated bone to sustain load without cracking.

On the structural properties, this study also reports on the lack of investigation on the intracortical structure (organization and porosity along the hierarchy). Intracortical porosity and lamellar structure are important features to investigate in OI bones as fractures of the cortical bone are typical for OI (Munoz et al., 2021). Bone fracture toughness is influenced by both the amount and architecture of the intracortical canals (Carriero et al., 2014b, 2014c; Yeni et al., 1997), and has been suggested to depend on an organized lamellar structure (Carriero et al., 2014c; Peterlik et al., 2006; Jepsen et al., 1996, 1997; Docaj and Carriero, 2021). Furthermore, although cortical bone fragility is the major clinical hallmark of OI, trabecular bone offers a high surface area, thus providing exposed surface of bone for the transplanted cells to potentially engraft and (re)model. Because of this, it is expected that trabecular bone would more rapidly exhibit the beneficial effects of stem cell therapy than cortical bone, however this has not yet been thoroughly investigated. This will also offer the possibility to study the influence of stem cell therapy on endochondral ossification and bone growth in OI. Similarly, compositional analyses of bone must be conducted at the tissue level to inspect the impact of stem cell therapy on OI bone mineralization, non-collagenous proteins, water content, as well as at the molecular and crystal level to assess collagen crosslinks and relationship with the mineral, which may ultimately affect the bone resistance to fracture.

Another important aspect to investigate is the mechanisms of action of the transplanted stem cells: Where do cells engraft? Do they effectively reach bone? What do they differentiate into? And how do they improve bone properties? The studies included in this meta-analysis largely showed transplanted cells, though of differing types and routes of transplantation, homed to bone and expressed OB genes (Li et al., 2007; Guillot et al., 2008; Vanleene et al., 2011; Jones et al., 2014; Ranzoni et al., 2016; Sinder et al., 2020). Li et al. (2007) described transplanted single cell progenitors (SPCs) homed mostly to bones and expressed OB-specific genes, later showing transplanted donor BMSCs within newly formed bone localized with osteocalcin expressing cells, suggesting OB contribution to bone formation (Li et al., 2010). Sinder et al. (2020) showed that with local femoral BMSC transplantation, donor progenitor cells differentiated into OB and osteocytes and engrafted on the femur endosteal surface and within the matrix. Guillot et al. (2008) showed transplanted cells clustered to areas of active bone formation, Jones et al. (2014) found cells homing preferentially to the long bone epiphyses, and Vanleene et al. (2011) and Ranzoni et al. (2016) showed cells migrated to bone. These four studies also all found these transplanted donor cells to be expressing OB genes (Ranzoni and et al., 2016; Guillot et al., 2008; Vanleene et al., 2011; Jones et al., 2014). Of note, Li et al. (2007), Ranzoni et al. (2016), and Guillot et al. (2008) identified, to a lesser degree, transplanted donor cells in other organs. For two studies included, histological observation of cells was used to determine OB differentiation, rather than genetic profiling (Panaroni et al., 2009; Otsuru et al., 2012). Controversially, another study reported that donor bone marrow cells systemically injected into OI mice do not become OB, but rather differentiate into the myeloid lineage such as osteoclasts and osteal macrophages lining endosteal and trabecular surfaces (Millard et al., 2015). Specifically, Millard et al. (2015) investigated bone-associated donor cells following intrauterine transplantation (IUT) of WT BM in *oim* mice. They reported that donor cells did not contribute the OB lineage cells, but were associated with osteoclasts or osteal macrophages (Millard et al., 2015). This study was excluded from our meta-analysis because their control PBS-treated *oim* mice did not show the expected bone fragility typical of OI bone. This study used sham operated mice as control for investigating the effect of stem cell treatment on OI bone quality. The authors described this uncharacteristic lack of fractures in OI sham operated control bones as possibly reflecting selection bias towards less fragile animals surviving the process of IUT. This study thus further underlines the importance of investigating the effect of stem-cell treatment in naïve OI mice controls as well as in sham operated mice.

Most of the studies included in this meta-analysis compared untreated *oim* mice to *oim* treated with stem cells+PBS, regardless differences in the delivery route of cell therapy. Guillot et al. (2008), Panaroni et al. (2009), Vanleene et al. (2011), Jones et al. (2012), Jones et al. (2014), and Ranzoni et al. (2016), and Li et al. (2007) compared their treated mice with naïve OI mice and did not use sham operated mice receiving placebo. Otsuru et al. (2012) injected MSCs via tail vein into *oim* mice and used PBS injected *oim* mice as their controls. The study of Li et al. (2010) investigated multiple treatment groups with local injections, comparing left vs. right femurs with varying combinations of cells, matrix, and PBS. Finally, Sinder et al. (2020) utilized local femoral injections and compared *oim* cell treated femurs with their contralateral leg as sham controls. Because of the complexity of the stem cell

treatments, particularly those involving irradiation and/or surgery, it is advised that both naïve mice and sham operated controls be compared to stem cell treated OI bone in order to fully evaluate the success of the stem cell treatment to improve OI bone quality.

Similarly, there is a need to examine the effect of stem cell treatment on the bone of different mouse models of OI. The studies meeting the inclusion criteria in this meta-analysis mostly used the *oim* mouse model, a murine model commercially available for decades and widely used to understand OI disease and its bone fragility. Although *oim* mice do not represent the genetic changes commonly observed in human OI, they model bone phenotype of severe OI and are therefore relevant for investigations of bone mechanics and the effect of therapies on bone quality. Only one study meeting inclusion criteria used the BrtlV/+ mice (Panaroni et al., 2009), a model that is instead still not publicly available. The use of diverse mouse models of OI to examine the short- and long-term effects of the engrafted cells on bone composition, structure and mechanics is needed to fully understand how to utilize stem cell treatment for rescuing bone fragility in OI.

Regarding the mechanism of action of stem cells, a new hypothesis in the field, as summarized by Prockop (2017), suggests that transplanted cells are able to produce beneficial effects in animal models and clinical trials by secreting paracrine factors, cytokines, extracellular vesicles, and other soluble factors which can deliver signals for intercellular communication (Phinney et al., 2015; Otsuru et al., 2012; Valadi et al., 2007). Yet, the mechanism behind their positive therapeutic effects is not yet fully understood. The ability to identify and then overexpress the responsible miRNA(s) or other trophic factors may lead to greater therapeutic effects.

As we move forward, genetically modified stem cell treatments, including gene insertion, deletion, and up-regulation, will need to be further explored as they have shown recent promising findings. These studies have built upon the knowledge gained from the aforementioned studies while seeking discovery of additional biological and mechanical improvements. Jones et al. (Jones et al., 2012) investigated the protein CXCR4, shown to influence MSC migration to fracture site, and found that up-regulation lead to increases in bone engraftment and improvements in bone mechanics. Recently, Liu et al. (2020, 2021) showed promising mechanical and structural improvements in the treatment of
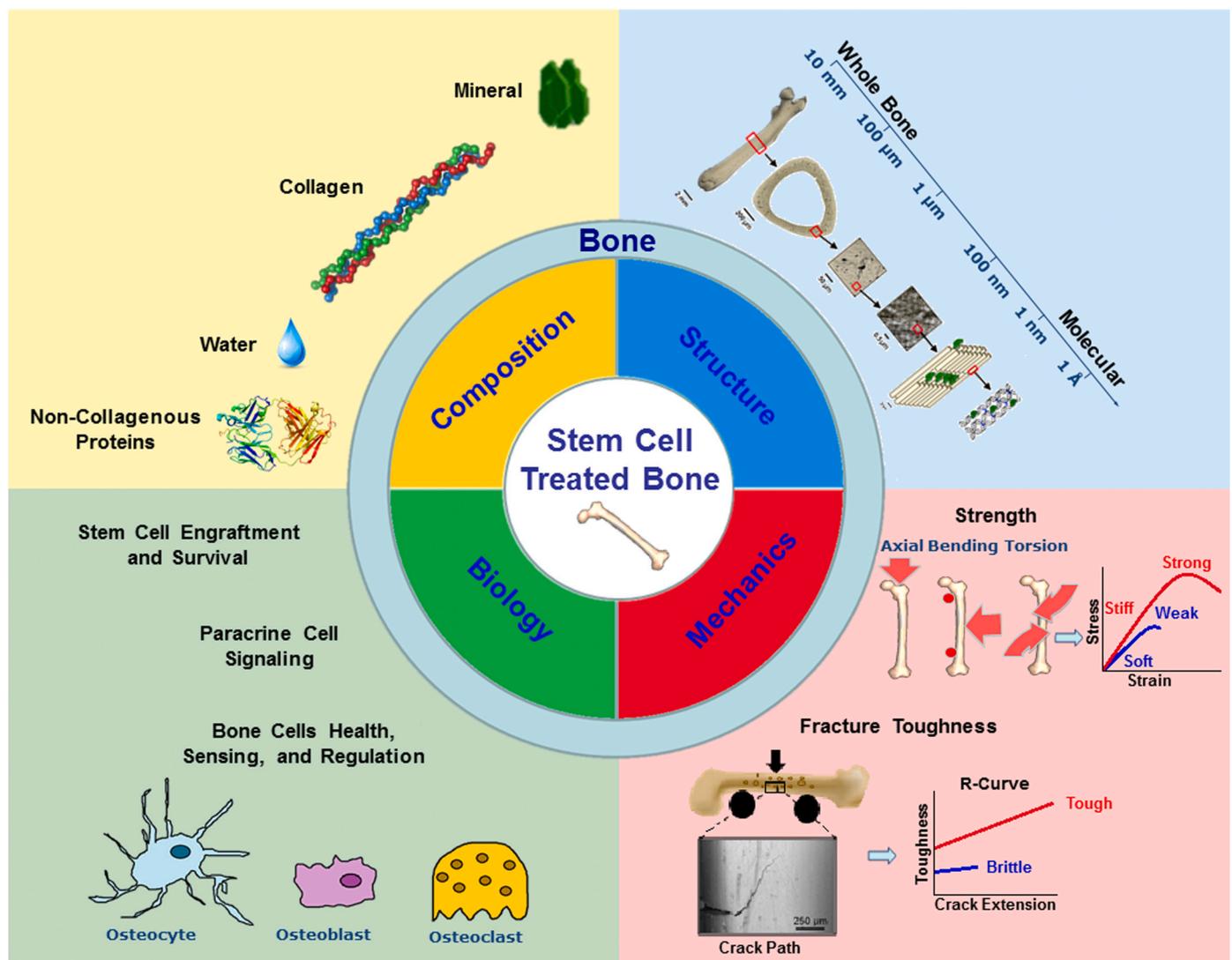

Fig. 3. Properties of bone health to be investigated to assess treatment efficacy. To assess the efficacy of stem cell therapy in bone, it is crucial to investigate bone health in its totality, and thus structural, compositional, biological, and mechanical properties of bone should be examined in detail and considering the relationship between them. Such a comprehensive and interrelated analysis of stem cell treated bone will reveal the mechanisms of stem cells in bone signaling and (re)modeling, and their efficacy to rescue bone brittleness in osteogenesis imperfecta. This interdisciplinary approach to medicine will ultimately guide stem cell therapy to improve bone quality and quantity in mice with brittle bone, and ultimately translate to clinical use to promote health in patients with osteogenesis imperfecta.

an OI type I mouse model with genetically modified adipose-derived stem cells. They investigated genetic modifications with recombinant mouse NELL1 protein and Nell1 gene as well as autologous COL1A1 gene modification (Liu et al., 2020; Liu et al., 2021). These results, among others, show the vast potential stem cell therapy for OI holds.

It is important to note that stem cell treatment for OI comes with limitations. These include amount of available stem cells, challenges related to stem cell identification, isolation and purification, and potential immunological rejection by the host, all of which may influence outcomes such as OI bone mechanical properties. Additionally, pre-conditioning irradiation treatment has been shown to increase osteoclast number, deteriorate material properties of cortical bone, and damage the bone marrow micro-environment for stem cells, implying that irradiation is a major challenge to overcome in stem cell therapy (Barth et al., 2011; Costa and Reagan, 2019; Willey et al., 2008; Cao et al., 2011).

Future research should continue to explore *how* and *why* stem cell therapy improves OI bone quality with the need to understand if these therapies are actually addressing the brittleness in OI bone. The effects of stem cell therapy on OI bone health in its totality must be explored through investigations on the inter-relationship between the biological, compositional, structural, and mechanical properties of bone (Fig. 3). Multiscale and multidisciplinary studies in the future will need to assess bone strength and fracture toughness, together to cell engraftment and mechanisms of action, and use appropriate sham controls, to provide a biological understanding of the mechanical changes that underlie the shown decrease in fracture incidence. As the field moves forward, standardization of research practices to conduct these studies must be implemented. While qualitative histological data has shown the potential of stem cell engraftment thus far, more quantitative results are necessary to refine and distinguish the optimal therapeutic effects with differing cell preparation and transplantation methods. Furthermore, the use of histological sections to count the overall donor cell engraftment over the overall number of cells can over- or under-estimate the engraftment as 2D slices of bone may not be representative of its entire volume. Therefore, new effective protocols and methodologies need to be developed for assessing and quantifying engraftment in the entire bone. In this sense, the development of new biomarkers and quantitative assays to predict the in-vivo efficacy of transplanted cells may greatly help (Prockop, 2017). Investigation of bone cells (osteocytes, OBs and osteoclasts) health, utilizing biomarkers of skeletal and cells cycle regulation, as well as exploring their shape, will aid in the understanding of the biological therapeutic effect of stem cells in OI bone. Moving forward it is important that reagents used in the preparation and culturing of stem cells be thoroughly defined in publications, as cells have the potential to change dramatically in cultures with varying conditions (Prockop, 2017). More studies are also needed to determine the best route of injection, age of injection, or follow up time effect outcomes, that were not possible to be established in this meta-analysis. Finally, due to the nature of these basic science studies, variability and lack of standardization of study characteristics were observed. This indicates the need for non-biased research techniques such as random housing and blinded analyses to be utilized and thoroughly described in the manuscripts reporting the stem cell studies (Hirst et al., 2014). These must be explicitly indicated, and when not met, appropriately discussed in the studies. Review-level limitations of this systematic review and meta-analysis include the lack of variation in the use of mouse models of OI included, as well as possible incomplete retrieval of all eligible studies.

In conclusion, this systematic review and meta-analysis shows the beneficial effects of stem cell therapy in mouse models of OI to reduce fracture incidence and increase maximum load, besides a low cell engraftment. This supports the need for further investigation of the mechanisms by which transplanted stem cells affect bone (re)modeling and bone quality. Particularly, being bone fragility the primary symptom of OI, there is a critical need to determine the fracture toughness of OI bone treated with stem cells so to assess the actual efficacy of the treatment to rescue OI bone brittleness.

Transparency document

The Transparency document associated with this article can be found, in online version.

CRediT authorship contribution statement

Lauren Battle: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Shoshana Yakar:** Conceptualization, Investigation, Writing – review & editing. **Alessandra Carriero:** Conceptualization, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

All authors state that they have no conflicts of interest.

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