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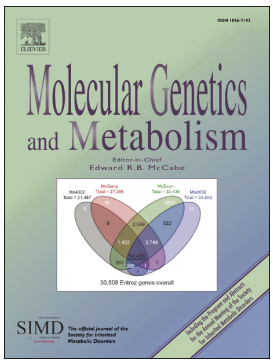

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Unraveling the mystery of Gaucher bone density pathophysiology

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Abstract

Gaucher disease (GD) is caused by pathogenic mutations in *GBA1*, the gene that encodes the lysosomal enzyme β-glucocerebrosidase. Despite the existence of a variety of specific treatments for GD, they cannot completely reverse bone complications. Many studies have evidenced the impairment in bone tissue of GD, and molecular mechanisms of bone density alterations in GD are being studied during the last years and different reports emphasized its efforts trying to unravel why and how bone tissue is affected. The cause of skeletal density affection in GD is a matter of debates between research groups. and there are two opposing hypotheses trying to explain reduced bone mineral density in GD: increased bone resorption versus impaired bone formation. In this review, we discuss the diverse mechanisms of bone alterations implicated in GD revealed until the present, along with a presentation of normal bone physiology and its regulation. With this information in mind, we discuss effectiveness of specific therapies, introduce possible adjunctive therapies and present a novel model for GD-associated bone density pathogenesis. Under the exposed evidence, we may conclude that both sides of the balance of remodelling process are

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altered. In GD the observed osteopenia/osteoporosis may be the result of contribution of both reduced bone formation and increased bone resorption

Keywords: Gaucher, bone, mineral density, resorption, pathophysiology

1. Introduction

Gaucher disease (GD) (OMIM ID: 230800) is an autosomal recessive lysosomal disorder, caused by pathogenic mutations in the *GBA1*, leading to a deficient activity of the lysosomal enzyme β-glucocerebrosidase (GCase) (Enzyme Commission 3.2.1.45). Deficiency of GCase results in the accumulation of glucosylceramide (GL-1) specially, but not limited, to macrophages, and its derivative glucosylsphingosine (Lyso-GL1). Different phenotypes (types I, II and III) and heterogeneity have been described, and signs and symptoms may include hepatosplenomegaly, haematological alterations, skeletal abnormalities and in some cases neurological affection [1].

Skeletal complications in type I and also type III Gaucher patients are the most relevant in terms of morbidity and life quality. Moreover, bone is the most refractory tissue to the specific therapy, such as enzyme replacement therapy (ERT), used for Type I GD. The complete explanation of these problems has not completely elucidated until now. Molecular mechanisms of bone density alterations in GD are being studied during the last years and different reports emphasized its efforts trying to unravel why and how bone tissue is affected. Normal bone density implies a delicate balance of bone homeostasis where bone formation and bone resorption are mutually regulated. Moreover, there is a delicate interaction between bone and immune system where any pathology in one of these systems affects the other. It is well known for many years the involvement of immune system in GD associated with the existence of a chronic inflammatory status that may have a deleterious influence in bone matrix homeostasis [2]

The cause of skeletal density affection in GD is a matter of debates between research groups. According to the mechanisms that participate in bone homeostasis: bone formation and resorption, there are two opposing hypotheses trying to explain reduced bone mineral density in GD. One hypothesis is the existence of increased bone resorption, while the other is an impaired bone formation. In this review, we discuss the diverse mechanisms of bone alterations implicated in GD revealed until the present, along with a presentation of normal bone physiology and its regulation. With this information in mind, we discuss effectiveness of specific therapies, introduce

possible adjunctive therapies and present a novel model for GD-associated bone density pathogenesis.

2. Skeletal manifestations in Gaucher patients

Bone complication in GD is characterized by the presence of Erlenmeyer flask deformity of the femur, atypical bone pain, osteopenia or osteoporosis, extremely painful bone crises, avascular necrosis and pathological fractures including vertebral collapse. Furthermore, osteopenia and osteoporosis occurs earlier and in severe manner in Gaucher patients as compared to healthy individuals and may cause many pathological fractures [3]. Painful episodes could be acute or chronic, predominantly in the pelvis and lower limbs. The presentation observed in some GD patients with mild fever, leukocytosis and a moderate inflammatory syndrome could be misdiagnosed with osteomyelitis [3].

Studies in untreated and treated Gaucher patients have shown a similar prevalence of bone lesions among groups, ranging from 80 to 94% of incidence [4]. In GD registries, the proportion of patients with any bone involvement has been estimated in more than 80%. Interestingly, almost all of the registered patients were on specific treatment. An Argentinian study with 32 patients on ERT revealed that although patients had achieved the therapeutic goals for splenomegaly and hepatomegaly, they presented a high prevalence of bone lesions (84%) where 70% of them were irreversible [5]. The most common bone lesions are medular infiltration and osteopenia [6]. Another research including 32 Gaucher patients, which most of them were on specific treatment, showed significant bone manifestations: 72% of subjects presented with radiological changes, 59% reported bone pain and 50% with diminished bone density [7]. A recent study has used the trabecular bone score to characterize bone micro-architecture and its macroscopic geometry, which revealed an altered score in all of the patients assayed [8], even in those who have normal bone mineral density assessed by densitometry. This result, suggests that disruption of bone trabecular architecture could be present even with normal bone density.

The adipose tissue is a preserved component of the microenvironment of the bone marrow that stabilizes this structure. It has been described that both the quantity and the quality of this tissue are relevant to preserve functional relationship between

marrow fat and bone [9]. When bone marrow fat in lumbar spine was evaluated in Gaucher patients, it has been observed that patients without treatment had a very poor content. However, after ERT treatment, fat fraction increased significantly already after one year [10, 11].

Despite the different methods to evaluate the bone status, there are scarce reports of bone histology from GD patients. In a report of bone biopsies from three patients, the heterogeneity of GD presentation is manifested. Two of these cases presented low bone turnover and resembles a decrease of bone formation, on the contrary, the third biopsy included showed signs of resorption with high number of osteoclasts, inflammatory infiltration and fibrosis [12]. The largest histological study undergone by Dr Zimran´s group from Israel recruited 26 femoral head biopsies, which most of them were from patients on specific therapy. In this study, osteonecrosis and osteoarthritis were a common finding, as well as infiltration with Gaucher cells. Remodelling lines and increased osteoid deposition were evident in most samples, which is evidence of varying stages of bone regeneration [13].

3. Bone homeostasis

3.1 Bone anatomy and physiology

The human skeleton is formed by cortical and trabecular bone. Cortical bone is compact and forms the outer envelope of skeleton, while the trabecular is porous and located in the interior of bone structure. During life, bone is in continuous remodeling process where the old bone is substituted by new one [14]. Trabecular bone houses the marrow cells in a microenvironment with soluble mediators including cytokines and growth factors that participate in the hematopoiesis process [15], indicating a close relation between bone, hematological and the immune system.

The ossification process is performed directly by the action of osteoblasts that proliferate from mesenchymal stem cells (MSCs) in response to the cytokines insulin-like growth factor-I (IGF-I) and transforming growth factor β (TGFβ). The differentiation of MSCs is mediated by the osteogenic cytokines bone morphogenetic protein (BMP) and the Wingless (Wnt) signaling. Wnt proteins are a family of secreted glycoproteins that bind to receptor complexes present at the plasma membrane including low-density lipoprotein receptor-related protein (LRP)-5/6 as well as frizzled proteins.

Binding of Wnt proteins to these membrane receptors acts on the most upstream intracellular component of the Wnt/ β-catenin pathway, the Disheveled (Dvl) proteins. This interaction promotes the phosphorylation and inactivation of GSK3β, which inhibits β-catenin degradation. Consequently β-catenin translocates to the nucleus [16], stimulating the expression of osteogenic genes Runt-related transcription factor 2 (Runx2) and Osterix (Osx) [17-19] (Fig. 1).

One of the osteoblasts functions is the production of bone extracellular matrix that is formed in part by organic components as type I collagen, osteopontin, and osteocalcin, among others. Consequently mineralization of organic matrix takes place by deposition of inorganic material, mainly hydroxyapatite [20]. The osteoblasts that remain embedded in this bone matrix reach the final state of differentiation: the osteocytes. Osteocytes establish a proper communication by their dendritic processes to form a network and participate in the regulation of bone remodeling with osteoblast and osteoclast [21, 22] through the production of both sclerostin and the nuclear receptor κB activated receptor ligand (RANKL). Sclerostin acts as antagonist of Wnt signaling pathway and inhibits BMP signaling, and RANKL promotes osteoclast formation, that is the osteoclastogenesis process [23-25].

Osteoclasts belong to the monocyte/macrophage lineage of hematopoietic origin and are the exclusive cells implicated in bone resorption. The osteoclasts differentiation process involves two key factors: the macrophage colony stimulating factor (M-CSF), generated by osteoblasts, and RANKL, generated by osteoblasts and osteocytes [26]. In this process, M-CSF binds to its c-fms receptor promoting the survival and proliferation of these cells [27, 28], while RANKL binds to its membrane RANK receptor to induce the fusion and differentiation of the precursors towards a mature osteoclast [29]. Bone resorption starts with its attachment to the bone matrix to be removed, acidification of extracellular microenvironment that mobilizes the mineral phase and secretion of matrix metalloproteinases (MMPs) and cathepsin K [30] which degrades organic matrix. The physiological bone remodeling process is schematised in figure 2.

3.2 MSCs differentiation into osteoblasts or adipocytes

MSCs can differentiate into multiple cell types: adipocytes, osteoblasts, myoblasts or chondroblasts. The commitment to each cell type is dependent on cytokines and

growth factors present in the microenvironment. Among these potential outcomes, differentiation towards adipocytes and osteoblasts has special significance in the preservation of bone homeostasis.

It has been demonstrated that age-related bone loss is associated with the increase in differentiation of MSCs into adipocytes with the consequent decline of osteoblasts number [31, 32]. This is in concordance with *in vitro* evidence that showed that adipocyte formation mediators inhibit osteogenesis and vice versa [33, 34]. Several transcriptional regulators participate in osteogenesis, among them Runx2 is the most relevant and is expressed by the osteoblast at all stages of development [18, 35, 36]. After commitment, Osx regulates the expression of many osteoblast differentiation markers [37]. On the other hand, adipocyte differentiation involves the expression of peroxisome proliferator-activated receptor gamma (PPARγ) and members of the CCAAT/enhancer binding proteins (C/EBP) family of transcription factors [38], which are known to be the vital transcription factors in the regulation of adipogenesis [39].

Moreover, transcription factors that regulate osteogenesis and adipogenesis are reciprocally controlled. In particular, Runx2 expression is inhibited by PPARγ thus impeding osteoblast differentiation [40, 41]. The regulation between these cell linages ensures the cross talk of intricate signaling pathways involving the parathyroid hormone (PTH), parathyroid hormone-related protein (PTHrP), TGFβ, BMPs, Wnt proteins [42, 43] and Sirtuin 1 (Sirt1) [44], among others.

The Wnt pathway stimulates osteogenesis through the regulation of expression of transcription factors including Runx2, Osx, and Distal-Less Homeobox 5 (dlx5), along with the inhibition of expression of adipogenic transcription factors C/EBP and PPARγ blocking adipocyte differentiation [45].

At physiological conditions Sirt1 expression is increased during the process of osteoblast differentiation inducing the up-regulation and activity of Runx2 [46, 47]. Additionally, Sirt1 inhibits adipocyte differentiation via interaction with PPARγ to recruit nuclear receptor co-repressor to its target promoters with the consequent inhibition of the expression of PPARγ-target genes [48]. However, in aged bone marrow, accumulation of ROS has the ability to repress the expression and also the enzymatic activity of Sirt1 modulating Wnt pathway favoring the differentiation from

MSC into adipocyte [49]. Other function related with Sirt1 is the ability of maintaining skeleton homoeostasis in inflammatory conditions by inhibiting NF-κB signal [50].

All these evidences showed that during MSC differentiation, osteogenesis and adipogenesis are mutually regulated processes (Fig. 3), but an imbalance could lead to a pathological condition.

3.3 Osteoimmunology

Bone tissue and immune system are closely related in physiological and pathological conditions. Bone and immune cells came from the same microenvironment and interact between each other to execute osteoimmunological functions. Studies performed in mice, have revealed the importance of immune system in bone development and homeostasis under physiological conditions [51].

Recent evidences indicate that osteoblast participate in the process of differentiation of B and T lymphocytes [52, 53]. IL-7 secreted by osteoblast contribute in this event and also is implicated in the maintenance of lymphoid progenitors in bone marrow, indicating the ability to regulate lymphoid lineages [54]. Furthermore, osteoblast-derived Dickkopf-1 (DKK1) acts as inhibitor of Wnt pathway in order to favor hematopoietic reconstitution via inhibition of hematopoietic stem cells senescence and the stimulation of bone marrow endothelial cells to produce epidermal growth factor (EGF) [55].

The overlapping between bone and immune cells is also evidenced by osteoclasts, bone cells that originate from the same precursor of macrophages and dendritic cells. As was mentioned, in physiological conditions, osteoclast formation occurs in the presence of both M-CSF and RANKL cytokines produced by osteoblasts and MSCs that induce osteoclast differentiation from precursor cells, stimulating its activity and survival [56-58]. The RANKL receptor (RANK) is expressed in osteoclasts and its expression is induced by the action of M-CSF on osteoclasts' precursors. The interaction between RANKL and RANK is inhibited by osteoprotegerin (OPG), that act as neutralizing decoy receptor that is secreted by osteoblasts and MSCs [59]. Other cytokines and growth factors could also induce osteoclast differentiation in an inflammatory pathological condition [56]. In this context, the function of RANKL can be replaced by mediators such as TNF-α, Proliferation inducing ligand (APRIL) A, receptor

expressed in the membrane of T lymphocytes (LIGHT), B lymphocyte activating factor (BAFF), insulin-like growth factor (IGF)-I and II, nerve growth factor, IL-6, IL-8 or TGF-β [56]. In the case that the stimuli of M-CSF is absent, osteoclast differentiation can be triggered by vascular endothelial growth factor, hepatocyte growth factor, placental growth factor and FLt-3 [56]. Remarkably, other factors such as IL-23, IL-17, IL-7 and IL-1 are involved in osteoclastogenesis indirectly by inducing RANKL secretion from a target cell [60, 61].

Th17 lymphocytes, not only contribute to osteoclastogenesis through the production of IL-17 promoting local inflammation, but also by expressing RANKL [62, 63] that is able to regulate immune response [64, 65]. The described non canonical pathways have a key role when these cytokines and growth factors are present as occur in the context of pathological bone injury during inflammatory diseases.

On the other hand, osteoblasts are bone cells specialized in bone matrix deposition and regulation of bone balance. In conjunction with MSCs, osteoblasts produce RANKL and OPG [55]. Osteoblast has also a role in hematopoiesis [66]. Another factor that has influence in bone immune system is sclerostin produced by osteocytes. It has been demonstrated in mice models that deficiency in sclerostin not only induced high bone mass, but also a reduction in mature B lymphocyte because of the reduction in the secretion of the chemokine CXCL12 in MSCs [67].

We can conclude that there is a close interaction between the immune and the skeletal systems, emphasizing the importance of the study of the interaction between them during bone pathology caused by infectious and chronic inflammatory conditions.

4. Molecular Mechanisms involved in skeletal GD

4.1 GD and osteogenesis

In this section, we summarized the molecular mechanisms involved in the reduction of bone formation associated to GD as revealed by research studies using animal models as well as *in vitro* cell line studies.

There have been many efforts to develop murine models of GD, starting with a knockout of *GBA1*. This mice presented deficient GCase activity and substrate accumulation in lung, brain and liver, however the animals die within 24 h of birth [68].

Other strategies were analyzed including the introduction of GD associated mutations in *GBA1*; chemical GCase inhibition with conduritol beta epoxide (CBE) and backcross between saposin C deficient mice and GCase V394L/V394L mice, but these models are suitable for the study of neuropathic GD [69-72].

In 2010 the first murine model for the non-neuropathic Type I GD has been developed [73], which recapitulates visceral and bone manifestations through conditional deletion of *GBA1* in hematopoietic and mesenchymal cells [73]. Mice of this model presented a low bone mineral density (BMD) showing a significant osteopenia and reduction in bone formation rate. Osteoblasts from these mice model showed an attenuated proliferation and differentiation due to reduced protein kinase C activity caused by the accumulated lipids glucosylceramide and lyso-GL1. Mistry's group had not detected an increment in osteoclasts formation that could contribute to the observed osteopenia in these murine model. Besides this mice model, two *in vivo* Gaucher models based in zebrafish were developed. One of these models used a morpholino-based gene knockdown for *Gba1* gene; while the other used a stable inheritable mutation generated by a forward genetic approach. Notably, both models lead to a truncated Gba1 protein and demonstrated a marked decrease in bone mineralization through the down regulation of canonical Wnt pathway and an increase in oxidative stress [74].

In the last decade, *in vitro* studies have been carried out using human MSCs from Gaucher patients as well as new cellular models originated by dedifferentiation of Gaucher patients' fibroblasts into induced pluripotent stem cells (iPSCs) or MSCs [75-79]. Using this approach, it was possible to analyze bone matrix formation *in vitro* by differentiating osteoblasts from MSCs in the appropriate culture media. It was observed that osteoblasts derived from Gaucher MSCs deposited less mineral matrix as well as type I collagen and also showed a decreased alkaline phosphatase activity [80-82].

Bone matrix formation activity could be affected by a reduced proliferation of MSCs or its increased cell death. Impairment in proliferation is confirmed in Gaucher patients' MSCs and in *in vitro* MSCs treated with CBE [75, 80], as well as in *in vitro* model of Gaucher MSCs derived from patients' iPSC [81, 82], showing an increased cell number in the G2/M phase of the cell cycle that indicates a decrease in proliferation rate.

Stromal cells cultures from mouse model of GD were also analyzed and showed a decrease proliferation evidenced by a MMT assay [56], however in Gaucher zebrafish model no global differences were observed in proliferation and apoptosis [74]. No changes or predisposition to cell death was observed [82] .

Another explanation of a reduced bone matrix production could rely on an alteration in osteoblasts differentiation. The expression of osteogenic key genes such as *Runx2* and *Osx* are significantly reduced in Gaucher animal models as well as downstream genes such as alkaline phosphatase (*Alp*) and bone sialoprotein (*Bsp*) [73, 74]. *In vitro* studies in Gaucher osteoblasts derived from Gaucher MSCs corroborate these expression abnormalities where alkaline phosphatase (*ALP*), Type I Collagen (*COLA1*), osteocalcin and *RUNX2* gene expression were lower than control cells [81, 82]. A described pathway associated to the regulation of these genes is the canonical Wnt signalling, whose activity is diminished in the Gaucher zebrafish model through demonstration of significantly reduced accumulation of β-catenin and increased levels of the negative regulators Gsk3β, Axin1 and Dkk-1 [74]. Besides, higher levels of Dkk-1 were detected in supernatants of *ex vivo* Gaucher MSCs compared to control cells [75]. Furthermore, Wnt pathway signaling was studied in *in vitro* models of Gaucher osteoblast derived from Gaucher iPSCs, in which a decreased β-catenin levels as a consequence of increased activity of GSK3β was demonstrated [81]. Even more, Dvl proteins levels are reduced in GD fibroblasts and in human fetal osteoblasts treated with CBE [83]. Another player in this pathway is adiponectin, who was recently shown that facilitates osteogenesis through Wnt/β-catenin pathway [84]. Interestingly adiponectin is reduced in Gaucher patients, likely associated to their low grade chronic inflammation [85].

Apart from Wnt pathway, a tight link between oxidative stress and reduced osteogenesis has been described [86], where molecular analysis and *in vivo* labelling in Gaucher models demonstrated the occurrence of an increased oxidative stress [74, 87]. These results confirm an early dysfunction of the osteoblast population associated to reduced osteoblast differentiation and bone mineralization in GD. Furthermore, this global and early cellular alteration could be the result of an increase in oxidative stress.

4.2 GD: osteogenesis vs adipogenesis

Most of the diseases that present bone loss with an inflammatory component evolve towards an alteration in the composition of adipose tissue of the bone marrow, where MSCs direct their differentiation towards adipocytes instead of osteoblasts [9, 88, 89]. *In vitro* model of osteoblasts differentiated from Gaucher patients' MSCs has demonstrated that the accumulation of lactosylceramide and glucosylceramide lead to a reduction in calcium deposition giving, in part, an explanation of low bone density observed in GD [81]. Indeed, a recent study showed that the accumulation of glucosylceramide stimulates adipogenesis and inhibited osteogenesis by direct interaction with PPARγ through its A/B domain [79]. This evidence, could suggest that the accumulation of glucosylceramide in Gaucher MSCs would be able to induce adipogenesis. Our group have cultured Gaucher MSCs in adipogenic media, and observed a higher expression of *PPARγ* gene and lower levels of *RUNX2* in Gaucher adipocytes than controls, suggesting that GD cells are preferentially induced to differentiate into adipocytes but accumulates lesser lipid droplets [82, 90], as also was observed by Campeau's group [76]. These observations are compatible with bone marrow fat fraction analyzed in Gaucher patients with quantitative chemical shift imaging (QCSI) before treatment [10].

4.3 GD and Osteoclastogenesis

Osteoclasts originate from the fusion of osteoclast precursors belonging to the monocyte/macrophage lineage, giving rise to multinucleated cells with tartrate-resistant acid phosphatase (TRAP) activity capable to resorb bone. A high number of circulating osteoclast precursors have been reported in several bone diseases associated with bone loss [91] whose precursors are recruited from peripheral blood mononuclear cells (PBMCs) [92] followed by osteoclast formation through stimulation by RANKL and M-CSF [93].

On one side, *in vivo* studies in Gaucher mice and zebrafish models have not shown differences in TRAP-positive osteoclast formation in comparison with controls animals, suggesting a normal osteoclast activity [73]. However, although histological studies from bone biopsies from GD patients are scarce, they can present evidence of bone resorption in human patients [12]. On the other side, *in vitro* evidence based on

chemical and Gaucher patient derived cell models unravelled alterations in osteoclastogenesis due to an increased osteoclast generation and bone resorption. The first *in vitro* chemical model based on the addition of CBE demonstrated that GCase deficiency in MSCs and PBMCs cause the secretion of proinflammatory cytokines as IL-8, IL-6, TNF-α and chemokines as MCP1 that induce osteoclastogenesis leading to formation of mature and active osteoclasts [80, 94]. Moreover, Mucci et al. have revealed the implication of TNF-α as well as T cells in osteoclastogenesis induction through RANKL. The presence of osteoclast was revealed by the expression of TRAP positive multinucleated cells that express vitronectin receptor and has the ability to secrete MMP-9 and resorb dentine [94, 95]. The knowledge that osteoblasts, osteocytes and MSCs can regulate bone resorption *in vivo* by the production of RANKL led to study their involvement in osteoclastogenesis. At the moment, it has been demonstrated that conditioned media from GCase deficient osteocytes and MSCs induce an increase differentiation of monocytes to osteoclasts mediated by RANKL [75, 96]; and the culture of Gaucher patients' PBMCs either with M-CSF or M-CSF and RANKL induced higher number of active resorptive osteoclasts than control PBMCs [97]. Moreover, a similar effect is observed when control PBMCs are cultured with glucosylceramide [98].

As it has been mentioned before, an inflammatory context could enhance osteoclastogenesis process through cytokines and GD is one of these disorders associated to an inflammatory context [2]. In this regard, the synergic effect of IL-1β with RANKL both secreted from Gaucher MSCs has been described [90]. Furthermore, the culture of Gaucher patients' PBMCs has evidenced a secretion of other pro-osteoclastogenic cytokines, including TNF-α and RANKL [97]. Finally, it is interesting to mention that a clinical correlation between *in vitro* osteoclast differentiation and bone mineral density has been revealed in GD1 patients [96, 98, 99]. Thus, these studies reinforce the idea there could also be an involvement of osteoclasts in bone density pathology of GD.

5. Novel model of GD bone density pathogenesis

Taking into account the reviewed evidence, we could be able to elaborate a novel model of pathogenesis that is schematised in Figure 4.

BMD in physiological conditions is maintained by a delicate balance between matrix formation and resorption. The evidence based on both animal and *in vitro* models of GD highlight the reduced bone formation activity because of a disturbed proliferation and differentiation into osteoblasts progeny of MSCs, along as a reduced osteoblast bone formation function. On the other side, osteoclastogenesis and concurrent resorption activity by osteoclasts has been demonstrated in *in vitro* studies. Also, *ex-vivo* studies using PBMCs from GD patients showed an increased number of osteoclast precursors and higher osteoclastogenesis rate that correlate with densitometric studies as assessed in clinical setting of the same patient. Bone histological studies from GD patients are scarce, and reveal heterogeneity of processes, where both altered formation and resorption activity has been shown.

Under the exposed evidence, we may conclude that both sides of the balance of remodelling process are altered. In GD the observed osteopenia/osteoporosis may be the result of contribution of both reduced bone formation and increased bone resorption. This knowledge could lead to the discovery of new therapeutic treatments that could be co-administered with the current therapy to improve patient's response.

6. Effect of therapies on skeletal manifestations in Gaucher patients

In the present time, when there are several specific treatments for Gaucher patients available, bone affection is among the main concerns for physicians who treat these patients due to the variability of bone manifestations, which leads to a high morbidity rate and reduction of life quality [8]. Despite the complications, bone affection in Gaucher disease could be alleviated by specific Gaucher therapies and/or bone-targeted ones.

There are two approved approaches of Gaucher disease therapies: enzyme replacement (ERT) (imiglucerase, velaglucerase alfa, and taliglucerase alfa) and substrate reduction therapy (SRT) (miglustat and eliglustat). The effectiveness of these treatments depend on several factors: the age of diagnosis, the age when the patient initiate the therapy, the bone affection at the beginning of the therapy and the type of bone affection (reversible vs irreversible)[100]. Lyso-GL1 analysis could be used to

monitor the outcome of therapies, which shows a significant reduction levels at the beginning of the therapy but becomes less robust over time [101].

ERT has been demonstrated to be effective in reducing bone crisis, pain and infarction, all complications associated with bone marrow infiltration, whereas the improvement of BMD require a very long treatment span. Despite treatment, avascular osteonecrosis is irreversible. Gaucher disease may affect the achievement of optimal peak bone mass during adolescence, leading to higher prevalence of osteopenia/osteoporosis and fragility fractures in adulthood [102]. Improvement of BMD and growth rates in pediatric patients on ERT has been observed. Moreover, patients suffer fewer bone crises and occurrences of skeletal events, although ERT does not completely prevent them [3].

Clinical trials results for SRT showed increases in BMD [103, 104]. Patients who were stable on ERT and were switched to SRT (miglustat) showed no change in bone pain [105]. Up to now, miglustat efficacy on bone symptoms remains poorly evaluated [106] and long term data for eliglustat is needed to fully evaluate its effects on bone disease. Another SRT drug, ibiglustat, is in clinical trials [107].

A new approach of specific treatment for GD is under clinical trial, and involves the use a chemical chaperone ambroxol, an FDA approved mucolytic drug. A pilot study of the use of Ambroxol showed improvement in hematological parameters in GD 1 patients [108], as well as bone manifestations in case reports [109, 110]. A recent comprehensive study on bone involvement using MRI revealed a reduction of bone marrow score in patients on specific treatment, both ERT and SRT. However, bone complications such as bone infarcts, avascular necrosis and bone crises are not completely eliminated [111], thus researchers introduce the idea of adjuvant therapies, targeted to correct a bone pathophysiological molecular mechanism. The first approach was the use of drugs typically used in osteoporotic patients, such as antiresorptive drugs or anabolic ones. Few studies on the effects of bisphosphonates have been reported in the literature (41, 42) but with heterogeneity of results and design of study [112]. The use of alendronate could be indicated as an adjuvant therapy in subjects with a marked reduction in bone mass [113], however there was no benefit toward improvement of the focal lesions typically seen in patients with GD [114]. Teriparatide was shown to increase BMD in a patient with Gaucher disease

[115]. Another proposal is a combination therapy with romosozumab, a monoclonal antibody that binds sclerostin, leading to its dual effect of increasing bone formation and decreasing bone resorption [116].

Therapeutic drugs directed to specific molecular target mechanisms have been proven in models of Gaucher disease. Activation of Wnt/β-catenin pathway by CHIR99021 was effective to induce bone formation *in vitro* [81]. Moreover, two anti-inflammatory drugs, anakinra and pentosan polysulfate were able to reduce osteoclastogenesis in Gaucher *in vitro* models [82, 117].

The effectiveness of these drugs in *in vitro* models of Gaucher disease or in osteoporotic patients may represent a proof of concept for the design of clinical trials in order to extrapolate these results into clinic.

Table I. Gaucher specific therapies. ERT: Enzyme Replacement Therapy; SRT: Sustrate Reduction Therapy

Therapy	Drug	Comments
ERT	Imiglucerase Velaglucerase alfa Taliglucerase alfa	FDA approved
SRT	Miglustat Eliglustat	FDA approved
	Ibiglustat	In clinical trial
Chaperone	Ambroxol	In clinical trial

Fig 1. Osteoblast maturation and differentiation mechanisms. Osteoblast precursors proliferate in response to IGF-I and TGFβ. IGF-I binds to the receptor tyrosine kinase IGFIR to activate MAPK pathway in osteoblasts and regulates RUNX2 transcription **(1.)**. Additionally, TGFβ regulates RUNX2 transcription by phosphorylating SMAD2 and SMAD3. Osteoblast differentiation is mediated by BMP-2 and Wnt signaling **(2.)**. Binding of bone morphogenetic protein (BMP) -2 to its receptor (BMPR) phosphorylating SMAD1 and SMAD5 proteins leads to the upregulation of RUNX2 and osterix (OSX), two transcription factors that control osteogenesis **(3.)**. WNT proteins interact with the receptor frizzled and co-receptor LRP5 or LRP6 and then activate Dvl proteins promoting the phosphorylation of GSK3β to activate a signaling pathway that stabilizes cytoplasmic β-catenin **(4.)**. Stabilized β-catenin is then translocated to the nucleus to induce genes that promote bone formation *RUNX2, OSTERIX* and *BMP-2* **(5.)**. Activation of RUNX2 and/or OSX leads to increased expression of osteoblast-specific genes, such as alkaline phosphatase and osteocalcin.

Fig 2. Osteoblast and osteoclast differentiation and interaction. Osteoblasts (Ob) and adipocytes (Ad) derived from mesenchymal stem cells (MSCs). Adipocyte differentiation involves the expression of the transcription factors PPARγ and C/EBPβ **(1.)**, and osteoblast differentiation involves the BMP and Wnt signaling that stimulate Runx2 and Osx expression **(2.)**. Megakaryocyte and mature osteoblasts secrete TGF-β and IGF-1 that induced pre-osteoblasts differentiation **(3.)**. IGF-1 and TGFβ also become incorporated into bone matrix and may be released later during resorption **(9.)**. Differentiated osteoblasts produce organic components such as type I collagen, osteopontin and osteocalcin; and inorganic components as hydroxyapatite, constituting the extracellular matrix. When osteoblasts remain embedded in the bone matrix get its final state of differentiation: the osteocyte (Os) **(4.)**. Osteoblast eventually become lining cells **(5.)** or undergo apoptosis **(6.)**. Osteoclasts are differentiated from monocyte/macrophage precursors of hematopoietic stem cells origin (HSC). M-CSF is secreted by osteoblast and RANKL produced by osteoblast and osteocytes promote the survival, proliferation and differentiation of osteoclast precursors to osteoclasts (Oc) **(7.)**. Osteoclasts constitute the exclusive cells involved in

bone resorption. They create an acidic microenvironment that mobilizes the mineral matrix through secreted enzymes, MMPs and cathepsin K, among others **(8.)**.

Fig 3. Interplay between mediators in mesenchymal stem cells (MSCs) differentiation to osteoblasts (Ob) or adipocytes (Ad). MSCs are pluripotent progenitor cells that are able to differentiate into several cell types, including adipogenic and osteogenic lineages depending on the stimulation with distinct growth and differentiation factors. The lineage-specific differentiation is a multiple-stage and well-coordinated process regulated by master regulators, such as Runx2 and Osterix for osteogenesis **(1.)**; and PPARγ and C/EBPβ for adipogenesis **(2.)**. These factors appear reciprocally regulated, RUNX2 expression is induced by SIRT1 and WNT and inhibited by PPARγ **(1a)**. Additionally, SIRT1 and WNT inhibits adipogenesis: Wnt signaling maintains preadipocytes in an undifferentiated state through inhibition of the adipogenic transcription factors C/EBP family transcription factors and PPARγ **(2a)**. Additionally adiponectin, secreted by differentiated adipocyte facilitates osteogenesis through Wnt/β-catenin pathway **(3.)**.

Fig 4. Altered mechanisms in Gaucher bone pathophysiology. **1.** Osteoclastogenesis induced by pro-inflamatory cytokines (TNF-α, RANKL, IL-1β) from Gaucher PBMCs, T cells, Os and MSC, as well as increased numbers of osteoclast precursors. **2.** Accumulation of GluCer (**2a)** induced PPAR-γ which is also overexpresed at genetic level in MSC (**2b)**. **3.** MSCs are mainly stuck in phase G2 of the cell cycle. **4.** Ad differentiation: **4a.** Lower lipid droplets were observed; **4b.** Subexpresion of RUNX2 and overexpresion of PPARγ. **5**. Reduced bone matrix deposition because of altered Ob differentiation caused by: **5a.** Inhibition of Wnt pathway leads to reduced expresion of DVL and high activity of GSK3 leading to phosphorilation of β-catenin, which is degraded by the proteasome; **5b.** Higher levels of ROS were detected; **5c.** Increment in lactosylceramide and GluCer; **5d.** Subexpresion of RUNX2/Runx2, COLA1/ColA1, ALP/Alp, Bsp and Ocn. PBMC: Peripheral blood mononuclear cell; Os: osteocyte; Oc:

osteoclast; MSC: mesenchymal stem cell; Ob: osteoblast; Ad: adipocyte; GluCer: Glucosylceramide

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